Studies on Dieback of Buffel Grass (*Cenchrus ciliaris*) in Central Queensland

A thesis by:

Sandrine Makiela

B.App.Sci. (Biol), B.Sci.Biol. (Hons)

Doctor of Philosophy

Central Queensland University

Faculty of Sciences, Engineering and Health

School of Biological and Environmental Sciences

March 2008

Abstract

Buffel grass (*Cenchrus ciliaris*) is an introduced, summer growing, perennial tufted tussock grass which is used extensively in improved pastures in the grazing industry. Since 1993 there has been an increasing level of dieback in buffel grass in districts of Central Queensland districts, involving red leaf symptoms and occurring in roughly circular patches. There is a potential for this condition to destroy large areas, ultimately resulting in loss of production for beef, dairy and sheep farmers who use this grass in improved pastures.

This is the first multifaceted study of buffel grass dieback (BGD). Areas showing signs of dieback have previously been the subject of extensive testing for soil fertility factors, soil chemistry, nematodes and phytoplasmas, with few conclusive results. Therefore, one of the aims of this project was to find the cause of buffel grass dieback. Specific objectives included describing the plant and field symptoms, determining factors responsible for plant death, and determining the method of spread.

A complete description of the symptoms was made at plant, patch and paddock levels. Symptoms of Buffel Grass Dieback (BGD) presented as a reddening of the leaves starting from the tip and progressively moving towards the ligule. The red symptoms range from bright red, to dark red, to bronze (RHSPCC red group 45: A, B; 46: A, B; greyed-orange group 166: A; 177; A) (The Royal Horticultural Society, 2001). Symptoms first appeared on the tips of the older leaves and progressively moved down the leaf. The next oldest leaf then showed symptoms, and so on, with the youngest leaf showing symptoms last. Any tillers followed the same pattern, regardless of whether symptoms on the primary shoot had progressed past the point at which the tiller was produced. The amount of time from new growth to the appearance of the red symptoms seemed to be directly proportional to the amount of rainfall. That is, the more rain, the longer it took for symptoms to develop. The amount of subsequent rainfall seemed to influence the time it took for plants to succumb to the condition. That is, when there was adequate water and lush growth plants grew faster than the spread of the condition. When plants became water stressed, the condition overtook growth and the plants succumbed.

Symptomatic leaves did not always have a clear red-green boundary. Occasionally, BGD symptoms progressed faster down one half of the leaf. Red symptoms were invariably more vivid on the adaxial surface of the leaves than on the abaxial surface. Roots of affected plants appeared stunted compared to roots of unaffected plants. Roots of affected plants often displayed soft, darker, ovoid sunken regions, which were possibly lesions.

The BGD condition appeared to become dormant as buffel grass became dormant. That is, if the dieback condition killed the plant before the onset of dormancy, no new shoots were produced subsequent to a rainfall event. However, if dormancy occurred before the plant succumbed to the condition, new shoots were produced after rain, and the cycle repeated with symptoms first appearing in the oldest leaf.

Patches were roughly circular and ranged from 2 m diameter to over 60 m diameter. Adjacent patches often coalesced and further enlarged. Symptoms first appeared on the periphery of an existing patch, where during the last cycle the plants had become dormant before succumbing to the condition. Symptoms progressively moved outwards from the periphery of the patch, at a rate of approximately 5 cm per week. Patch spread was irregular and did not correspond with soil compaction or land slope, though the condition may spread more rapidly downhill due to runoff.

BGD affected plants weighed approximately two thirds that of unaffected plants. They were noticeably shorter and had shorter leaves and internodes, with the difference in height attributed to internodes rather than leaf length. BGD affected plants also had fewer tillers than unaffected plants of the same age. Although the numbers of leaves per tiller were the same as unaffected plants, the overall result was a decreased amount of foliage available for grazing, thereby decreasing productivity of livestock. In fact, the loss of productivity was twofold, since cattle had been observed to selectively graze unaffected plants.

BGD affected plants had fewer seed heads, shorter seed fascicles, and a higher proportion of non-viable embryos compared to unaffected plants. Therefore, not only did BGD affected plants succumb and die, but there were fewer seedlings to replace them. This could have detrimental consequences for the sustainability of an improved pasture.

At the cellular level, there was no discernable difference in cell size between BGD affected plants and unaffected plants in either roots or leaves. However, the roots of BGD affected plants were more damaged at the cellular level, with the cortex mostly sloughed off and the mesophyll cells disrupted.

The bulliform and mesophyll cells of BGD affected leaves were more irregular in shape. The bundle sheath cells of BGD affected leaves appeared disrupted, with chloroplasts not in their usual alignment. There also seemed to be a breakdown of chloroplasts.

The leaf pigment data concurred with the premise of a breakdown of chloroplasts. Red symptomatic leaves had lower concentrations of chlorophylls *a* and *b* compared to green leaves on the same plant. Red symptomatic leaves also had higher concentrations of anthocyanins and carotenoids. It appears that, in red symptomatic leaves, chlorophylls were being destroyed and anthocyanins were being excessively produced.

There was no discernible difference in the phloem vessels of BGD affected and unaffected plants, both in the roots and the leaves. However, the xylem of both roots and leaves was partially occluded by structures tentatively identified as tyloses. These structures could also have been local accumulations of phenols or polyphenols, or in some cases the remnants of partially decomposed cells. These occlusions seemed more severe in the roots than in the leaves. Possible inclusion bodies were also found in the mesophyll cells of BGD affected leaves. Inclusion bodies are usually a sign of pathogen infection. However, there were no pathogens detected in the histology work.

Chemical analyses were made of BGD affected plants, as well as of the soil in which they were growing, concluding that both plants and soil in the BGD affected paddock surveyed were deficient in nitrogen, phosphorus, sulfur and zinc.

A survey was made of other plant species present in the vicinity of the dieback condition, with particular attention given to those species which have reported allelopathic effects. In addition, a study was made on other plant species which also appear to be affected by the dieback condition. Microbial isolations were regularly made from both plant and soil material. The isolates obtained were tested for proof of pathogenicity using Koch's Postulates, but none proved to be the causal agent of BGD.

The mode of transmission of the condition was studied, and BGD was found to be soilborne. Whether root contact is necessary for successful transmission was not established.

Possible methods of controlling the condition were investigated. While none of the treatments successfully controlled the condition, one of the treatments investigated, Amistar (a systemic fungicide), greatly reduced symptom severity.

Although the cause of BGD was not found, several important discoveries were made concerning its effect and spread, and many possible causes of the condition were eliminated. It is likely that BGD is caused by a disease complex, with potential pathogens including soilborne fungi and/or viruses. Several abiotic factors such as water and nutritional stress may be contributing causal agents, weakening the plants and making them more susceptible to a pathogen.

More work is needed to conclusively identify the primary causal agent of this potentially costly condition.

Table of Contents

List of Tables

List of Figures

Publications Arising From the Thesis

Makiela, S., Harrower, K.M. & Graham, T.W.G. (2003) Buffel grass (*Cenchrus ciliaris*) dieback in Central Queensland. *Agricultural Science*. **16**(2): 34-36.

Makiela, S. & Harrower, K.M. (2008) Overview of the current status of buffel grass dieback. *Australasian Plant Disease Notes*. **3**(1): 12-16.

Acknowledgments

I would like to thank my supervisors, Associate Professor Keith Harrower and Dr Gavin Graham, for patiently guiding me through the project. Also, Professors David Midmore and Kerry Walsh for their valued advice, and the technicians: Charmain, Tanya, Rob, Barry, Paul and Graeme, for their assistance with methods and equipment.

I need to thank my sponsors, Meat & Livestock Australia, for funding this project. The assistance of the QDPI is greatly appreciated in lending me equipment. I also thank Maurie Conway and Peter Agius of QDPI for their help with field work.

I am very grateful to the primary producers who gave me free access to their properties. I also wish to thank them for lending me equipment and assisting me in every way.

Thank you to David Reid, QDPI, for statistical support. Last but certainly not least, thanks to all the volunteers who helped in this project: Noel Sammon, Elen Klop, Hannah Abbott, Wendy Dockerty, Helen Morison, Pamela Malyzec and Anthony Parr. Without these people much of my work would not have been possible.

Special thanks to my family and friends.

Declaration

I declare that the main text of this thesis, unless otherwise stated, is my own work and has not been submitted in any other form at any University or institution. Information derived from other sources has been acknowledged in the text and a list of references is given.

Name: Sandrine Makiela

Signed:

Date:

Table of Abbreviations

AS	Australian Standards
BGD	Buffel Grass Dieback
CEC	Cation Exchange Capacity
CQ	Central Queensland
CQU	Central Queensland University
CSBP	Cumming Smith British Petroleum (company)
cv.	Cultivar
DPX	Dibutyl phthalate, Polystyrene resin, Xylene
EC	Electrical Conductivity
ESP	Exchangeable Sodium Percentage
GLM	Generalised Linear Model
ICP	Inductively Coupled Plasma (Spectrophotometer)
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectroscopy
ISTA	International Seed Testing Association
LSD	Least Significant Difference
RHS	Royal Horticultural Society
RHSPCC	Royal Horticultural Society Plant Colour Chart
SE	Standard Error (of the mean)
s.e.d.	Standard Error of Difference
TTC	1,3,5 triphenyl tetrazolium chloride

Chapter 1

Introduction

1.1 Purpose of Research

The significance of this research is that large areas of sown pastures planted with buffel grass (*Cenchrus ciliaris*) were observed to be dying. Primary producers who use buffel grass as a part of their improved pastures are losing valuable grazing land and consequently suffer a loss in animal production. Buffel grass is adapted to five million hectares across northern Australia and is the dominant sown pasture species for the most productive sector of the beef industry. Failure to diagnose and correct this problem could jeopardise the production potential of a significant part of the beef industry.

The aim of this project was to find the cause of Buffel Grass Dieback (BGD). Specific objectives were:

1. to describe the plant and field symptoms of BGD, including its spread,

2. to determine the effects of BGD on buffel grass,

3. to identify the causal agent(s) of BGD, in addition to factors responsible for symptoms and plant death.

1.2 Literature Review

1.2.1 Buffel Grass History

Buffel grasses are native to drier regions of Africa, Saudi Arabia, India, Indonesia and Southern Asia. Two species, *Cenchrus ciliaris* (buffel grass) and *C. pennisetiformis*

(Cloncurry buffel), were accidentally introduced into Australia on the north-west coast of Western Australia in the harnesses of Afghan camels between 1870 and 1880. In the 1920s *Cenchrus setiger* (Birdwood grass) was introduced from India (Humphreys, 1967; Cavaye, 1991; Eagles *et al.*, 1992; Hall, 2001). Since then, over 300 accessions of these three species have been introduced by Government agencies and pastoralists. No new cultivars have been released since 1970 and there are now only three or four commercially available.

Buffel grass was first introduced to Central Queensland (CQ) in 1928, when a State Department of Agriculture officer, G.B. Brooks, sowed buffel grass seed obtained from Port Hedland at "Archer", Rockhampton. The three main cultivars currently in use in CQ are 'Gayndah', 'American' and 'Biloela'. The Gayndah cultivar was introduced from Kenya in 1930 and has since been sown throughout tropical Australia. The American cultivar was imported from Texas A. & M. University in 1960. The Biloela cultivar was released in the mid 1950s after trials at the Biloela Research Station (CQ) (Humphreys, 1967; Paull and Lee, 1978; Cavaye, 1991).

1.2.2 Species Description

Morphology

Buffel grass (*C. ciliaris*) is a summer-growing, tufted perennial grass which grows to 12 - 120 cm tall, although up to 170 cm is possible under good conditions. Tillers originate from the subterranean crown, and stems are often branched. The seed head is cylindrical, 2.5 - 15 cm long and 8 - 16 mm in diameter. Leaves are generally 7 - 30 cm long and 3 - 8 mm wide. The rachis of the seed head is a serrated stalk to which clusters of one to three spikelets are attached by very short stalks. The spikelets are attached and the

pedicels are never swollen (Paull and Lee, 1978; Food and Agriculture Organisation of the United Nations, Accessed 2001).

Distribution

Buffel grass is now found throughout sub-humid and semi-arid Australia as it is well adapted to a wide range of soils and climates. It is naturalised in approximately five million hectares across northern Australia (Hall, 2001) (refer Figure 1, p.5), and is also gaining popularity in some southern states. Buffel grass pasture development mainly takes place in brigalow, poplar box, gidgee, softwood and mulga communities (Cavaye, 1991).

In Queensland buffel grass is well adapted to over 2.4 million hectares of land. The three main buffel grass regions in Queensland are:

- The southern region which includes the Western Downs, Maranoa and Warrego. Most development has been in brigalow or poplar box communities. An estimated one million hectares are under buffel grass in this region.
- The central west from Tambo to Aramac and Longreach to Jericho, including Blackall, Barcaldine and Isisford. Most development has been in gidgee scrub, brigalow, ironbark and poplar box woodlands. About half a million hectares are under buffel grass in this region.
- The central Queensland region from Rockhampton to Alpha and Nebo to Taroom. Almost all development has been in brigalow and softwood scrub areas. An estimated one million hectares are under buffel grass.

Less extensive areas of buffel grass occur from Gayndah to Monto, and in the south-west region centered on Charleville (Paull and Lee, 1978; Cavaye, 1988, 1991).

Soil Composition, Rainfall and Climatic Requirements

Buffel grass establishes well on loose, friable soils. It is more difficult to establish in heavy clay soils or soils with a hard setting surface (Paull and Lee, 1978; Cavaye, 1988, 1991; Muller, 2001). The optimum soil pH for buffel grass is 7 - 8, but it can grow on soils with a pH as low as 5.5 (Paull and Lee, 1978; Food and Agriculture Organisation of the United Nations, Accessed 2001). Reasonable fertility is necessary for good pasture development. Soils higher in nitrogen and phosphorus are best (Cavaye, 1991; Muller, 2001). Many cultivars of buffel grass are resistant or slightly resistant to soil salinity (Graham and Humphreys, 1970; Russel, 1976).

Buffel grasses are very drought resistant. The main buffel grass areas in Queensland are in the 375 – 750 mm rainfall zones. It does not grow as well in higher rainfall areas (Paull and Lee, 1978; Food and Agriculture Organisation of the United Nations, Accessed 2001).

Growth of buffel grass seedlings is checked by frost but death occurs only in areas of prolonged frost. Established plants are unaffected at temperatures of -5 to -10ºC, but the minimum temperature for growth is 5 – 16ºC. Optimum growth temperature is around 30ºC (Paull and Lee, 1978; Food and Agriculture Organisation of the United Nations, Accessed 2001).

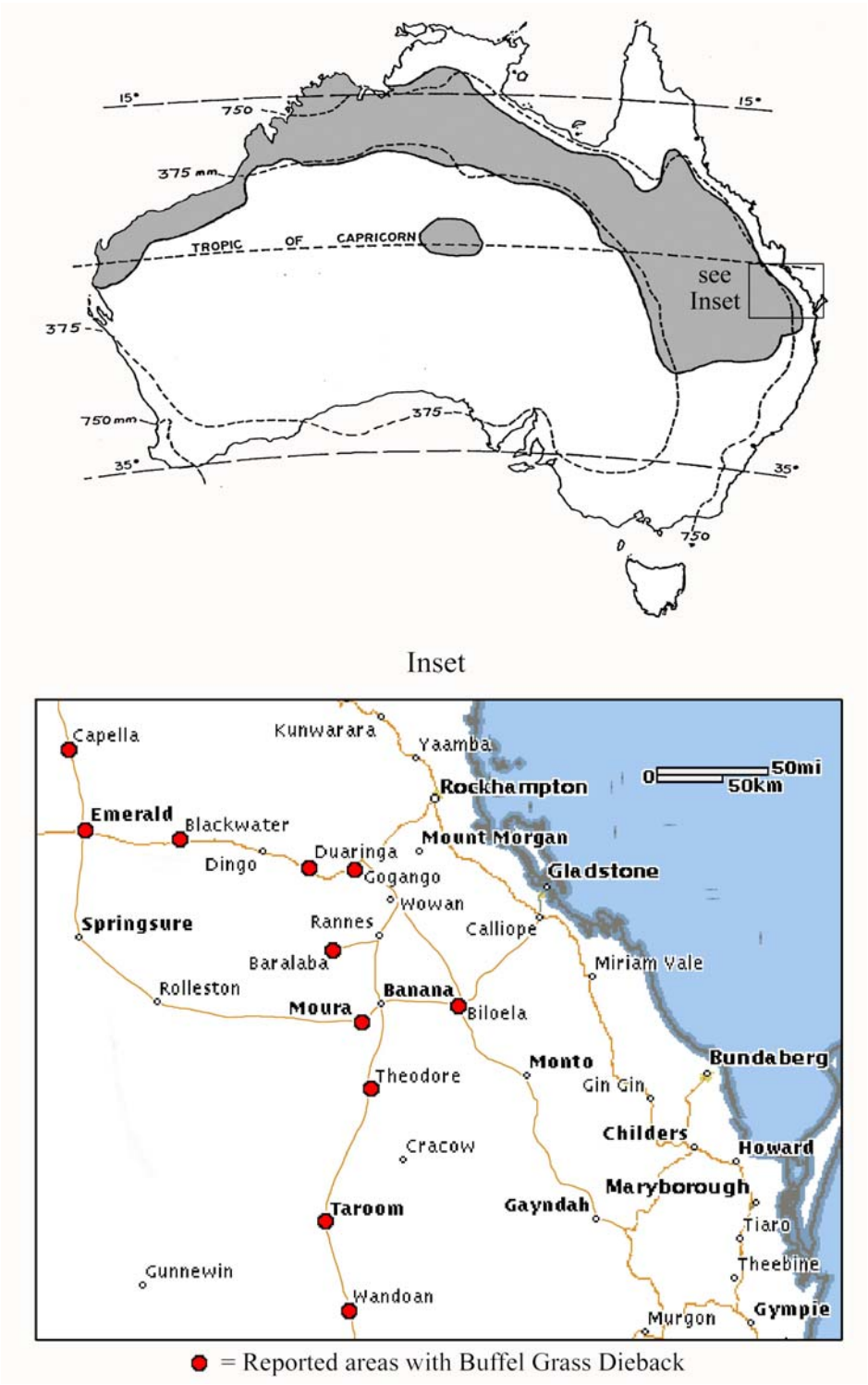


Figure 1 – Estimated areas with buffel grass (*Cenchrus ciliaris*) pastures across northern Australia (shaded areas). Areas with the dieback condition (as reported by Graham and Conway, 1998) are highlighted red in the inset.

(adapted from Silcock, pers.comm. and Google maps)

Reproduction

Buffel grass is grown commercially for seed, but is usually also grazed at the same time. Generally, a pasture is grazed until spring, when the livestock are removed from the paddock and the plants are slashed, to ensure that an even height seed crop develops. Once seeds develop, the paddock is harvested (this can occur over several cycles of growth and seed harvest, depending on the cultivar), and cattle are brought back in for grazing (Paull and Lee, 1978).

Buffel grass is a very prolific seed producer. When commercially grown for seed, seed yields range from 10 - 50 kg/ha every harvest (Paull and Lee, 1978; Cavaye, 1991). It has a prolonged flowering period and up to three harvests are possible during summer (Cavaye, 1991). Bristles around the fascicle ensure that seed dispersal ability is high, either by wind dispersal or by adherence to passing animals. Seed dormancy is generally long, potentially lasting for 12 months or longer (Hacker and Ratcliff, 1989; Cavaye, 1991).

Buffel grass was believed to be an obligate apomict with the suggested mechanism of apospory followed by pseudogamy (Humphreys, 1967). Therefore, seedlings would be genetically identical to the parent plant. While this is an advantage in terms of agriculture, it can also be a disadvantage as a lack of genetic diversity would increase the susceptibility of a population to disease. However, Bashaw (1962) discovered that sexuality does occur in buffel grass, although only in a small percentage of the population. Therefore, buffel grass can be described as generally apomictic.

Polyploidy commonly occurs in buffel grass, with different levels of polyploidy found amongst the different accessions. Chromosome numbers range from 2n = 18 to 2n = 56 (Visser *et al.*, 1998c), with a basic chromosome number of x = 9 (Visser *et al.*, 1998a). Polyploidy in nature is often associated with hybridisation, and is one of the most rapid

methods known of producing radically different genotypes. It is often used in plant breeding to create new accessions, and it is believed that polyploids are more resistant to changing environmental conditions (Stebbins, 1967). Aneuploidy has also been found in buffel grass, resulting in new aneuploid chromosome numbers. It is believed that this is caused by uneven segregation of chromosomes (Visser *et al.*, 1998b, c).

A species which is generally apomictic, thereby decreasing genetic variation and increasing susceptibility to disease, is usually at a higher risk of extinction. However, because of the levels of polyploidy and aneuploidy found in this species amongst the various accessions, being generally apomictic is not a great threat as there is a large gene pool available from which to potentially create new resistant accessions.

Uses

The main use of buffel grass is in the improvement of pastures in the cattle and sheep industries. Its wide adaptation and tolerance of drought, fire and over-grazing has brought great financial benefits to many individual producers and companies (Hall, 2001). Compared with native grasses, buffel grass is more competitive and more productive, especially in drier conditions. Another advantage is that buffel grass responds to out of season rain, whereas native grasses remain dormant. It also produces a rapid growth response to early summer rain and remains greener longer than native grasses in cooler autumn conditions (Hall, 2001).

One of the major benefits of buffel grass pastures is increased carrying capacity (Paull and Lee, 1978; Cavaye, 1988, 1991; Hall, 2001). Buffel grass also provides useful feed reserves in dry periods. Established buffel grass pastures on good fertile soil can give a fivefold increase in stocking rate, and can support continuous stocking rates of one beast

(cattle) to 3.3 ha or a breeding sheep to 0.6 ha (Paull and Lee, 1978; Cavaye, 1991). Improved buffel grass pastures also increase productivity. While the nutritional value of buffel grass is seasonal, producing lower quality grazing than native pastures in Winter, it provides better grazing than native pastures for the rest of the year. When a buffel grass pasture is fertilised, or mixed with legumes, it consistently provides better grazing than native pastures. A grazing trial on a fertilised, acid red earth at Charleville compared the productivity of sheep on two buffel grass accessions (cv. Biloela and Q10077) and native pasture ('tMannetje and Jones, 1990). At one sheep per 1.25 ha in a 485 mm rainfall environment, Biloela buffel grass generated the production of the most wool, followed by Q10077 and then native pastures. Another grazing trial in the Burnett district of south-east Queensland recorded an average live weight gain per year of 117 kg from steers grazing buffel grass pastures on a fertilised red soil ('tMannetje and Jones, 1990). At a similar stocking rate, steers on unfertilised native black speargrass (*Heteropogon contortus*) pasture gained only 78 kg per year ('tMannetje and Jones, 1990).

Buffel grass pastures also play a role in soil erosion protection and outcompete unwanted grasses. Without buffel grass, less palatable grasses such as wiregrass (*Aristida* spp.) would dominate significant areas of pastures due to overgrazing in dry years. Producers would consequently have to reduce stocking rates resulting in decreased productivity, and would probably also lose valuable topsoil due to erosion (Hall, 2001).

Weed Potential

Buffel grass is easily naturalised in most Australian soils and climates, and forms self-sustaining populations. It has a rapid growth rate, prolonged flowering period, high seed production and dispersal, and long seed dormancy. It is also very drought resistant, is

encouraged by burning, and is able to tolerate constant grazing. These characteristics make buffel grass a very desirable pasture species. However, these same characteristics have led to its unsolicited establishment in uncleared native vegetation (Franks *et al.*, 2000), including national parks.

The invasive nature and dominating ability of buffel grass over native species has led to a loss of biodiversity of both flora and fauna (Hall, 2001). Buffel grass also causes more extensive fires and tends to burn hotter and later in the season when compared with native grasses. Fire thereby destroys native plants and the buffel grass regenerates quickly, replacing the plants killed by fire. This is a particular threat to dry rainforest remnants, as buffel grass grows along the edges of such areas and transmits successive hot fires that progressively kill the rainforest (Low, 1997).

Buffel grass produces seeds prolifically, but the seeds are not available to seed-eating birds such as finches and parrots as they do not eat these seeds, and so those bird numbers decline (Low, 1997; Franks *et al.*, 2000). The removal of native plants by buffel grass also impacts on those fauna which have specialised shelter and dietary requirements (Fensham and McCosker, 2001). Some animals, such as the galah (*Cacatua roseicapilla*), appear to benefit from an increase in buffel grass pastures.

The positive impacts of buffel grass pastures, such as increased production for primary producers, needs to be weighed against the negative impacts, such as loss of biodiversity and the cost of controlling it in areas where it is not wanted (Esdale, 2001). Any diseases of buffel grass could also be useful as biological control agents in areas where it is not wanted. Balancing production and conservation within buffel grass pastures is becoming an increasingly important issue to all concerned.

1.2.3 Known Disorders and Diseases of Buffel Grass

Nutrient Deficiency/Toxicity

There has been very little work done on nutrient disorders of buffel grass; the majority of the nutrient-related work investigates the nutritional value of buffel grass for various livestock species. The main limiting nutrients for buffel grass pastures are nitrogen and phosphorus (Muller, 2001). Other nutrients are generally adequately supplied by most soils. Buffel grass is relatively tolerant to slightly higher nutrient levels found naturally in some soils. Most toxicities, such as manganese toxicity, are usually a result of soils being chemically altered by human interference (Schwartz and Safaya, 1978).

The symptoms of many pathological diseases and damage caused by insects closely resemble symptoms due to certain nutrient disorders (Smith, 1974). Therefore, an accurate diagnosis requires the pooling of data from as many diagnostic methods as possible, including plant and soil chemical analyses, local knowledge of deficiencies and the history of introduced chemicals the area, and knowledge of the symptoms caused by pathogens of the plant (Smith, 1974).

Pests

The only commercially significant pest of buffel grass is the indigenous seed head caterpillar or paralid moth, *Mampava rhodoneura* (Cavaye, 1991). The female moths lay their eggs into the seed head, and, after hatching, the larvae feed on the developing seed. This moth first became a problem in Queensland in 1980. As the caterpillars prefer dense seed heads, the pest is only a problem in the higher rainfall regions of eastern Queensland (Cavaye, 1991).

Coccids (scale insects – order Homoptera, superfamily Coccoidea) have been known to attack the crown of buffel grass plants but cause no commercial damage (Perrott, 2001).

Diseases

The most economically important diseases of buffel grass are buffel grass blight and ergot (Perrott, 2001). Buffel grass blight is caused by the fungal pathogen *Pyricularia grisea* (Loch, 1998; Perrott and Chakraborty, 1999; Rodriguez *et al.*, 1999; Perrott, 2001) (teliomorph *Magnaporthe grisea*), which is also responsible for the destructive impact of rice blast disease worldwide (Loch, 1998). Buffel grass blight was first observed in 1990 in the US and Mexico before reaching epidemic proportions in those countries in 1996. Symptoms are usually small, elliptical dark brown to tan coloured lesions (1 – 2 mm) with tapering ends, formed along the length of a leaf. Older leaves tend to die off and some plants succumb completely (Perrott, 2001), thereby reducing pasture productivity.

Recently, *P. grisea* was isolated from necrotic foliar lesions of buffel grass in the Central Queensland area, with plants displaying very similar symptoms to those affected by buffel grass blight in the US and Mexico (Perrott and Chakraborty, 1999). However, this condition is not currently in epidemic proportions in Australia, possibly due to a different strain or race of *P. grisea* to that which causes buffel grass blight in the US and Mexico. Nonetheless, the perfect stage of the fungus (*M. grisea*) occurs on some alternative hosts in Australia, potentially generating further genetic variability (Loch, 1998).

Ergot is a fungal disease caused by *Claviceps* spp. (Cavaye, 1991; Craig and Hignight, 1991; Perrott, 2001). The symptoms present as a yellow, honey-like substance called honey-dew which exudes from the seed head. This contains the spores of the fungus

which are then transmitted to healthy seed heads by insects. Secondary fungi develop on the honey-dew, causing it to turn black (Cavaye, 1991). Ergot can be found in all buffel grass areas in Australia but is favoured by warm, wet conditions. It is particularly a problem with seed production both in Australia and overseas.

Minor pathogens of buffel grass include *Bipolaris* spp., which causes minor leaf spot diseases, and *Cerebella* spp., a secondary invader on ergot affected florets (Perrott, 2001). Other minor pathogens are listed in Lenne (1990).

1.2.4 Buffel Grass Dieback

The following is a description of the condition reported before this study was undertaken.

Symptoms

A dieback condition of buffel grass in areas of Central Queensland (CQ) has been a cause of growing concern to graziers since 1993. It is currently estimated to be scattered over hundreds of hectares in the CQ area (refer Figure 1, p.5) (Graham and Conway, 1998). The term 'condition' is used as yet there is no conclusive proof of a fully characterised pathogen (disease) or of a fully understood disorder due to abiotic factors. There is potential for this condition to destroy large areas of sown pasture, ultimately resulting in loss of production for beef, sheep and dairy farmers.

The dieback condition presents as a discolouration of healthy leaves with a marked tip necrosis, generally red or bronze in colour, which spreads towards the ligule. If rain has occurred within approximately three weeks beforehand and the plants are lush, the necrotic areas are bright red in colour (RHSPCC red group 45: A, B; 46: A, B) (The Royal Horticultural Society, 2001). There is generally a sharp boundary between healthy and

symptomatic leaf tissue. The entire plant eventually succumbs and dies within two or three months. Roots of symptomatic plants appear stunted and rotted (Graham and Conway, 1998).

The condition appears to affect the middle height varieties of buffel grass, American and Gayndah, more so than taller varieties, such as Biloela. Native grasses grow unaffected in the dieback affected patches. Cattle may avoid grazing the affected pastures (Graham and Conway, 1998).

Buffel grass dieback occurs in roughly circular patches with distinct boundaries that may reach up to 60 m in diameter, and substantial areas of paddocks may lose buffel grass by convergence of adjacent patches. These patches have been reported on many different soil types and soil compositions (Graham and Conway, 1998), including alluvial duplex soils, sandy loams, and heavier clay loams. Recolonisation of affected areas by less palatable plants renders those areas less useful for grazing. Self-sown buffel grass seedlings succumb to the condition before reaching the third leaf stage.

Previous Work on Buffel Grass Dieback

Field studies, pathological studies and chemical analyses were done in an attempt to find the cause of this condition. Field studies included field observations and field trials. Field trials were conducted to investigate the effects of soil-plant nutritional factors, root nematode invasion and soil chemistry. This was done by treating BGD affected areas with various fertilisers, a nematocide, combinations of these, as well as using tyne renovation, and then measuring the yield of these treated areas compared to control areas. Plant yield measurements from the field suggested that neither root nematodes nor soil nutrient deficiencies are the cause of BGD. The application of fertiliser, especially nitrogen,

improved the growth of buffel grass plants but affected plants continued to show the red symptoms and did not regain normal growth (Graham and Conway, 1998).

Pathological studies included the screening of soils and plants for nematodes, phytoplasmas and other pathogens. In the nematode studies, pasture plots (control and treated with the nematocide *Temik*©150G) were sampled for the root nematode *Rotylenchulus parvus* by extracting 60 cm deep x 50 mm diameter soil cores from the condition interface/boundary, from diseased buffel grass patches and from areas with healthy buffel grass. Samples were analysed at the Leslie Research Centre, Toowoomba, Queensland. The nematode control chemical *Temik*© did not improve growth or diminish symptoms. Changes in nematode populations were difficult to interpret (Graham and Conway, 2000).

For phytoplasma analysis, affected buffel grass plants were submitted to Northern Territory University (NTU), Darwin, and Central Queensland University (CQU), Rockhampton. However, no evidence of the presence of phytoplasmas was detected (Graham and Conway, 2000).

Affected buffel grass plants were submitted to DPI Plant Pathology, Brisbane, and to the Cooperative Research Centre for Tropical Plant Pathology, University of Queensland, Brisbane, for pathology examination. Subsequent field inspections, pathology tests, isolations and glasshouse studies were conducted by Dr K. Harrower, W. Payne and S. Makiela, Central Queensland University, Rockhampton. Field grown plants showing obvious signs of wilting and reddening of leaves typical of the dieback syndrome were examined in the laboratory where attempts to isolate a disease-causing microorganism were carried out (Payne, 2000). The two Brisbane groups isolated several fungi, but none were thought to be pathogenic. A virulent strain of *Pyricularia grisea*, which was known to

cause leaf blight disease in buffel grass, was isolated. However, the leaf blight symptoms were visually different from those of buffel grass dieback. The Rockhampton group isolated cultures of *Fusarium oxysporum* from affected roots. These cultures were identified using an interactive key (Seifert, 1996). The leaves with lesions and red symptoms yielded cultures of *P. grisea*. However, *P. grisea* was not isolated from affected red leaves which did not display lesions (Payne, 2000). Buffel grass plants were also grown in a polyhouse. Various soil regimens were used including field soil, a low fertility soil, nitrogen deficient soil and a saline soil. A spore suspension of phialoconidia of *F. oxysporum* (50 mL) of 10^5 cfu.mL^{-1}, obtained from cultures derived from *F. oxysporum* naturally infected plants, was applied to each pot and then watered in using 100 mL sterile distilled water. Pot grown plants most frequently yielded isolates of *F. oxysporum* (15%) when grown under saline conditions. An infection rate of just less than 10% was found in the low fertility soil and nitrogen deficient soils. Other soil regimes did not yield infected plants. In no case was the leaf reddening symptom of the BGD syndrome observed (Graham and Conway, 2000).

Chemical analyses were performed on both plants and soil. Samples of buffel grass shoots were collected from unaffected and affected areas of buffel grass pasture and were submitted to Agritech Laboratories, Toowoomba, for complete chemical analysis (% N, P, K, and mg/kg trace elements). BGD affected buffel grass shoots appeared to contain higher concentrations of nitrogen, and lower concentrations of phosphorus and potassium. The data on other elements were inconclusive (Graham and Conway, 2000).

Soil profiles (cores) from areas of unaffected and affected buffel grass, as well as from the interface/boundary areas, were sent to the Department of Natural Resources Analytical Centre Laboratory, Indooroopilly, Queensland for chemical analyses. Soil

samples were analysed for pH, EC, CEC, Cl, Ca, Mg, Na, K, and ESP. However, soil tests to a depth of 1 m did not provide clear evidence of soil problems causing buffel grass dieback (Graham and Conway, 2000).

From all of these trials it was suggested that soilborne microorganisms may be the major contributors to the condition. Since Koch's Postulates were not fully satisfied because the leaf reddening symptoms were not re-created in the polyhouse study, it was suggested that additional biotic or abiotic factor(s) may also be involved. As *P. grisea* was isolated only from affected red leaves with lesions, it was suggested that this was a secondary infection, and may not be involved in causing the dieback condition in CQ (Graham and Conway, 2000; Payne, 2000).

1.2.5 Similar Conditions in Other Plants

Patch conditions can occur as a result of plant stress brought on by extreme air temperatures, low soil moisture content, water saturated soil, certain nutrient deficiencies or toxicities, or improper fertilisation practices. These mostly occur in cereals and turfgrasses where intensive farming practices are used (Couch, 2000). However, most patch conditions are caused by disease-causing organisms.

Diseases which occur in patches are relatively common, especially in cereals and turfgrasses. Take-all patch has been identified as a major disease of cereals since the mid 1800s (York, 1996). Symptoms include a black crust around the stem base, root rot, seed heads which are either empty or have shrivelled grain, and in more severe cases death of the plant. Usually it appears in patches several metres in diameter. The causal organism is a fungus, *Gaeumannomyces graminis*. The movement of the fungus to previously unaffected soils is likely to be by air-borne spores, wind-blown soil particles and the

movement of infected plant or soil material (York, 1996). Take-all patch of cereals is known to be affected by the nutritional status of the soil (Reis *et al*., 1982; York, 1996). As the soil becomes deficient in nutrients, the severity of take-all patch increases. In an attempt to control this disease, both a range of fungicides and several biological control agents have been used with limited success (York, 1996).

The soil fungus *Rhizoctonia solani* is one of the most important plant pathogens, causing bare patch disease in many cereal and lupin species (O'Brien and Zamani, 2003). Symptoms include damping-off of seedlings, root, crown and stem rots, and foliar blights (Moen and Harris, 1983; Ryley *et al*., 2004). Similarly, a thin, binucleate *Rhizoctonia* causes Eradu patch in cereal and lupin crops. Symptoms include stunting and ill-thrift (MacLeod and Sweetingham, 1997; MacNish and O'Brien, 2003).

Patch diseases make up a large and important segment of the major diseases of grasses, especially turfgrasses. They are generally caused by fungi, but occasionally are caused by bacteria. Many of these diseases have symptoms in common, for example, the frogeye pattern, that is, roughly circular patches of diseased grass with centre tufts of green resistant species (Couch, 2000).

One of the more common bacterial species which cause patch diseases in grasses is *Xanthomonas* spp.. Fungal agents include *Leptosphaeria*, *Gaeumannomyces*, *Rhizoctonia*, *Fusarium*, *Ascochyta*, *Pythium*, and *Myriosclerotinia* spp. (Couch, 2000; Settle *et al.*, 2001), and many others. For example, *Gaeumannomyces graminis* causes severe patch disease in Tifdwarf hybrid couch grass (*Cynodon dactylon*) (Wong *et al.*, 2000), *Rhizoctonia solani* causes brown patch on creeping bentgrass (*Agrostis pulustris*) (Blazier and Conway, 2004), and *Ascochyta phleina* causes patch disease of Kentucky bluegrass

(*Poa pratensis*) (Smith *et al.*, 2001). The symptoms of most of these include foliar lesions, blighting, foliar discoloration, and eventual plant death.

Conditions favouring disease development usually include wet weather, with heavy outbreaks of the diseases being noted after prolonged periods of rainfall. Optimum temperatures are often warm (25 – 30ºC), but can be cooler for some pathogens, with optimum temperatures of 0 – 2ºC. Higher soil nutrient levels encourage certain patch diseases while inhibiting others. A few pathogens require periods of sunshine to initiate a diseased patch, others require that the plant host becomes dormant, as in cooler weather, before they can infect the plant. Some pathogens produce resistant resting bodies to survive periods of unfavourable conditions (Couch, 2000; Agrios, 2005).

Control of patch diseases is usually by cultural or management practices or by the use of fungicides. Some management practices include removal of the diseased plants and replanting, maintaining specific conditions and the instigation of a balanced fertilisation program. Good surface and subsurface drainage will generally decrease the severity of the disease, as will the removal of dead plant material (Maloy, 1993; Gardner *et al*., 1998; Couch, 2000; Settle *et al*., 2001; Truscott and Gilligan, 2001; Agrios, 2005).

Common fungicides include azoxystrobin, fenarimol, flutolanil, quintozene, triadimefon and iprodione. In some cases synergistic fungicide combinations are used. To reduce the likelihood of fungicide resistance, rotating fungicides with different biochemical modes of action and utilising other means of control simultaneously is encouraged (Maloy, 1993; Gardner *et al*., 1998; Parry, 1990; Couch, 2000; Babadoost and Islam, 2004; Agrios, 2005).

Although there are differences between the above mentioned patch diseases and buffel grass dieback, there are also many similarities; for example, the presence of roughly

circular patches, the frogeye symptoms, more rapid spread after rain and a blighting of the leaves. These similarities may provide important clues to the cause of buffel grass dieback, and the genera of bacteria and fungi which cause turfgrass and cereal patch diseases should be considered as possible causal agents or contributors to buffel grass dieback.

1.3 Outline of Research

As shown in the literature review, much of the previous work done on this disorder was either inconclusive, provided no clear evidence, or provided results which were difficult to interpret. Only a small amount of research generated definitive results. Therefore, most of the possible causes of BGD which were investigated in the past cannot be irrefutably eliminated as possible causes, and may for that reason require further investigation. As a result of this, a decision tree was formulated (Figure 2, p.20) showing possible causes of BGD, and possible areas of research.

However, investigating possible causes of BGD was only one of the aforementioned aims of this study. The other two involved compiling a description of the condition and discovering the effects that the condition has on buffel grass plants. Subsequently, a schematic diagram of these three areas of research was formulated (Figure 3, p.21), outlining the research undertaken in this study. The diagram also represents the structure of this thesis.

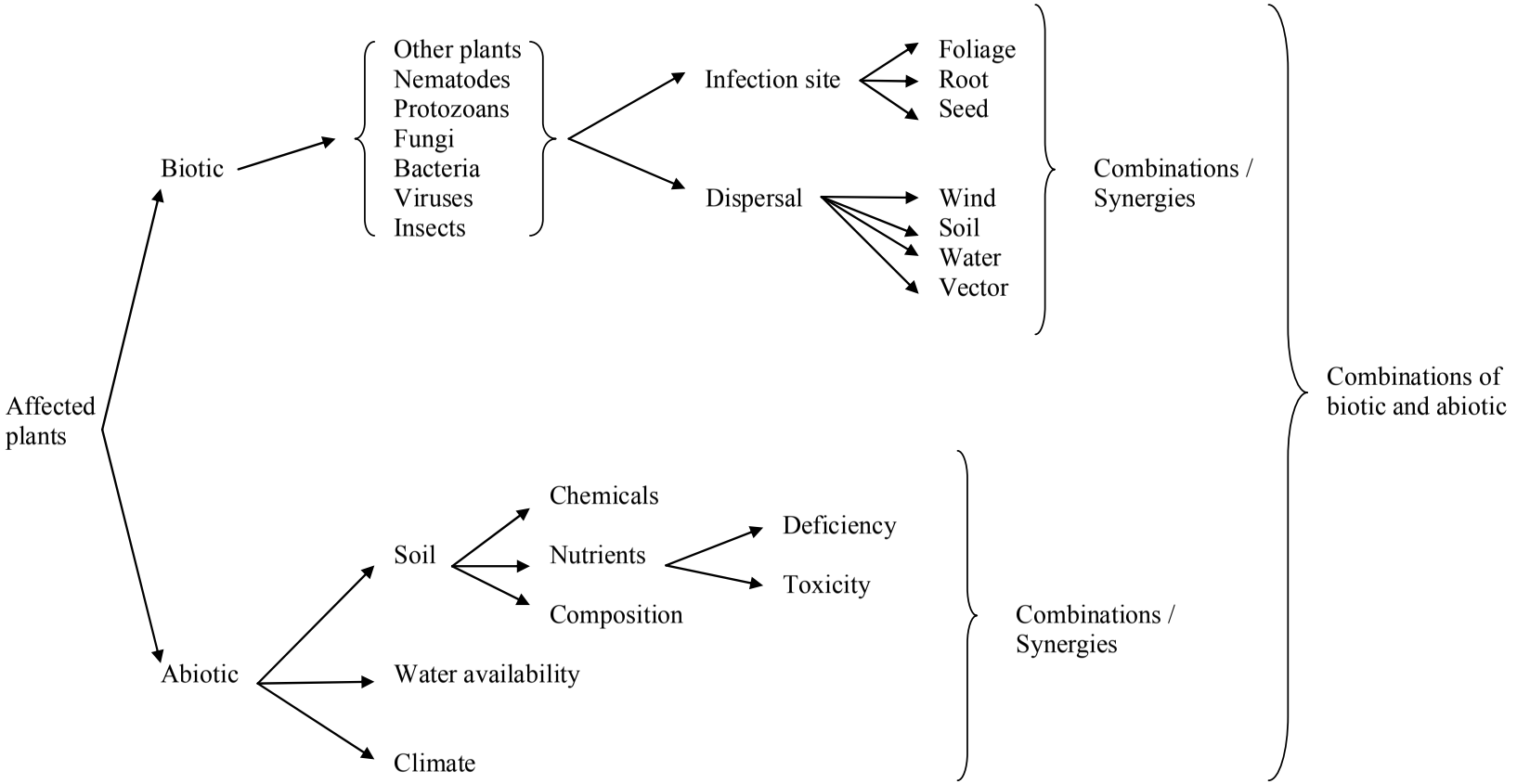


Figure 2 – Flow chart of possible causes of buffel grass (*Cenchrus ciliaris*) dieback

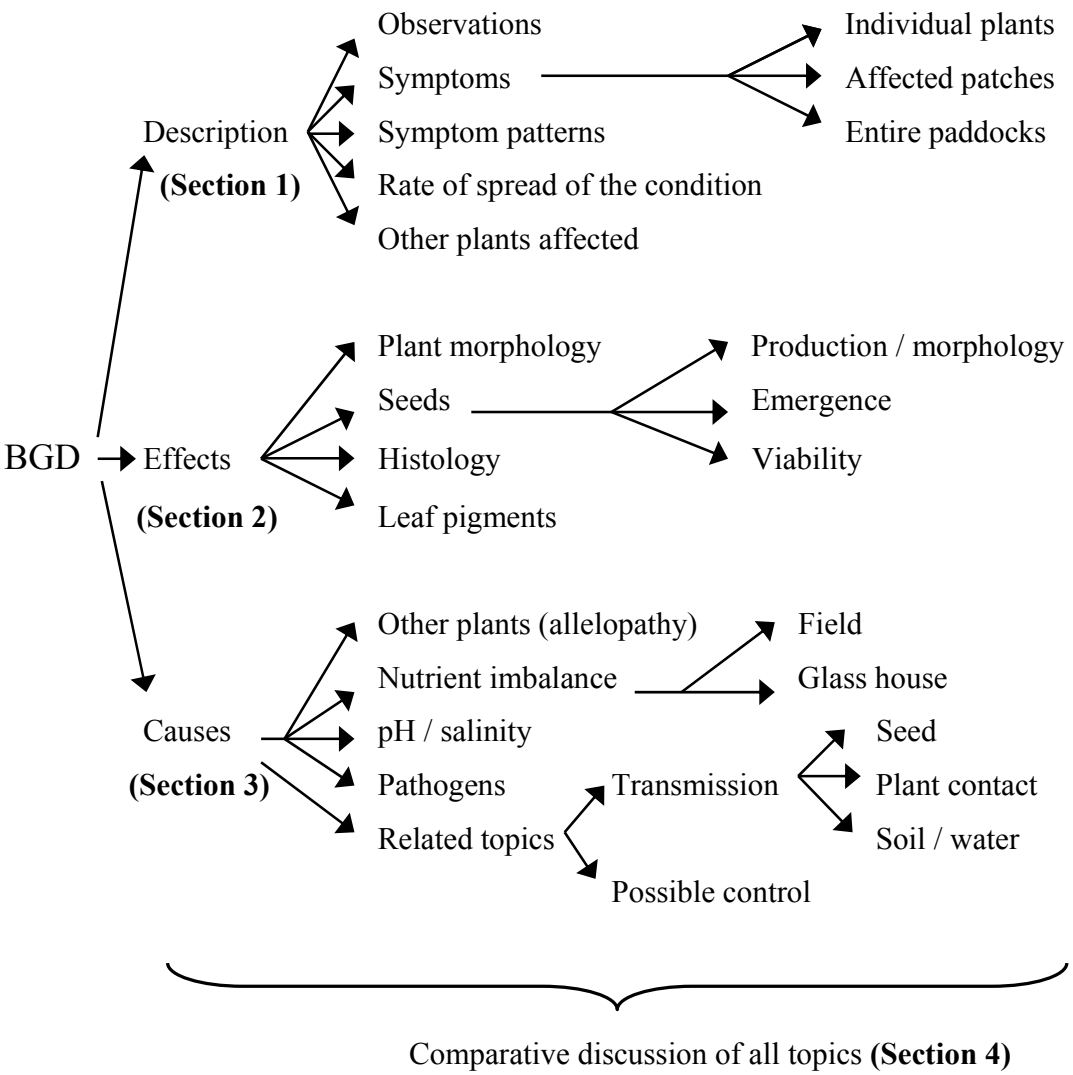


Figure 3 – Thesis schematic displaying areas of research studied

Due to the time constraints of a study of this nature, as well as restrictions due to growth seasons, weather patterns (including a prolonged period of drought), and distance (the field site was a 400km round trip from Rockhampton), with the exception of some preliminary investigations, the research areas outlined in Figure 3 were studied simultaneously in what might be termed a multifaceted approach. While the experiments in each chapter are reported in chronological order, the chapters themselves are not in

chronological order but rather are grouped by subject area. This thesis is, therefore, predominantly not written in chronological order. Because of this structure, most comparisons between chapters are kept for the final discussion.

Some research areas outlined in Figure 3 reached an outcome with one or two experiments, whereas other areas required subsequent experimentation. Therefore, some chapters are substantially larger than others.

A Note on Field Sites

It was reported by Graham and Conway (1998) that BGD occurred and that symptoms were the same at all sites indicated in Figure 1 (inset) (p.5). However, most property owners do not want to admit to the presence of BGD on their property, for fear that it will decrease the potential sale value of the property (Graham, pers. comm.). The readiness of two property owners at Baralaba to have their properties used for this study led to their properties being the sole off-campus sites. Furthermore, BGD was first described on these two adjoining properties (Graham and Conway, 1998) which are roughly central of the reported BGD affected areas in Central Queensland.

The field sites for this study were at Baralaba, a rural township situated in Central Queensland. All field work was carried out on two properties, the names of which will not be disclosed for ethical reasons and to satisfy the wishes of the owners. Property 1 and Property 2 are neighbouring properties located at approximately 24º 20' E and 149º 51' S. Property 2 was used only in the first two sections below, conjointly with Property 1. The majority of field work was carried out on Property 1 as symptoms on this property were more easily discernible. With the exception of the first two sections of Chapter 2, any mention of field work or field sites can be assumed to mean from Property 1.

The grazing paddocks on both properties consisted primarily of the American cultivar of buffel grass, with some Biloela buffel grass and a few other species, both grasses and others. The American and Biloela cultivars are easily discernible in the field. American buffel is of medium height (up to 1 m), semi-prostrate, has narrow leaves and purplish seed heads. In compassion, Biloela buffel is tall (over 1 m), erect, has broad leaves and straw coloured seed heads (Paull and Lee, 1978; Eagles *et al.*, 1992).

1.3.1 Note to Readers on Thesis Structure

The thesis is segregated into four sections, as illustrated in Figure 3. Section 1 aims to describe the condition, Section 2 outlines the effects that BGD has on the host plants, Section 3 investigates possible causes of the condition, and Section 4 is the final discussion which integrates the findings of the other three sections. Each section is made up of several chapters, each reporting one or more experiments in a given study area.

Since many research areas are investigated in this study, each chapter has its own introduction, materials and methods, results and discussion. Chapters in which multiple experiments are reported generally have one introduction followed by a set of materials and methods, results, and discussion for each experiment in turn.

The introduction of each chapter gives some background information and provides reasons as to why the work was undertaken. It also includes the aims of the chapter. The materials and methods are divided into two sections: methodology and methods. The methodology explains why that particular method was chosen and possibly gives some background information, whereas the methods simply outline what was done. The results display the findings in the form of tables, graphs and summaries; raw data were not included. The discussion attempts to explain the results. At the end of each chapter is a

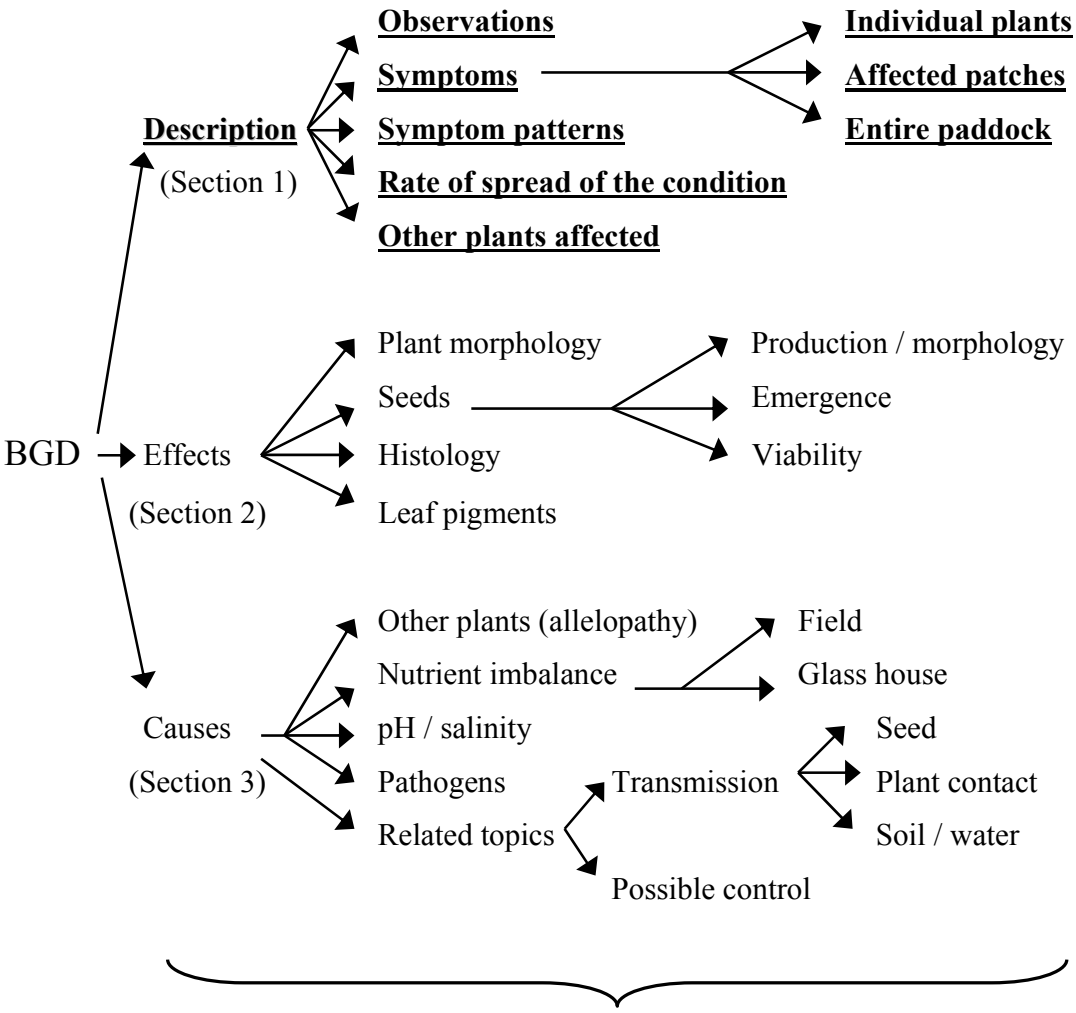
section called ‘Concluding Statements’, which outlines in a few sentences the major outcomes or conclusions of the chapter, relating them back to the aims.

All statistics were done using GenStat for Windows, version 7 (VSN International). All error bars on graphs are Standard Error of the Mean, not Standard Deviation. References which apply to an entire paragraph are generally cited once at the end of that paragraph.

The property owners, on whose properties all field work was carried out, wished to remain anonymous. Therefore, they are not named, but are referred to in the text as ‘property owners’.

Section 1

Description of Buffel Grass Dieback



Chapter 2

Observations and Characterisation of the Dieback Condition

2.1 Introduction

Most symptoms are the outwards visible signs of a distinct malfunctioning in a plant, brought about by pathogens or abiotic causal agents. When distinct, symptoms can often help to monitor the prevalence and spread of a condition. The type of symptom can, in many cases, also indicate the nature of the causal agent (Fox, 1993; Narayanasamy, 2001). For example, in grasses, nutrient disorders often produce characteristic foliar symptoms, as evidenced by Smith's work on buffel grass (Smith, 1974) and on *Panicum maximum* (Smith, 1972). Diseases of grasses can also cause distinct symptoms, as displayed by *Pyricularia* leaf blight of buffel grass (Perrott and Chakraborty, 1999), and by Cocksfoot mild mosaic virus in New Zealand pasture grasses (Guy, 2006).

The majority of previous work carried out on BGD was reported as "inconclusive" or "difficult to interpret". The cause of BGD could, therefore, be one or a combination of factors, either biotic or abiotic. Therefore, it was considered essential that a complete characterisation of the symptoms of the condition be made before any other work was carried out. Not only would this make the condition easier to identify, but it could also provide clues as to the causal agent(s).

This chapter includes all visual preliminary studies made. Further data collection based on these observations, as well as conclusions and questions arising from this chapter will be addressed and expanded on in later chapters.

2.2 Interviews / Discussions With Primary Producers

2.2.1 Materials and Methods

Methodology

Primary producers are often the first people to establish contact with since they generally know the history of the land, including past problems. They habitually have information which is useful in scientific investigations. Discussions were held with two primary producers to obtain information such as when and where BGD was first sighted on their properties, their observations and opinions on the matter, and if there were any attempts to combat the condition.

Method

Over the course of this study, numerous informal discussions were held with the two primary producers on whose properties the field work was carried out. Facts as well as opinions were noted. The information gained was collated into tables.

2.2.2 Results

The following three tables list the observations from primary producers. The first table lists information which is common to both properties. The second and third tables list information exclusive to property 1 and 2 respectively.

Common Points:

- The pastures consist of predominantly American buffel, with some Biloela buffel and other species.
- In relation to the spread of *Fusarium* diseases of cotton, cotton was never grown on the property and cotton seed was never fed to cattle (Ragazzi *et al.*, 1995).
- The condition was first sighted around 1993.
- The condition became established around 1996.
- Affected plants are often found along cattle tracks and/or vehicle tracks.
- Other plant species appear to colonise BGD affected patches once the buffel grass starts to die off.
- The condition seems to spread more/faster with rain and just after rain.
- The condition was first sighted near a cattle camp (resting location for cattle).
- Affected plants have shrivelled roots compared with unaffected plants.
- Affected plants still produce seed, though in lower quantities. The germination of seed from affected plants is severely reduced.
- The condition recedes after large amounts of rain, and re-emerges when weather dries off.
- Cattle generally will not eat affected buffel plants. They appear to take a few bites and move away.

Table 1 – Primary producer information common to both properties regarding buffel grass (*Cenchrus ciliaris*) dieback

Property 1 Points:

- The original American buffel seed came from Banana in the early 1980s.
- The condition was first sighted on a creek bank in the southern corner of the paddock.
- Affected patches appear to spread faster downhill.
- Affected plants become yellowish after rain.
- The spread of the condition seems related to grazing. If the pasture is grazed, the boundary spreads faster than if it is not grazed.
- The condition is possibly spread by stormwater, water-wash, creeks, etc.
- The condition is possibly wind blown into other paddocks.

Table 2 - Primary producer information from Property 1 regarding buffel grass (*Cenchrus ciliaris*) dieback

Property 2 Points:

- The property uses rotational grazing.

Table 3 - Primary producer information from Property 2 regarding buffel grass (*Cenchrus ciliaris*) dieback

The owners of Property 1 attempted to control the condition using different treatments on strips of land. Ploughed strips had a slight effect, in that the condition temporarily disappeared, but eventually came back. The grass on the fertilised strips grew better but still had BGD symptoms.

2.2.3 Discussion

Both property owners agreed on the year that BGD was first sighted on their respective properties, which was expected as they are adjacent. Both also agreed that

affected patches were colonised by other plant species, suggesting that these are resistant to the condition.

The primary producers observed that the condition spreads faster after rain. This implies that the condition is primarily caused by a disease-causing organism (Agrios, 2005) or by the mobilisation in the soil of certain elements which require more moisture, such as nickel, lead and cobalt (Durbin, 1978), which are toxic to plants in higher concentrations. Both of these implications are supported by the farmer's opinions that the condition could be spread via creeks or water runoff, including the observation that the condition spreads downhill. However, the condition was reported as travelling up from a gully, indicating that the mode of spread of this condition is not restricted to water runoff. The causal agent(s) of this condition may be soilborne, with the causal agent(s) moving both uphill and downhill. As soil is often moved by water runoff, this substantiates the downhill spread and the increased rate of spread in wet weather. Wind will also move soil, so it is possible that the condition was windblown into other paddocks, that is, if it were transmitted through soil. Wind may also spread inoculum, which may be spread both uphill and downhill depending on wind speed and direction. Windborne inoculum may also be spread by water runoff (Parry, 1990). Either mode of spread is plausible at this point.

The condition recedes immediately after large amounts of rain then re-emerges once the paddock dries, indicating that water stress may be a factor. This supports both hypotheses of a disease-causing organism or a harmful element which requires added water for mobilisation.

The condition was first sighted around cattle camps and creeks, and occasionally seems to be on cattle trails, suggesting that BGD is either spread by cattle, or exacerbated by soil compaction. Corroborating this is the observation that the spread of BGD seems

related to grazing. Also supporting this is the grazing regimes of the two properties and the fact that Property 2, which uses rotational grazing, has a lower incidence of BGD than Property 1. Rotational grazing involves less cattle movement, and generally only rotates cattle on a specified route (McCosker, 2000). It is possible that the condition is spread via soil on the cattle's hooves, again suggesting that soil is the mode of transmission. However, it is not impossible that BGD is spread by cattle through other means, whether by deposited faeces, a skin disorder, or even parasites which may carry a plant pathogen.

Cotton is a crop which is subject to *Fusarium* wilt or blight (Ragazzi *et al.*, 1995). *Fusarium* species are widely known as plant pathogens, and could easily be transmitted to other plants through the dropping of infected plant material on the soil. Cotton seed and meal are often fed to cattle in times of drought, therefore providing a possible route of infection to buffel grass plants. Cotton is grown in the Baralaba area and small amounts are occasionally dropped by the roadside from passing trucks. However, as cotton seed and meal were never fed on the property, it is unlikely that BGD originated from cotton. This does not discount *Fusarium* as a possible causal agent, but discounts the possibility that it was sourced from cotton.

The morphological observations of plants affected with BGD (shrivelled roots, yellowing leaves after rain) may indicate that either stunting or rotting of the roots occurs, which in turn decreases the uptake of certain nutrients, resulting in the yellow symptoms. Conversely, the root and leaf symptoms could be unrelated and the leaf yellowing could be due to a chemical or hormonal imbalance in the plant, possibly due to the presence of a pathogen as reported by Andel and Fuchs (1972).

Affected plants produce fewer seeds, which in turn have a poor germination rate. This implies that fewer nutrients are being partitioned into seed production. Whether or not

the condition is spread by seeds needs to be investigated, as this could dramatically affect the rate of spread, particularly regarding the sale of seeds.

Cattle have been observed to selectively graze unaffected buffel grass and avoid BGD affected buffel grass. A study by Marten (1978) showed that diseased plants are less palatable to livestock. This may suggest that BGD is caused by one or more disease-causing organisms.

The trials performed by primary producers suggest that nutrient deficiency was an unlikely cause of BGD. Soil disturbance temporarily halted the condition, suggesting that the primary causal agent is soilborne.

While the primary producer observations provide many clues, they will need to be substantiated before any conclusions can be made.

2.3 Field Observations of BGD Symptoms and Symptom Progression

2.3.1 Materials and Methods

Methodology

Previous descriptions of BGD were adequate for the purpose of identifying the condition, but they were not a complete portrayal of symptoms. To obtain a full description, observations should be made over time, over a wide variety of weather conditions, with particular emphasis on colour changes and symptom progression.

Method – progression of symptom severity

Affected field plants were observed on numerous (26) field trips throughout this study in order to obtain a complete description of symptoms and their development at the

plant level, affected patch level, and paddock level. Observations were also made weekly over several weeks following rainfall events. One set of observations was made after a large rainfall event following a 3 month period of drought, and the progression of symptoms from new growth was noted. Particular attention was given to colour changes and their progression throughout the plant, as these patterns can be unique to a particular condition, and may, therefore, be used as an identifying feature. Any differences in plant symptoms in dry periods and in wet periods, i.e. after rainfall, were also noted, since this could be indicative of a biological causal agent(s).

Several major observations from each field trip were tabulated, comprising of:

- the paddock type – there were three paddocks surveyed, one unaffected, one slightly BGD affected, one heavily BGD affected, each of which was *ca.* 81 ha (200 acres),
- the nature of the crop – whether the paddock in general was lush, drying, or dry,
- a description of the plants which included:
  - the type of plant, that is, whether or not they were previously affected by BGD,
  - the current severity of the BGD condition, and
  - estimated percentages of these plants within the paddock. These were derived by driving through the paddocks along transects 20 m apart, stopping every 20 m, and visually appraising (and noting) symptom severity.

A comparative ranking of the overall severity of symptoms per field trip (combining the two BGD affected paddocks) was formulated based on the above results, taking into account the estimated percentages of each severity level. A scale of 1-15 was chosen for these rankings rather than a more conventional 1-10 scale since there were more than 10 discrete stages of the severity of the condition. The scale can be summarised as follows:

1. 1-2 red leaves in few previously affected plants
2. 1-2 red leaves in several previously affected plants
3. 50% of plants in the 'ring' symptomatic
4. 75% of plants in the 'ring' symptomatic
5. Symptomatic 'ring' apparent on most patches in the paddock
6. Plants in the 'ring' have 50% of leaves symptomatic
7. All previously affected buffel grass now symptomatic
8. The 'ring' spreads outwards
9. The 'ring' spreads to self sown seedlings in the patch
10. Previously healthy growth have 50% symptomatic leaves
11. Self sown seedlings dead, established plants near the 'ring' are all affected
12. Patches continue to spread outwards
13. Many established plants are dying
14. Many established plants are dead
15. Most plants have died.

These rankings (26 total, one per field trip) were then graphed against monthly rainfall data and maximum/minimum average monthly temperatures (obtained from the Bureau of Meteorology, Baralaba station, two paddocks away from my field site) (Figure 4), to discern any possible patterns.

All of these results were on American buffel only.

Method – collective description of symptoms

During the abovementioned field trips, individual BGD affected plants as well as BGD affected patches were meticulously scrutinised for symptoms, both foliar and root, symptom progression, and other factors such as the presence of insects.

Individual BGD affected plants (>30) were selected at random and their symptoms both noted and photographed. Leaf colouration was compared with the Royal Horticultural Society Plant Colour Charts (2001) and the matching colour codes were noted. These were also visually morphologically compared with unaffected plants growing within a 10 m radius of the affected plant. Ten BGD affected plants selected at random, each from a different patch, and ten unaffected plants also selected at random were carefully exhumed by digging around the plant (distance *ca.*30 cm), and 50 cm deep. Their roots were carefully washed in water to remove the soil and examined. Any differences between BGD affected and unaffected plants were noted and photographed.

Two transects were drawn in each of ten different patches, and BGD affected plants were compared at different levels of symptom progression. Twenty plants in early stages of symptom development were also tagged, and symptom progression was noted over several field trips. Ten quadrats (each 1 m^2) were randomly thrown within BGD affected areas, and BGD affected plants within the quadrats were examined for the presence of insects and evidence of insect damage. Ten quadrats were also thrown in unaffected areas, and unaffected plants were examined.

All of the above methods were done using American buffel grass. However, as Biloela buffel grass also grew in these paddocks (reported to be unaffected by BGD by Graham and Conway, 1998), these plants were similarly examined, using ten plants from

within a BGD affected patch, and ten plants growing in nearby areas with unaffected American buffel grass.

The above observations were reported under three categories:

1. Symptoms at the plant level

2. Symptoms at the affected patch level

3. Symptoms at the paddock level

It should be noted that these results are based on visual observations only. Subsequent experimentation based on these observations, such as actual measurements of plant weight, height, etc, are reported in later chapters.

2.3.2 Results

Progression of symptom severity

Tables 4 and 5 are a key to Table 6, which presents data collated from various field trips.

Plant Type	Definition	Description
HBG	Healthy buffel grass	Areas never affected with buffel grass dieback
RABG	Recently affected buffel grass	Areas including patch edges and self-sown seedlings
PABG	Previously affected buffel grass	Affected buffel grass in established patches

Table 4 – Codes representing different plant symptom types of buffel grass (*Cenchrus ciliaris*) according to previous history with buffel grass dieback

Code	Definition	Images
HE	Plants healthy Green leaves No discoloration	
EM	Red symptoms emerging Older leaves turning red Younger leaves healthy	
MO	Moderately affected Older leaves may be turning bronze Intermediate leaves turning red Younger leaves may have reddening tips	
CO	Completely affected Older leaves undergoing a senescence-like process All leaves red or bronze	

Table 5 – Codes representing symptom severity of buffel grass (*Cenchrus ciliaris*) affected with buffel grass dieback

Code	Definition	Images
DY	Plants dying Previously red areas of leaves turning bronze Most leaves undergoing a senescence-like process	
DE	Plants dead Previously bronze sections of leaves remain dark Most leaves have abscised	
HP	Paddock of healthy buffel grass Mostly buffel grass with some other species (lower leaves of plants in foreground senescent)	
AP ↓ Two types ↓	Paddock of affected buffel grass Patches of buffel plants affected with buffel grass dieback Recolonisation of central area of patch by other plant species (★)	
H-AP	Heavily affected paddock	Affected patches coalesced, covering >80% area
S-AP	Slightly affected paddock	A few small distinct affected patches

(Table 5 continued)

Field Trip date	Paddock	Nature of Crop	Description of Plants			Other Major Observations
			Est. %	Type	Code	
23-Mar-01	HP	lush	100	HBG	HE	Patches seen along cattle trails and vehicle tracks.
	H-AP	lush	45	PABG	HE	No recently affected buffel grass.
			50	PABG	EM	
			5	PABG	MO	
03-May-01	H-AP	drying	30	PABG	MO	Both healthy and affected buffel producing seed.
			70	PABG	CO	Leaf lesions seen on ~ 2% of affected buffel.
31-Aug-01	H-AP	drying	30	PABG	CO	Patches seen along cattle trails and vehicle tracks.
→			30	PABG	DY	No recently affected buffel grass.
01-Sep-01			40	PABG	DE	
28-Feb-02	HP	lush	100	HBG	HE	Dying plants turned bronze in colour.
	H-AP	lush	5	PABG	MO	No recently affected buffel grass.
			10	PABG	CO	
			85	PABG	DY	
30-Mar-02	H-AP	dry	20	PABG	DY	Difficult to distinguish healthy buffel from affected buffel when
			80	PABG	DE	dead.
12-Jun-02	HP	lush	100	HBG	HE	Leaf lesions seen on ~ 2% of both affected and healthy buffel.
	S-AP	lush	100	RABG	EM	Previously dormant plants have reemergence of fresh foliar
			85	PABG	HE	material.
			15	PABG	EM	Many new seedlings in affected patches, most are affected.
	H-AP	lush	100	RABG	EM	Symptoms start on the boundaries of previously affected
			90	PABG	HE	patches.
			10	PABG	EM	
17-Jun-02	S-AP	lush	70	PABG	HE	Patches seem to appear in random locations throughout a
			30	PABG	EM	paddock.

Table 6 – Field trip observations of buffel grass (*Cenchrus ciliaris*) dieback.

Note: Tables 4 and 5 are the keys to the code.

Field Trip date	Paddock	Nature of Crop	Description of Plants			Other Major Observations
			Est. %	Type	Code	
17-Jun-02	H-AP	lush	70	PABG	HE	
(cont)			30	PABG	EM	
20-Jun-02	HP	lush	100	HBG	HE	Pronounced 'ring' effect seen on many patches.
	S-AP	lush	65	PABG	HE	Red symptoms often start as mottling.
			15	PABG	EM	If leaf blade is damaged, symptom development is delayed at
			20	PABG	MO	the site of damage.
	H-AP	lush	65	PABG	HE	
			15	PABG	EM	
			20	PABG	MO	
26-Jun-02	HP	drying	100	HBG	HE	Pronounced 'ring' effect seen on many patches.
	S-AP	drying	55	PABG	HE	
			10	PABG	EM	
			35	PABG	MO	
19-Jul-02	S-AP	drying	100	HBG	HE	All previously affected dormant buffel now showing symptoms.
			50	PABG	MO	
			50	PABG	CO	
26-Jul-02	HP	drying	100	HBG	HE	All self-sown buffel seedlings in affected patches have
	S-AP	drying	20	PABG	CO	symptoms.
			80	PABG	DY	
16-Aug-02	HP	drying	100	HBG	HE	All previously affected self-sown buffel seedlings dead.
	S-AP	drying	100	PABG	DY	
21-Feb-03	HP	lush	100	HBG	HE	Pronounced 'ring' effect seen on many patches.
	S-AP	lush	70	PABG	HE	
			30	PABG	EM	

(Table 6 continued)

Field Trip date	Paddock	Nature of Crop	Description of Plants			Other Major Observations
			Est. %	Type	Code	
27-Feb-03	S-AP	lush	90	PABG	HE	Leaf lesions seen on a few plants.
			10	PABG	EM	
13-Mar-03	S-AP	lush	75	PABG	HE	Leaf lesions seen on ~ 2% of both affected and healthy buffel.
			20	PABG	EM	Slight 'ring' effect.
			5	PABG	MO	
28-Mar-03	S-AP	lush	60	PABG	HE	Leaf lesions seen on ~ 2% of both affected and healthy buffel.
			10	PABG	EM	Pronounced 'ring' effect seen on many patches.
			30	PABG	MO	
25-Apr-03	S-AP	lush	30	PABG	HE	Symptoms progress from oldest to youngest leaves.
			10	PABG	EM	
			60	PABG	MO	
16-May-03	HP	lush	100	HBG	HE	Leaf lesions seen on ~ 2% of both affected and healthy buffel.
	S-AP	lush	20	PABG	HE	All self-sown buffel seedlings in affected patches have
			10	PABG	EM	symptoms.
			30	PABG	MO	If leaf blade is damaged, symptom development is delayed at
			40	PABG	CO	the site of damage.
11-Jun-03	S-AP	drying	65	PABG	CO	Both healthy and affected buffel producing seed.
			35	PABG	DY	
21-Oct-03	S-AP	drying	60	PABG	CO	Both healthy and affected buffel producing seed.
→			25	PABG	DY	Central areas of dead affected buffel patches recolonised by
22-Oct-03			15	PABG	DE	other species.
09-Jan-04	HP	lush	100	HBG	HE	Pronounced 'ring' effect seen on many patches.
	S-AP	lush	80	PABG	HE	Many self-sown buffel seedlings in patches.
			20	PABG	EM	Both healthy and affected buffel producing seed.

(Table 6 continued)

Field Trip date	Paddock	Nature of Crop	Description of Plants			Other Major Observations
			Est. %	Type	Code	
30-Jan-04	S-AP	drying	5	PABG	HE	Both healthy and affected buffel producing seed.
			10	PABG	EM	All self-sown buffel seedlings in affected patches have
			50	PABG	MO	symptoms.
			35	PABG	CO	
20-Feb-04	S-AP	lush	10	PABG	EM	All self-sown buffel seedlings show red symptoms and are
			45	PABG	MO	dying.
			45	PABG	CO	
12-Mar-04	S-AP	drying	5	PABG	EM	Both healthy and affected buffel producing seed.
			35	PABG	MO	Affected self-sown buffel seedlings dead.
			20	PABG	CO	
			25	PABG	DY	
			15	PABG	DE	
03-Apr-04	H-AP	lush	15	PABG	HE	Leaf lesions seen on ~ 2% of both affected and healthy buffel.
			10	PABG	EM	If leaf blade is damaged, symptom development is delayed at
			30	PABG	MO	the site of damage.
			45	PABG	CO	Both healthy and affected buffel producing seed.
	S-AP	lush	10	PABG	HE	
			10	PABG	EM	
			50	PABG	MO	
			30	PABG	CO	
07-Jun-04	S-AP	drying	35	PABG	MO	Pronounced 'ring' effect.
			25	PABG	CO	
			30	PABG	DY	
			10	PABG	DE	

(Table 6 continued)

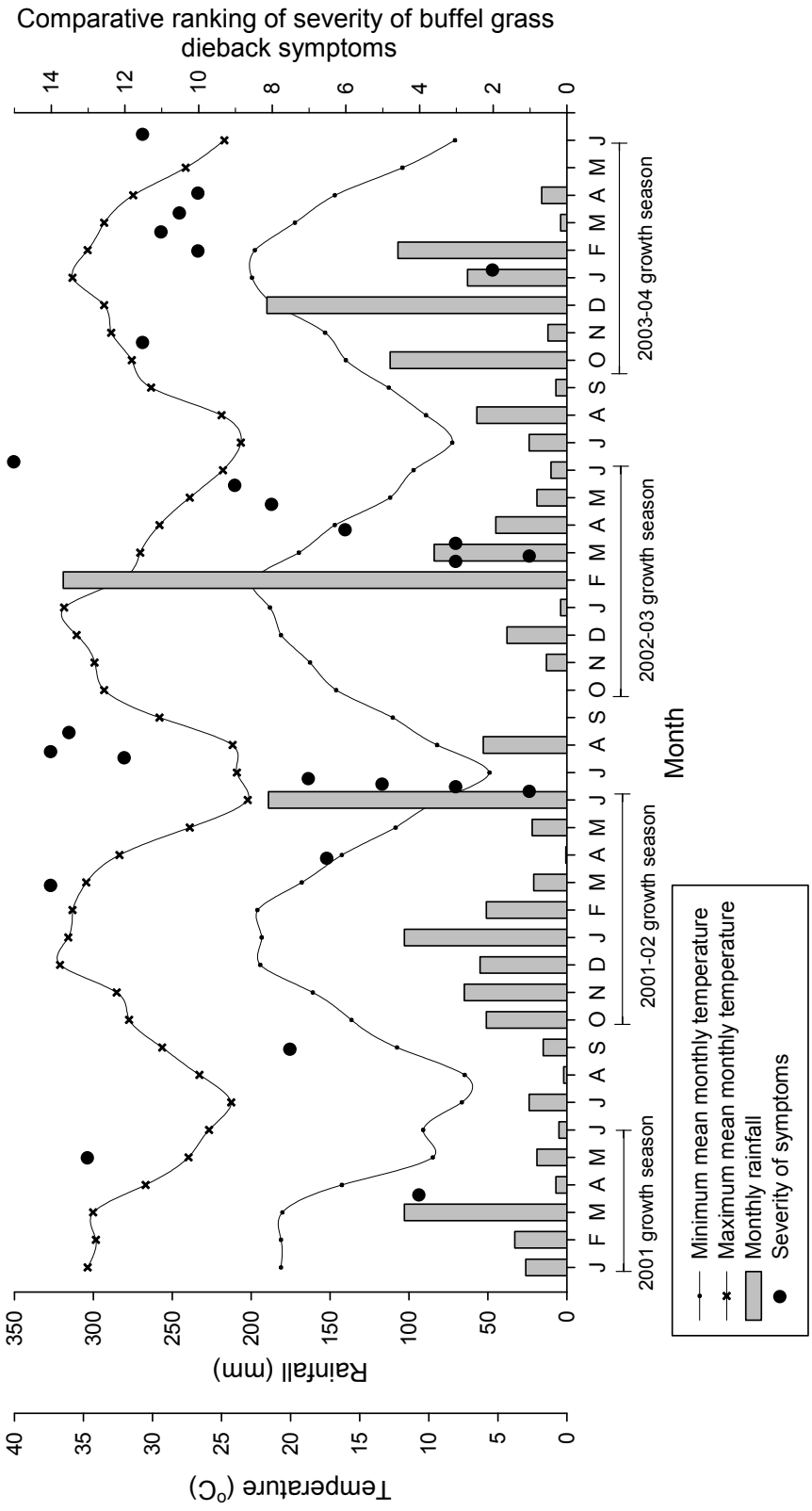


Figure 4 - Comparative schematic of monthly rainfall and severity of buffel grass dieback symptoms

Collective description of symptoms

The following is a collective description of BGD symptoms and their progression based on Table 6 and Figure 4 results, and on the closer observations obtained during the field studies.

Symptoms at the Plant Level

Symptoms of BGD present as a reddening of the leaves starting from the tip and progressively moving towards the ligule. The red symptoms range from bright red, to dark red, to bronze (RHSPCC red group 45: A, B; 46: A, B; greyed-orange group 166: A; 177; A)(The Royal Horticultural Society, 2001) (Figure 5). Symptoms first emerged on the tips of the older leaves. The next oldest leaf then showed symptoms and so on with the youngest leaf showing symptoms last. Any tillers followed the same pattern, regardless of whether symptoms on the primary shoot had progressed past the point at which the tiller was produced. It was not uncommon to see an almost completely affected primary shoot with several new tillers, some of them completely unaffected and others with the oldest leaves beginning to show symptoms. The progression of symptoms did not change when the affected plants produced seeds.

Figure 5 – Red symptoms of buffel grass (*Cenchrus ciliaris*) dieback

Affected buffel grass plants rarely produced seed heads and produced fewer seed heads per plant than unaffected plants. The seed heads were also generally shorter and darker in colour. The seed fascicles were thinner than those of unaffected plants giving the seed head a rough appearance since there was space between the fascicles (Figure 6).

Figure 6 – Seed heads from buffel grass (*Cenchrus ciliaris*) unaffected with dieback (top) and affected with dieback (bottom)

After a dry period, followed by a rainfall event, new green growth started after approximately three days. The red symptoms first appeared on average two weeks after the most recent rainfall event. The amount of time from new growth to the appearance of symptoms seemed to be directly proportional to the amount of rainfall, that is, the greater the rainfall, the longer it took for symptoms to develop. Buffel grass plants affected with BGD before maturity were approximately half the height of unaffected plants, and also formed thinner clumps. There were no tumours or other abnormal growths.

When there was lush growth the plants produced new leaves faster than the condition spread, such that the youngest leaves were generally free of symptoms. However, symptoms were usually apparent in the second or third youngest leaves. Once the plants were stressed (through lack of water or otherwise), new growth was slower and

the condition overtook the plants so that even the youngest leaves were affected. Once the condition took over the plants, growth was again slowed, leading to the eventual death of the plant. The length of time from the first sign of symptoms to plant death was impossible to determine, as it was entirely dependent on the amount of rainfall. However, Figure 5 gives a rough estimate of the time line.

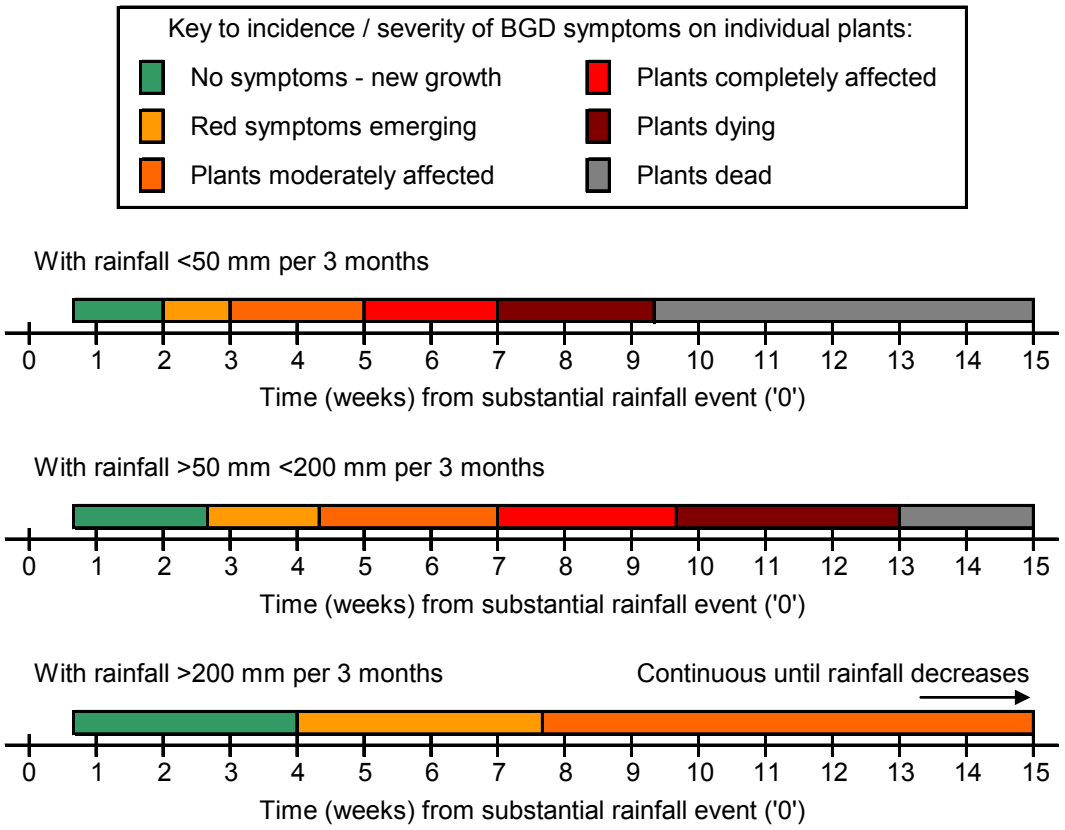


Figure 7 – Time lines showing severity of buffel grass (*Cenchrus ciliaris*) dieback symptoms with various amounts of rainfall

Contrary to Graham and Conway's (1998) work, symptomatic leaves did not always have a clear red/green boundary. Often symptoms began with red mottling of the leaves, which eventually coalesced to produce entirely red regions (Figure 8). Occasionally BGD symptoms progressed faster down one half of the leaf (Figure 8). Symptoms were invariably more vivid on the adaxial surface of the leaves than on the abaxial surface. If the

leaf vasculature was damaged, by cattle or vehicles passing through, the progression of symptoms down the leaf was delayed at the site of damage. Previously affected dead leaves remained bronze in colour, instead of the usual straw colour of unaffected dead buffel grass plants. There was never any 'rust' or powdery substance on the leaves.

Figure 8 – Buffel grass (*Cenchrus ciliaris*) dieback symptoms showing mottling (left) and uneven spread of symptoms down the leaf (right)

Occasionally, large necrotic foliar lesions were present, although only in a small percentage of affected plants (estimated 2%). Lesions were also occasionally found on unaffected buffel grass plants. Lesions were usually ovoid, elongating linearly but more pronounced at the tip. The lesions had a firstly grey, then brown margin with the centre of the lesions darkening. The periphery of the lesions often turned beetroot to plum red (RHSPCC red group 45: A, B; 46: A, B)(The Royal Horticultural Society, 2001) (Figure 9). This colouring was more conspicuous on the veins but spread to the interveinal areas with age. Lesions often coalesced with chlorotic zones which turned red. The red pigment was usually linearly distributed. Mature lesions were entirely necrotic with minute black structures, possibly fungal reproductive structures. These were isolated and identified as reproductive structures of *Pyricularia grisea* (described in Chapter 9).

Figure 9 – Necrotic lesions found on both dieback affected and unaffected buffel grass (*Cenchrus ciliaris*) leaves (left), and the black structures found in the lesions

Roots of affected plants appeared stunted compared with roots of unaffected plants. The roots were approximately half the length of unaffected buffel grass roots, though their diameter was similar. There were also fewer roots (Figure 10). When cleaned of dirt, roots of affected plants occasionally displayed soft, darker, necrotic regions, and sporadically displayed ovoid lesions (Figure 11). There was no evidence of any insect damage such as chewing, rasping or stab wounds.

Figure 10 – Roots of unaffected (left) and dieback affected (right) buffel grass (*Cenchrus ciliaris*) plants

Figure 11 – Necrotic areas (left) and lesion (right) of roots of buffel grass (*Cenchrus ciliaris*) affected with buffel grass dieback

BGD affected and unaffected buffel grass plants were examined for the presence of insects and insect damage. In each quadrat in both unaffected and BGD affected areas, 2-4 plants showed signs of minimal insect damage such as chewed leaves or leaf spots. Though the occasional grasshopper was found, they were present in low numbers. No aphids or other sap-sucking insects were found.

Buffel grass has a tendency to become dormant in times of water stress. While the above ground parts of the plants die, the roots are still alive. New shoots emerge once adequate rainfall has fallen. If the dieback condition killed the plant before the onset of dormancy, clearly no new shoots were produced subsequent to a rainfall event. However, if dormancy occurred before the plant succumbed to the condition, new shoots were produced after rain, and the cycle repeated with symptoms first appearing in the oldest leaf.

A comparison of the Biloela and American cultivars showed that the American cultivar is the most susceptible to BGD. The Biloela buffel plants, whether growing in the middle of a BGD affected patch (of American buffel) (Figure 12) or growing in an unaffected area, displayed no dieback symptoms. In fact, the Biloela buffel plants in both

areas appeared to be healthy, with comparable plant and root size and no root abnormalities or lesions.

Figure 12 – Apparently healthy Biloela (cv.) buffel grass (*Cenchrus ciliaris*) growing within a dieback affected patch of American (cv.) buffel grass (white tags show patch boundary)

Symptoms at the Patch Level

Patches were roughly circular and ranged from 2 m to over 60 m diameter. Adjacent patches often coalesced and further enlarged (Figure 13). Red symptoms first appeared on the periphery of an older existing patch, where during the last cycle the plants had become dormant before succumbing to the condition. All plants that succumbed and died before dormancy left a bare central area which was usually recolonised by native species and introduced species (other plant species will be addressed in a later chapter). Symptoms progressively moved outwards from the periphery of the patch: slowly if moving into previously unaffected areas, but more rapidly if moving into previously affected areas. Previously affected buffel grass plants, while having formed new shoots simultaneously after rain, did not all exhibit symptoms at the same time, but progressively

from the periphery of the existing dead patch, that is, symptoms progressed back towards the centre of the patch, but did not begin there. The progression of symptoms outwards and inwards from the periphery of the patch created a 'ring' effect.

Figure 13 – Large area of coalesced patches of buffel grass (*Cenchrus ciliaris*) dieback. The greyer areas on the right are dead buffel grass, having succumbed to the dieback condition. The red patch boundary can be seen winding through the middle.

After a high rainfall event, self-sown seedlings often recolonised the bare central area. These subsequently succumbed to the condition in the aforementioned pattern, depending on their location in the patch. Generally, they succumbed before reaching the third leaf stage.

Symptoms at the Paddock Level

Patches appeared to be randomly distributed throughout a paddock. However, in some instances they appeared to be associated with cattle or vehicle trails (Figure 14). It was unclear as to whether or not the patches originated from the trails.

Figure 14 – Buffel grass (*Cenchrus ciliaris*) dieback patches along a cattle trail

Concerning the development of symptoms (following the June 2002 rainfall event), approximately 10% of previously affected buffel grass were showing symptoms 17 days after rain, this rainfall event being subsequent to a period of drought. This increased to 30% after 22 days, 35% after 25 days, and 100% at 54 days.

2.3.3 Discussion

Figure 4 shows two distinct increases in symptom severity to the point of plant death: from May to July 2002, and from December 2002 to March 2003. These do not correspond with the mean monthly temperatures (Figure 4), since one symptom severity increase occurs in Autumn/Winter and the other in Summer. Therefore, it appears that ambient temperature does not contribute to the BGD condition. However, both increases occurred after a large monthly rainfall (which followed a dry period) followed by low monthly rainfall, such as in June 2002 and February 2003, that is, symptoms emerged

following a period of lush growth, and increased in severity when monthly rainfall decreased.

From Figure 4, it appears that a larger amount of rain increased the time needed for symptoms to overcome the plants. In both cases (June 2002 and February 2003) there was little subsequent rain. In comparison, following a continuous supply of moderate rain, such as the late 2003 to early 2004 rains, symptom severity did not increase to the stage where most plants were dying (severity ≥13). Available water seems to be of great importance with the dieback condition, since when adequate water is available, the condition is firstly delayed from starting, and secondly cannot overcome the plant (refer Figures 4 & 5). The fact that plants only completely succumb in dry times may indicate that stress of some kind, whether water stress or water-related stress (such as the unavailability of water-soluble nutrients) is a contributor to the BGD condition (Ayres, 1978; Schoeneweiss, 1978). Buffel grass is reported as having "outstanding drought tolerance" (Loch, 1999), mainly because it has several mechanisms which aid it to cope with water stress, such as osmotically adjusting its leaves and becoming dormant in even drier times (Wilson *et al.*, 1980). These mechanisms are common with a number of other semi-arid, tropical C_4 grass species (Ng *et al.*, 1975; Wilson *et al.*, 1980). As such, it is unlikely that water stress is the primary cause of BGD but, if present, makes the plants more susceptible to other causal agent(s) of BGD (Boyer, 1995).

The bright red symptoms are intriguing. Colour changes in non-senescent leaves are usually produced by a nutrient imbalance, as evidenced by Smith's work (1974) on nutrient deficiency in buffel grass, by a chemical imbalance (eg. herbicides), or the presence of a pathogen, for example, *Rhizoctonia* bare patch ('purple patch') of wheat and

oats, causing yellow and purple foliar symptoms (Hynes, 1933; Kirkegaard *et al.*, 1999; O'Brien, and Zamani, 2003). These colour changes are usually from the accumulation or destruction of various photosynthetic pigments (Roberts & Whitehouse, 1976; Salisbury & Ross, 1992; Matile *et al.*, 1999; Close and Beadle, 2003), and may explain the mottling, often seen before a red/green boundary is produced.

The progression of red symptoms from older leaves to younger leaves and from the tip to the ligule may signify vascular involvement of the causal agent(s). This may indicate a nutrient imbalance, whereby nutrients are deliberately translocated throughout the plant (Matile *et al.*, 1999; Close and Beadle, 2003), or that the agent causing the red symptoms may be accumulating in the leaves. The question is by which pathway would this causal agent travel?

In grasses, transport through the phloem usually travels from source to sink, that is, from the mature leaves to the younger growing leaves, fruiting structures, storage organs and roots (Forde, 1966). While phloem transport explains the progression of symptoms from older leaves to younger leaves, it does not explain how the older leaves showed symptoms in the first place. It could not have been from the roots, since these act as a sink. One explanation would be that the causal agent(s) specifically and preferentially affects older leaves, which seems unlikely. Also, in grasses, phloem transport from the older leaves tends to go to the roots, whereas phloem transport from younger leaves tends to go to the growing tips (Forde, 1966; Salisbury & Ross, 1992), which again opposes the idea of phloem transport. The exception to this is that, under certain environmental stresses, such as after heavy grazing, fire, or at the end of a dormant period, the roots may act as a carbon source, with the phloem flowing from roots to leaves. However, this flow would tend to be directed to younger leaves, not the older leaves (which first display symptoms), which

again opposes the idea of phloem transport. It is also possible that the symptoms in the older leaves indicate a nutrient deficiency, whereby nutrients are translocated (by the phloem) out of the older leaves to the younger leaves, resulting in colour changes in the older leaves.

Another argument in relation to phloem transport is bioaccumulation and the partitioning of resources and/or harmful substances. The causal agent(s) may be taken up by the plant and is being translocated and partitioned to the older leaves, whereby a critical accumulated amount above a threshold level causes visible symptoms. As the next oldest leaf matures, partitioning occurs to that leaf and so on up the plant. However, the red symptoms of BGD are not restricted to older, mature leaves. Younger leaves also displayed symptoms once growth was slowed.

The transportation of the causal agent(s) through the xylem is another possibility, especially when in conjunction with bioaccumulation. In theory, the larger mature leaves would have a higher rate of transpiration than the younger leaves. Therefore, proportionally more of the xylem contents would reach them, and while water would be transpired, other components would be used or accumulated presumably first at the leaf tip, as occurs with boron toxicity (Nable *et al.*, 1997). This is, of course, assuming that the causal agent(s) enters the plant via the roots or the stem below the first leaves.

The uneven spread of symptoms down an affected leaf supports the idea of bioaccumulation. The more vivid colours on the adaxial surface of the leaf indicate that the accumulation (if any) occurs in specific cell types, such as chlorenchyma cells. It may also signify that the red symptoms are a product of the accumulation or destruction of photosynthetic pigments, that is, either anthocyanins and/or carotenoids are being produced

in higher amounts, or chlorophyll is being destroyed. This is discussed further in Chapter 5.

The bronze colour of dead leaves from an affected plant, compared with the usual straw colour of dead leaves from unaffected plants, may signify that a chemical change has taken place or that something, such as anthocyanins, was left behind in the leaf (Lee *et al.*, 2003). Ordinarily most leaf components are translocated from a dying leaf before senescence occurs.

The length of time from the first appearance of red symptoms to plant death is an important piece of information as it may provide important clues to the cause of BGD. While this time appears to be dependent on the amount of available water (Figure 5), an approximation of this time could be established in controlled conditions (section 2.4).

The absence of a powdery substance or rust on affected leaves indicates that a powdery mildew or rust is not the primary causal agent(s). The lesions observed (with minute black structures) on some affected plants may be a secondary symptom of BGD; therefore the causal agent of the lesions may also be the causal agent(s) of BGD. However, since lesions are only present on a small percentage of affected plants and are also present on unaffected plants, it is more likely that the causal agent(s) of the lesions has nothing to do with BGD, and is simply an opportunistic pathogen. Nevertheless, microbial isolations were attempted from lesions and other plant parts (refer Chapter 9).

The smaller and fewer seed heads on plants affected with BGD may indicate that there are insufficient resources for seed production or that resources are partitioned elsewhere. This is usually caused by chemical/nutritional stress (Welch, 1986) or disease, for example, as with *Rhizoctonia* root-rot of wheat (Kirkegaard *et al.* 1999). The darker

colour may be related to the leaf colour change, especially to the 'bronzing' of red leaves when dead, since this is a similar colour.

The stunted roots of BGD affected plants may be caused by a nutritional imbalance or by a decreased carbohydrate source, since the above ground plants were also stunted. However, the stunted roots could have caused the stunted plants since there would be a restricted supply of minerals and water. Irrespective of which caused the stunting of the other (or possibly both simultaneously), the causal agent would have eventuated outside the plant. Often stress of some kind causes stunting, whether relating to water, nutrients or disease.

Severe water stress results in wilting, which is not a symptom of BGD. Minor water stress could stunt the plant (Ng *et al.*, 1975; Ayres, 1978; Durbin, 1978). However, this would also be reflected in the growth of other plants in the paddock since water stress is generally not localised or restricted to patches. It is unlikely that water stress is the main cause of the stunting of affected plants.

A lack or excess of certain nutrients can cause stunting, often accompanied by various leaf discolorations (Smith, 1974; Roberts & Whitehouse, 1976; Salisbury & Ross, 1992). In relation to buffel grass, Smith (1974) reported that deficiencies of nitrogen, phosphorus, magnesium, sulfur, and zinc resulted in red foliar symptoms. Whereas this seems to fit the profile concerning BGD red symptoms, a nutrient imbalance would also be apparent in adjacent unaffected plants, especially in relation to leaf symptoms, which tend to appear gradually. While some nutrient disorders, such as boron toxicity, do occur in 'patches' and can vary even between cultivars of the same species (Nable *et al.*, 1997; Reid *et al.*, 2004), it is unlikely that, in a paddock which comprises *ca.*90% American buffel grass, symptoms would only appear in small (3-4 m) patches which spread and converge.

Since the plants are of essentially the same age, especially following a drought, symptoms would be expected to show simultaneously in a much larger area.

Plant diseases often cause stunting and have been known to occur in patches with defined boundaries (Couch, 2000; Smith *et al.*, 2001; O'Brien and Zamani, 2003; MacNish and O'Brien, 2003). The stunting can be due to numerous factors, including the pathogen:

- feeding off the host thereby reducing resources available for growth,
- occluding the vasculature thereby restricting the flow of resources,
- interfering with plant growth regulators through toxins or other products,
- producing toxins which damage cells (Agrios, 2005).

In the case of BGD, disease is a likely explanation for stunting, although the stunting observed in BGD affected plants could be caused by a combination of stresses.

The observed root necrotic areas and root lesions on BGD affected plants are most probably caused by one or more disease causing organisms, since there were no signs of insect attack. A number of organisms cause root rot, such as *Rhizoctonia* (MacNish and O'Brien, 2003), *Gaeumannomyces* and *Fusarium* (Cromey *et al.*, 2006). Similarly, several organisms can cause lesions, such as rhizobacteria (Suslow and Schroth, 1982) and *Phytophthora* (Matheron and Matejka, 1993). However, whether the necrosis and lesions are caused by (one of) the causal agent(s) of BGD or by an opportunistic pathogen is unknown.

Observations of insects showed no obvious pests and no signs of large-scale insect damage or colonisation. Minor insect damage was seen on both affected and unaffected buffel grass. While this suggests that insects are not the main causal agent of BGD, it is possible that they are a contributor to plant stress or are involved in the spread of the

condition. For example, some insects could be involved with the movement of soil, such as dung beetles, or in some cases act as vectors for plant diseases (Vanderplank, 1982; Khan & Pathak, 1993).

The relationship between BGD and buffel grass dormancy could signify one of two possibilities. Firstly, the primary causal agent(s) could be biological and become dormant when the host is dormant. This occurs in many agricultural soils where nutrients are limiting, with dormancy of the pathogen being broken when in the presence of chemicals from germinating seeds or growing roots (Nelson, 1990). Secondly, the primary causal agent(s) could be chemical and the uptake of this chemical(s) ceases when dormancy is achieved. This could also be true of a toxin produced by a biological agent(s).

The occurrence of patches of affected grass gives a clue to the nature of the dispersal of BGD. If the causes were windborne, it would be expected that affected patches would mostly occur in the direction of the prevailing winds (McCartney, 1991; McCartney and Fitt, 1998). At the field site, prevailing winds are southerly (Bureau of Meteorology, pers.comm.). The condition was first sighted in the southern corner of the paddock (primary producer observation, section 2.2, p.28), and subsequently spread to a paddock SE of the original paddock. Therefore, it is possible that the causal agent(s) of BGD is dispersed by wind. Sap-sucking insects or other insect vectors could spread the causal agent(s). In these cases disease dispersal is a result of chance contamination of insects visiting plants, sometimes preferentially visiting diseased or healthy plants (McElhany *et al.*, 1995). Therefore, while these diseases occur in patches, they do not usually spread in circular spreading patches (McElhany *et al.*, 1995; Jones, 1996; Ogle and Brown, 1997). Waterborne conditions which occur in patches are generally caused by foliar pathogens dispersed by rain splash (McCartney and Fitt, 1998). Any causal agents in the soil which

were spread by water would be expected to spread in the direction of water runoff, and while they may form patches where there is lying water or possibly from rising water tables, they would not be expected to spread in circular patches. Conversely, soilborne conditions frequently occur in patches, especially when the cause is a disease-causing organism (Murray and Davis, 1996; Couch, 2000; Agrios, 2005).

In several instances, BGD affected patches were associated with cattle or vehicle trails. This suggests that the condition may be spread through plant contact (as cattle or vehicles move through) or through soil (soil in hooves and tyres).

So far, a soilborne and/or windborne causal agent(s) is the most likely method of spread, though the condition may also spread by plant-to-plant contact. However, what is causing the patches?

Chemical or nutritional problems would not normally be expected to occur in circular patches, but more often in large and/or irregular shaped areas, as occurs with boron toxicity (Nable *et al.*, 1997) and salinity (Cordoba *et al.*, 2001). Occasionally they occur in smaller irregular shaped areas as in the case of a chemical spill (fertiliser, pesticide, herbicide, etc.). It is unlikely that BGD is caused by pesticide residues. Since buffel grass is generally apomictic, the plants are essentially 'clones', and would therefore have the same or similar pesticide resistance. Symptoms would appear in all or most plants, and not be restricted to enlarging circular patches. Therefore, while a chemical imbalance may be a contributing factor to BGD, it is unlikely that it is the primary causal agent(s). Patches in crops or grasses are most commonly caused by pathogens (Parry, 1990; Couch, 2000; Smith *et al.*, 2001; MacNish and O'Brien, 2003; O'Brien and Zamani, 2003), which seem to be the most likely causal agent(s) of BGD.

Another argument for a biological primary causal agent(s) is the 'ring' effect, also called the frogeye pattern (Nable *et al.*, 1997; Couch, 2000; Agrios, 2005), that is, a 'ring' of affected plants, surrounded by unaffected plants, with dead plants in the middle of the 'ring'. This is a symptom of several plant pathogens including *Rhizoctonia* (MacNish and O'Brien, 2003) and *Gaeumannomyces* (Wong *et al.*, 2000). If the cause were primarily chemical, most of the previously affected plants, once dormancy was broken, would show symptoms almost simultaneously, as the chemicals causing the condition would probably still be present in the soil, thereby being taken up by all plants growing in the old patches.

The recolonisation of affected patches by other plant species suggests that the condition is specific to buffel grass, or more precisely, to American buffel grass, since Biloela buffel grass appears to be unaffected. This suggests that the primary causal agent is a disease-causing organism, as these are often host specific (Parry, 1990). A nutrient or chemical imbalance is not usually specific to a particular species, though some species may be more tolerant than others. Usually, other species in the vicinity would also display some degree of symptoms.

The rapid re-emergence of BGD after a period of drought shows that it has the potential to spread quickly (all re-affected 54 days after rain). This could be detrimental to graziers. The rate of spread of BGD needs to be determined to obtain a better estimate of how fast this condition can destroy established pastures.

From the above results, it seems that the most likely primary cause of BGD is biological and that other stresses are minor contributors. The condition is probably soil or water-borne, entering through the roots, and possibly spreading through the xylem of the plants. The possibilities of the condition spreading via plant-to-plant contact or being windborne have not been eliminated.

2.4 Observation of Field Plants in Controlled Conditions

2.4.1 Materials and Methods

Methodology

While the above study gave a general time line of symptoms and plant death (Figure 5), it was considered necessary to determine the length of time from early symptom emergence to plant death under more controlled conditions. These data would act as a baseline for future field and shadehouse work. For this purpose, field plants were collected and kept in a shadehouse for observation. Observing these plants at more regular intervals may also reveal any subtle changes in symptoms not evident in the field due to the length of time between field trips.

Method

Twenty buffel grass clumps (mature plants with 15-20 tillers) (American cultivar), ten unaffected, the other ten just starting to show BGD symptoms, were collected from the field, ensuring minimal root disturbance, that is, they were dug out with the surrounding soil (20 cm radius, 60 cm deep). They were collected from the same field to minimise differences in soil type. These were then transplanted into large pots (40 cm diameter x 60 cm height) with field soil, and brought back to CQU (Rockhampton) where they were kept in a shade house. While it is standard practice to arrange plants in a randomised design, it was believed that this may cause a shading effect unlike that which is found in the field, since the unaffected plants are larger than the BGD affected plants (refer to observations of previous section). Therefore, the BGD affected plants were arranged into a roughly circular shaped ‘patch’, with the unaffected plants forming a circle around them. The

leaves of the plants, both BGD affected and unaffected were in contact as they are in the field.

This experiment ran for six months (September 2001 – March 2002). The plants were watered in accordance with the amount of rainfall which usually falls over that growing season, that is, 350-400 mm (data obtained from Bureau of Meteorology). Therefore, the plants were watered (with a sprinkler system) once a week with the equivalent of *ca.*15 mm rain.

The plants were observed for changes weekly over a six month period.

2.4.2 Results

All plants suffered slightly from transplant shock (some minor wilting) but recovered after the first watering.

The unaffected plants never displayed BGD symptoms and continued growing and developing, including seed production. The BGD affected plants recovered somewhat (some symptoms disappeared) with the first watering but symptom progression continued one week later. Symptoms progressed as observed in the field (Section 2.3). The BGD affected plants did not grow much, each only producing 2-3 new tillers. The BGD affected plants never recovered again and all eventually died between 16-17 weeks (4 months).

2.4.3 Discussion

The slight recovery of the affected plants after watering concurs with the field observation that the BGD condition seems to disappear after rain. This again suggests that water availability has some involvement with the BGD condition, though whether it affects the plants or the causal agent(s) is unknown.

While four months is only an approximation, it shows that BGD can kill rapidly. This factor coupled with a possible fast rate of spread could be devastating to primary producers.

2.5 Other Plant Species Potentially Affected

2.5.1 Materials and Methods

Methodology

Patch diseases of cereals and grasses are relatively common (Parry, 1990; Couch, 2000), and are usually host specific. Therefore, if the causal agent(s) of BGD is biological, it is possible that species which are related to buffel grass, or are otherwise similar, may be susceptible to BGD. Conversely, patchiness in a lawn or pasture, caused by a localised chemical imbalance, often affects several other species, depending on their tolerance levels. Therefore, if a chemical imbalance is the causal agent(s) of BGD, it would be expected that several other species also exhibit BGD symptoms or signs of stress, especially within patches of BGD affected buffel grass. Hence, the purpose of this study was to provide clues as to the causal agent(s) of BGD.

Method

Two studies were done on other plant species. The first study was done in the early stages of the research (2001). Unfortunately, the plant species of interest (see Results below) was only present in very low numbers (in BGD affected areas), and all of them died soon after this study. It was not until the end of this research (late 2007) that BGD patches had reached other areas where this other plant species grew. Therefore, the second study is not as extensive as the author would have liked.

The first study was done using the same paddock as in Section 2.3, at a time when BGD symptoms were prevalent. Observations were taken by walking along a series of transect lines 10 m apart and stopping every 10 m, thus taking observations in a 10 x 10 m grid pattern. This area included 10 patches of BGD affected buffel grass. These patches were 3-4 m in diameter and were separated from each other by several metres of unaffected buffel grass and other plant species.

Plant species other than American buffel grass were observed not only for symptoms of dieback, but also other signs of stress, such as wilting or colour change. Particular attention was given to those plants which recolonised patches of BGD affected buffel grass.

The second study was done in the same manner as the first, with the following exceptions:

1. the study was done in two paddocks, the one mentioned above and an adjacent paddock, since the BGD condition had spread to an area where the plant species identified in the first study was prevalent.

2. twenty symptomatic plants of the species identified in the first study (*Urochloa mosambicensis*) were closely examined and their symptoms documented. This included exhuming the plants and taking note of any root symptoms. Twenty plants which were not displaying symptoms were also examined, including the roots. The sizes of both symptomatic and asymptomatic plants were measured.

2.5.2 Results

For a complete list of other plant species present, refer to Tables 27 and 28 (Section 9.2, p. 215). In both studies, *Urochloa mosambicensis* was the only plant found exhibiting

any symptoms within patches of BGD affected buffel grass. Other plant species showed no unusual symptoms or signs of stress.

In the first study, *U. mosambicensis* was found in five of the ten patches, and in each case was showing various symptoms (Table 7).

Patch number	Condition of *Urochloa mosambicensis*
2	Leaves orange-yellow from the tip down, colour mottled. Necrotic tips.
4	Leaves yellowing from the tip down, some mottling. Occasional brown necrotic lesions with yellow halo.
7	Mottled yellow leaves, slightly orange on either side of the midrib. Necrotic tips.
8	Leaves yellow from the tip down, in some places greenish. Some necrotic lesions.
10	Leaves yellow-orange, mottled. Necrotic tips.

Table 7 - Presence and condition of *Urochloa mosambicensis* growing within patches of BGD affected buffel grass (*Cenchrus ciliaris*)

The leaves of *U. mosambicensis* were chlorotic from the tip towards the ligule, ranging in colour from greenish-yellow, to yellow, to orange (RHSPCC: yellow-green group 145 C, 149 D, 150 D, 154 C & D; yellow-orange group 21 B & C, 23 B; orange-red group 30 B, 32 A)(The Royal Horticultural Society, 2001). The colour was often mottled, and was occasionally further down one side of the leaf than the other. Leaf tips were necrotic (RHSPCC greyed-orange group 177 D)(The Royal Horticultural Society, 2001). The symptoms were more prevalent on the mature leaves, and to a lesser extent on the next oldest leaves. These symptoms were observed on the older tillers; the younger tillers were unaffected (Figure 15).

Figure 15 - Symptoms of *Urochloa mosambicensis* (left) growing in a patch of dieback affected buffel grass (*Cenchrus ciliaris*) (right)

Small lesions (*ca.*2 mm) were seen on many leaves. These had a necrotic centre and yellow halo. The halo was often uneven, and coalesced with other chlorotic areas of the leaf (Figure 16).

Figure 16 - Lesions on leaf of *Urochloa mosambicensis* growing in a patch of dieback affected buffel grass (*Cenchrus ciliaris*)

In the second study, there were 32 instances where *U. mosambicensis* was found growing in a BGD affected patch of American buffel grass. In all of these cases, *U. mosambicensis* displayed the above symptoms. In many cases the symptoms appeared more severe than in the first study, with plants showing more red symptoms rather than

yellow (Figure 17). Affected plants also followed a similar pattern to BGD symptom progression in Buffel grass, in that symptoms progressed from older to younger tillers.

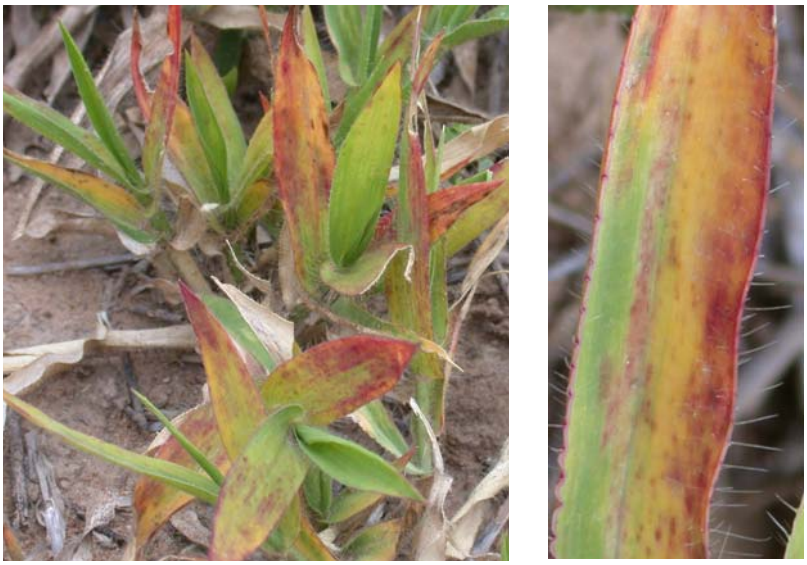


Figure 17 – More severe symptoms of *Urochloa mosambicensis* growing in a patch of dieback affected buffel grass (*Cenchrus ciliaris*)

When not within patches of BGD affected buffel grass, *U. mosambicensis* appeared healthy. The exceptions were two roughly circular coalescing patches (each *ca.* 4 m diameter) of *U. mosambicensis* which were displaying the above symptoms (Figure 18). The patches were situated within a slightly larger area of *U. mosambicensis* pasture, along a dirt vehicle trail. There was an emerging patch of BGD affected buffel grass across the trail from these patches.

Symptomatic plants of *U. mosambicensis* were slightly smaller (approximately 80%) than asymptomatic plants. There were no obvious differences in the roots (Figure 19), and no signs of root lesions or damage. Symptomatic plants seemed to produce similar numbers of seed heads to asymptomatic plants (3-4 per plant), and seed heads were similar in size.

Figure 18 – Symptomatic patch of *Urochloa mosambicensis* growing along a vehicle trail (bottom left)

Figure 19 – Roots of asymptomatic (left) and symptomatic (right) *Urochloa mosambicensis*

2.5.3 Discussion

The similarity in the pattern of the symptoms displayed by *U. mosambicensis*, as compared with the symptoms of BGD affected buffel grass, opens the possibility that both species are affected by the causal agent(s) of BGD. Both *U. mosambicensis* and buffel grass exhibit a colour change from the tip down, progressing from the older leaves to the

younger leaves. Both have a marked tip necrosis, and both have the symptoms showing first on the older tillers.

While the symptom progression pattern was the same in both species, the foliar colour change was somewhat different, with *U. mosambicensis* showing more yellow symptoms than red. This is a common occurrence in pathogens that have more than one species in their host range. For example, *Rhizoctonia* produces purple foliar symptoms in wheat (Kirkegaard *et al.*, 1999), but produces sharp eyespot in other cereals (Cromey *et al.* 2006).

There were some other differences between *U. mosambicensis* and buffel grass symptoms, such as in the roots. There was no obvious difference in the roots of asymptomatic and symptomatic *U. mosambicensis*, whereas there is a marked difference in the roots of BGD affected and unaffected buffel grass. This may be due to a pathogen causing dissimilar symptoms on different host (as mentioned above), or that the two conditions have different causal agents. Further tests will be necessary to determine which of these is correct.

Other plant species did not display any symptoms, whether a colour change, wilting or morphological changes. This seems to suggest that the main causal agent(s) of BGD is biological rather than chemical. It is unlikely that of all the species present, all but two are tolerant to a particular chemical condition. It is more likely that the cause is a host-specific biological agent.

There are relatively few diseases of *U. mosambicensis*, most of which are viral, for example, *Digitaria* striate virus (Greber, 1979) which causes foliar striations, and sugar-cane mosaic virus (Teakle and Grylls, 1973) which causes mosaic or necrotic reactions. However, none of the reported diseases produce the symptoms depicted above, suggesting

that the symptoms observed on *U. mosambicensis* are either caused by the same causal agent(s) of BGD, or that it is a new unreported condition.

Pathogens tend to have a host range which includes closely related species. Therefore, further clues as to the identity of this causal agent(s) could be obtained by comparing buffel grass and *U. mosambicensis*. Both species are in the family Poaceae; in fact, both are in the sub-family Panicoidea and Tribe Paniceae. Both are also C_4 grasses, so it is likely that they have many other similarities. Both species are predominantly apomictic. This would mean that a population would be more susceptible to disease due to the low genetic diversity (Czapik, 2000). However, Biloela buffel grass also has these attributes, and is more closely related to American buffel than *U. mosambicensis*, and yet it appears to be unaffected by BGD. If the causal agent(s) of BGD are also producing the above symptoms in *U. mosambicensis*, it is either a pathogen with a very specific host range, or several causes which, together, affect both buffel grass (American) or *U. mosambicensis*. Clearly, more work is needed regarding *U. mosambicensis*.

2.6 Concluding Statements

In the above sections a complete description of BGD was made. It seems likely that the primary causal agent(s) is biological. Other stresses, possibly environmental ones such as water availability or a nutrient imbalance, are probably minor contributors to the condition, weakening the plants and making them more susceptible to disease.

Chapter 3

Patch Dynamics and Factors Responsible for Patch Spread

3.1 Introduction

As previously mentioned (section 2.3.3, p. 60), both chemical and biological agents can cause patch disorders. Those patches with chemical causal agents tend to appear in large areas rather than spread from one point, with the rate of symptom emergence being dependent on factors such as the amount of the chemical/nutrient available to the plant, the tolerance levels of the different plant species present, water availability (if it is water soluble), and soil physical properties (if it is soilborne) (Durbin, 1978; Jones, 1998). Patches with biological causal agents spread according to the movement and/or dispersal of the causal agent. Similar to patches caused by chemical agents, the amount of spread can also be dependent on water availability and soil physical properties. In addition, it can also be dependent on weather patterns, the presence of vectors (if applicable), the presence of other microbes and the resistance of the host plant species (Parry, 1990; Jones, 1996; Murray and Davis, 1996; Couch, 2000).

In Chapter 2 the occurrence and mode of development of patches of buffel grass affected with the dieback condition were noted and discussed. While new patches appear randomly within a paddock, the rate of lateral spread of individual patches needed to be determined, as well as factors responsible for the spread of these patches. This information would be valuable to the grazing industry to determine a rate of pasture loss (under normal environmental conditions).

3.2 Screening of an Affected Paddock

3.2.1 Materials and Methods

Methodology

Before attempting to measure the lateral spread of patches, factors which may influence this spread and/or patch location should be identified, for example, the patches may spread downhill or along cattle tracks, as observed in Chapter 2. The distribution of patches may also be correlated with other paddock features, such as areas where cattle rest, commonly known as 'cattle camps'. The distribution of patches and other paddock features could be compared in a map. This map would have to be of a reasonably BGD affected paddock, and for practical purposes be done as a grid map, with a series of transects.

Method

A grid map was made of an 81 ha. (200 acre) buffel grass paddock (coordinates 24^{o} 20′ 55.8″ E, 149^{o} 51′ 33.0″ S), which was heavily affected by BGD, but was originally almost a monoculture of American buffel. The mapped paddock was the paddock in which BGD was first sighted on Property 1, as mentioned in Chapter 2, part 2.2. The paddock was screened in a grid pattern for the distribution of the dieback condition. Transects were placed at 20 m intervals, stopping every 20 m along a transect line. Each grid square was noted as containing predominantly affected buffel grass (either heavily (>70% affected) or moderately (<70%) affected), unaffected buffel grass, or other plant species. Cattle tracks and vehicle tracks were also noted.

3.2.2 Results

(refer Figure 20)

Figure 20 - Grid map of a predominantly buffel grass (*Cenchrus ciliaris*) paddock severely affected with the dieback condition

Notes: Tracks and trough not to scale.
Trees and large shrubs are not shown.
Moderately affected areas include areas with small patches (<10 m diameter)
Cattle camps are in the central large patch and along the south-eastern boundary fence.

The condition was first sighted (by primary producers) in the southern corner and in the central cattle camp of the paddock. The southern corner is the downhill side, though the slope of the land is very gentle (1-2°). Many smaller patches (in moderately affected areas) were along cattle and vehicle trails, although not exclusively so. All of the large coalesced patches were in contact with either a vehicle or cattle trail, and in about 50% of cases were orientated along them. Areas with predominantly other plant species (other than buffel grass) were mostly (*ca.*70%) found surrounded by heavily affected areas. They were also found in moderately affected areas but never surrounded by unaffected buffel grass.

3.2.3 Discussion

While patches were found along cattle and vehicle tracks, and in about 50% of cases were orientated along these tracks, there was no strong correlation with them, that is, patches were not exclusively found along tracks. Therefore, it is probable that the condition is not exclusively spread by cattle or vehicles. However, the results suggest that cattle and vehicle movement may be involved with the spread of the condition.

Certain plant diseases are spread by cattle when infected plants are eaten, digested, and the remains (including the causal agent) are excreted. For example, cattle feeding on root crops such as turnips infected with clubroot (*Plasmodiophora brassicae*) spread the propagules in their dung (Ogle and Brown, 1997). However, these are not likely to be spread by vehicles driving along a track. Insect vectors of diseases may be moved by cattle or vehicles, as can infective propagules on infected leaves when cattle or vehicles move through infected plants (Ogle and Brown, 1997). Soil containing a pathogen can also be moved either on hooves or tyres. It is unlikely that a chemical causal agent would be spread by these means.

The possible link between the BGD condition and cattle or vehicle tracks suggests that the condition is caused primarily by a disease-causing organism.

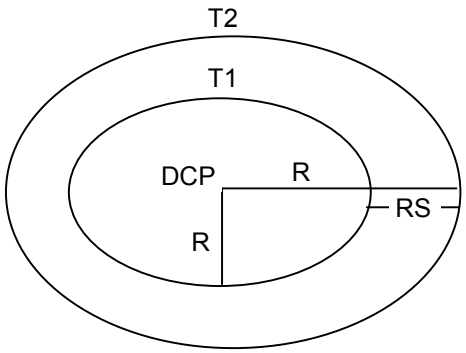
3.3 Individual Patch Spread – Pilot Study

3.3.1 Materials and Methods

Methodology

A pilot study was done to determine the rate of lateral spread of a BGD affected patch. Since not all patches are regular in shape, this could also identify any variations in patch spread.

Given that the patches are not perfectly circular, a line segment from the designated centre point to the periphery of the patch will be called a 'ray', not a radius. The increase in patch size over time will be called 'radial spread' (refer Figure 21).



Legend	
DCP	Designated centre point
T1	Time 1
T2	Time 2
R	Ray
RS	Radial spread

Figure 21 – Diagrammatic representation showing terminology used to describe a patch of buffel grass (*Cenchrus ciliaris*) affected with the dieback condition

Method

A relatively isolated BGD affected patch of American buffel grass was chosen at the field site, in the same paddock as the mapping exercise (section 3.2). A wooden peg was driven into the ground at the estimated centre of the patch. It should be mentioned that a

cattle track crossed through the patch at the northern side and that the land sloped in a S-SW direction (*ca.*1.5°). Rays were measured in four places, avoiding obstructions such as trees and shrubs. The measurements were taken from the central wooden peg to the furthermost plant displaying symptoms. The measurements were repeated six weeks later. This trial was undertaken during autumn 2001 (March – May), with normal temperature ranges and average rainfall (50 mm spread over this period).

3.3.2 Results

Direction of radii	Initial Measurements (cm)	Measurements after 6 weeks (cm)	Increase (cm)
7° W of N	1000	1030	30
West	900	950	50
South-West	1800	1980	180
South-East	2300	2450	150

Table 8 - Radial spread measurements of a patch of buffel grass (*Cenchrus ciliaris*) affected with the dieback condition

Average radial spread (over all directions): 17.1 cm per week

Average radial spread (North and West sides): 6.7 cm per week

Average radial spread (South-East and South-West sides): 27.5 cm per week

Unfortunately, the chosen patch converged with a nearby patch at *ca.* 8 weeks, therefore further measurements could not be made.

3.3.3 Discussion

Over the 6 week period of this particular trial, it can be seen that the dieback condition spreads relatively quickly. However, the spread of the patch was uneven, favouring the south-west and south-easterly directions. Several possible hypotheses arise.

The uneven spread of the patch aligned with the slope of the land, suggesting that the dieback condition spreads more rapidly downhill. Assuming that this is correct, this might imply that the main causal agent of BGD is waterborne via rainfall runoff. The property owner stated that the dieback condition tended to spread more rapidly in or immediately after wet weather. During the course of this trial there was enough rainfall to cause slight runoff, so this hypothesis is plausible.

Another possibility for the uneven spread of the condition is soil compaction. A cattle track crossed the path on the northern side, which was the side with the least growth. Repeated trampling by cattle, such as along a cattle track, produces soil compaction (Mullholland and Fullen, 1991). Increased soil compaction restricts the growth of plant roots, stunting plant growth, and can impede the movement through the soil of microorganisms, water, and some nutrients (Kozlowski, 1999). The decreased porosity of heavily compacted soils would also inhibit many aerobic microorganisms and some plant species (Kozlowski, 1999). Conversely, compaction encourages anaerobic microorganisms and can increase the phytotoxicity of some herbicides (Rahman *et al.*, 1978). While the dieback condition has been reported on many soil types (Chapter 1), its rate of spread on different soil types has not been measured. Consequently, it is feasible that the causal agent, whether microbial, water related, or chemical, is hindered by soil compaction.

Obviously, more work is required in the area of patch spread, firstly to determine an average rate of radial spread, and secondly to clarify the uneven nature of spread. Factors such as land slope and soil compaction need to be taken into consideration.

3.4 Spread of Individual Patches

3.4.1 Materials and Methods

Methodology

A more extensive experiment was done to obtain an average rate of radial spread and to ascertain whether or not the uneven spread of patches is prevalent. The slope of the land and soil compaction also needed to be measured, in an attempt to find a cause for any uneven spread. These will be measured along the four cardinal points as a standard, as many of the patches slope in more than one direction.

The 81 ha. paddock in which the pilot study was done was, by this time, completely overrun with BGD, and all patches had coalesced. This process, from when symptoms were first sighted, to the paddock being completely overrun, took 10 years. Therefore, an adjacent paddock in which symptoms were just appearing was used for this experiment.

Due to weather difficulties, as well as to encompass the warmer growing season, this experiment was run for a longer period of time than the pilot study.

The chosen paddock was not grazed for two months prior to this trial, nor was it grazed during the trial. It should also be noted that isolated patches of BGD are rare; most are close to or converged with nearby patches. Therefore, there were difficulties in obtaining sufficient replicates for relevant statistics to be performed (refer 3.4.2. Results).

Method

Ten relatively isolated BGD affected patches were chosen and wooden pegs were driven into the ground at the estimated centre of each patch. Due to the lack of discrete patches transected by cattle tracks and in the interest of consistency, none of the patches chosen were intersected by cattle tracks. Rays of the patches were measured in four

directions: North, East, West and South, from the central wooden stake to the furthermost plant displaying symptoms. The measurements were repeated 42 weeks later. Intermediate measurements were not possible due to lack of rainfall, which is required for symptom development. This trial was undertaken from September 2001 to June 2002, with normal temperature ranges and average rainfall for that time of year.

The prevailing slope of the land in each patch was measured using two 2 m long straight aluminium flat rods, a tape measure, a ruler and a spirit level. To determine the prevailing slope, one of the aluminium rods was placed on the ground, passing through the centre of the patch, with the spirit level resting on top. A mark was made on the spirit level at the top of the air bubble to indicate the extent of deviation from level position. This was repeated at several radial directions. The direction with the largest deviation was considered to be the prevailing slope of the patch. To determine the angle of the slope (of the prevailing slope of each patch), one aluminium rod was placed on the ground in the middle of the patch, in the direction of the prevailing slope. The second aluminium rod, with the spirit level resting on top, was placed on top of the first and the downhill end was raised until the spirit level registered a level plane. The ruler was suspended from the top aluminium rod, 1 m from the point at which the two rods met. The three sides of the 'triangle' were measured, and the angle of the slope was calculated using $a^2 = b^2 + c^2 – 2bc \cos A$.

Soil compaction was measured using a cone penetrometer, using the Australian Standards method AS 1289.6.3.2 (Standards Australia International Limited, 1997). Since the field soil was dry and hard, the maximum allowable number of blows (8) was used on each site, and the depth attained was recorded. The soil compaction was measured at the visible periphery of each of the 10 replicate patches mentioned earlier, at four points:

North, East, South and West. Triplicate measurements were taken from each location, and the calculated average was considered to be an accurate reading. The soil compaction of cattle and vehicle tracks was also measured for comparison, with five replicate sites of each, all within the same paddock. In all cases, measurements were taken from the surface only. This was to encompass the top 20 cm of soil which not only includes much of the root system and associated microbial biomass (Pagliai, 1987), but is also the layer most affected by compaction from cattle, which are reported as producing very dense zones at depths of 7-10.5 cm (Mulholland and Fullen, 1991).

Since none of the penetration readings reached 300 mm, the penetration resistance could not be calculated, as described by Australian Standards method AS 1289.6.3.2 (Standards Australia International Limited, 1997). As such, the penetrometer data are presented as the average total penetration achieved after the maximum 8 blows.

The data were analysed using single factor ANOVAs to determine differences in radial spread and soil compaction between the four directional points, and to compare soil compaction of the paddock to that of cattle and vehicle tracks.

3.4.2 Results

Table 9 shows the average growth of the ten patches in the four directions, as well as the range of measurements. The overall average radial spread was 194.1 cm, which equates to a growth rate of 4.6 cm per week.

Direction	N	E	S	W
Mean (cm) (± SE)	192.2 (27.07)	191.1 (14.48)	158.3 (13.52)	214.4 (33.59)
Range (cm)	110 - 380	120 - 260	130 - 200	80 - 370

Table 9 – Mean difference in radial spread after 42 weeks of patches of buffel grass (*Cenchrus ciliaris*) affected with the dieback condition

Radial spread did not differ significantly at the different directions (l.s.d. = 70.4).

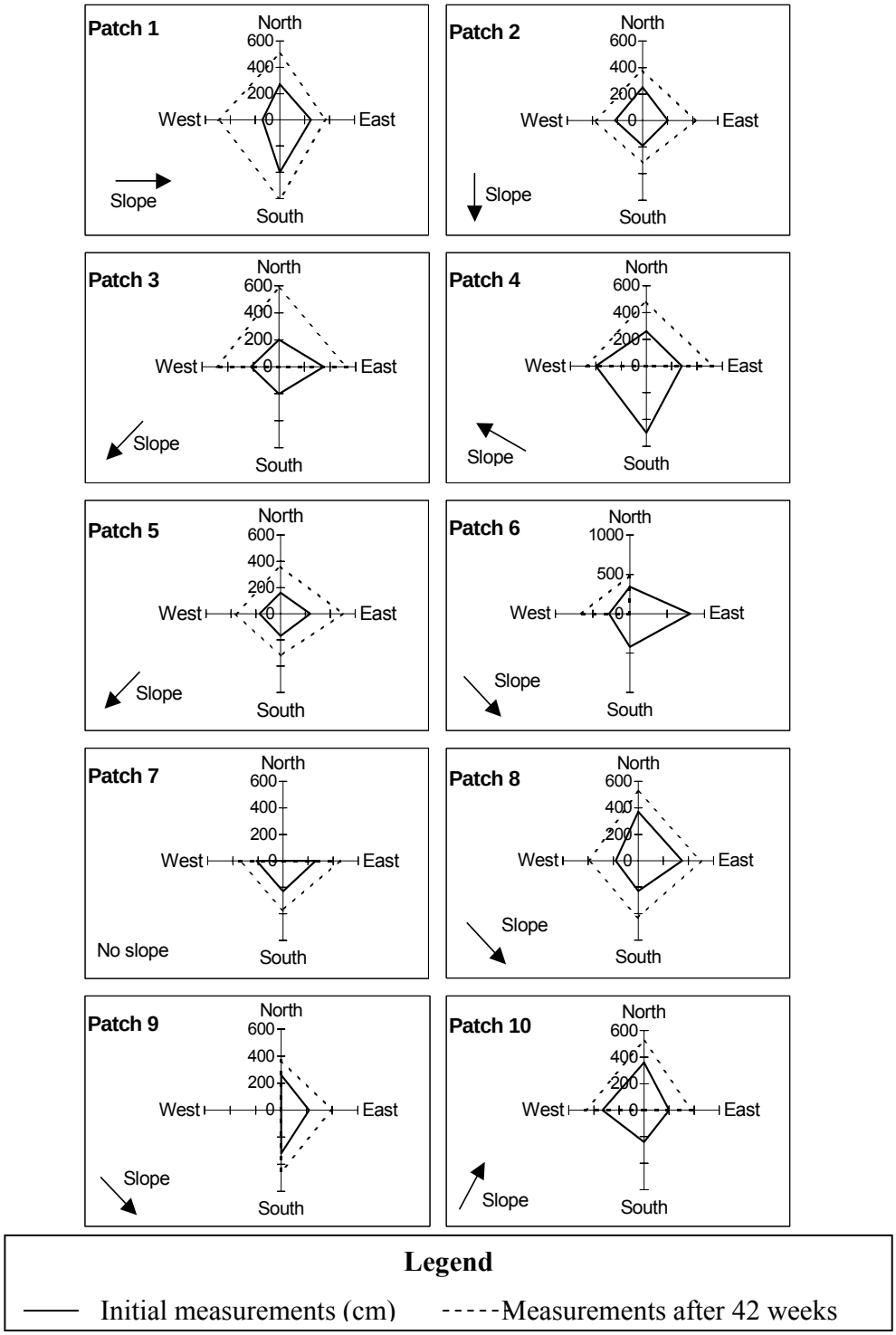


Figure 22 – Radial spread of patches of buffel grass (*Cenchrus ciliaris*) affected with the dieback condition

The radial spread of individual patches, as well as the slope of the land, is represented in graphical form in Figure 22. Missing values are as a result of convergence with another patch. No matter the direction, the slope of the land in all patches (except the one which was level, patch 7) was between 1-2°. The prevailing slope of each patch varied greatly. Only four patches had not converged with another.

Table 10 shows the average depth of penetrometer penetration taken from the patches (broken down into patch directional measurements and total), cattle and vehicle tracks, as well as the range of values obtained.

Area measured	N	E	S	W	All patches	Cattle tracks	Vehicle tracks
Mean (mm) (± SE)	79.4 (4.55)	81.3 (4.78)	73.2 (3.96)	76.5 (2.44)	77.6 (1.33)	88.8 (4.08)	65.5 (2.32)
Range (mm)	60 - 98	64 - 106	58 - 98	66 - 87	60 - 106	78 - 102	58 - 73

Table 10 – Mean depth of penetrometer penetration from the periphery of patches of buffel grass (*Cenchrus ciliaris*) affected with the dieback condition

There was no significant difference ($P = 0.15$) between soil compaction at the four directional points. There was significant difference ($P = 0.019$) between areas, *i.e.* patches and cattle and vehicle tracks, with the l.s.d. (16.2) showing that vehicle tracks < cattle tracks but neither are significantly different from patch compaction measurements.

Table 11 compares the maximum and minimum directions of spread, slope and compaction for the four patches which had not converged with others.

Patch number	Growth		Slope	Compaction	
	Least	Most		Least	Most
1	E	W	E	W	E
2	N & S	E	S	E	S
5	S	E	SSW	S	E
8	E & N	W	SE	N	S

Table 11 – Comparison of directions of maximum and minimum radial spread, slope and soil compaction of patches of buffel grass (*Cenchrus ciliaris*) affected with the dieback condition

Due to the patch convergence, there were limited data (4 patches) with which to investigate correlations or other statistical relationships. As such, it was concluded that there were not enough replicates to perform meaningful statistics. Table 11 shows that, concerning the four patches reported, there does not appear to be any relationship between patch growth and slope of the land or soil compaction.

Unfortunately, due to the scarcity of discrete BGD patches throughout this research, this experiment could not be replicated.

3.4.3 Discussion

The average rates of radial spread of the BGD affected patches are slower than in the initial pilot study (Section 3.3). However they could be more accurate since they span a longer period of time. Radial spread was irregular in every patch, indicating that uneven spread is commonplace.

There was no significant difference in the soil compaction of the four different directions but patch growth was irregular, suggesting that there is no direct relationship between patch spread and soil compaction. The penetrometer readings from the BGD affected patches were comparable with those from cattle and vehicle tracks. The readings from cattle tracks in particular were fairly average compared with patch values, suggesting that the paddock itself was ‘trampled’ and compacted after many decades of grazing. Vehicle tracks were, as expected, more compact than cattle tracks, but were not out of the range of the patch readings. The similar readings suggest that tracks probably do not hinder the spread of BGD due to soil compaction. However, the general compaction of the paddock may still contribute to the condition.

Soil compaction can have several effects on the soil profile and soil biota. While compaction has been shown to not interfere with some specific bacterial populations, and even increase the counts of some bacterial species (Ikeda *et al.*, 1997), it does decrease the general microbial population (Li *et al.*, 2002). It also decreases soil aeration, encouraging anaerobic microorganisms, and can interfere with the carbon and nitrogen cycles (De Neve & Hofman, 2000), as well as the nutrient uptake of plants (Alves *et al.*, 2003). While soil compaction has a negative effect on many plant species, weakening them, it does not always have an effect on pathogens. For example *Phytophthora* sp., which causes root rot of American chestnut (Rhoades *et al.*, 2003), is unaffected by soil compaction. Therefore, the similar compaction readings from tracks and paddocks may indicate that compaction is weakening the plants, possibly making them susceptible to BGD.

As for the apparent hindrance of BGD by cattle and vehicle tracks, observed in Section 3.3, a possible explanation is that, due to the constant traffic, tracks are denuded of plant life, which could hinder the spread of a pathogen which requires close proximity to a host (Burdon and Chilvers, 1982; Otten and Gilligan, 2006).

From Figure 22 and Table 11, there is no apparent relationship between radial spread and slope of the land. Therefore, surface water runoff down a slope is probably not the main mode of spread of BGD. Table 11 also shows no relationship with soil compaction, at least not in the top 20cm of soil. The uneven spread of patches is apparent, but the cause remains unknown.

It is unrealistic to compare the rate of spread of BGD with that of other conditions, since most depend entirely on factors such as weather and farming practices. However, most patch conditions are reported as having irregular spread (Burdon and Chilvers, 1982; Couch, 2000; Agrios, 2005; Otten and Gilligan, 2006). In many of these cases, where a

soilborne pathogen is involved, the reasoning is that pathogens move at different rates due to the heterogeneous soil environment (Otten and Gilligan, 2006). In other cases, where there is vector involvement, irregular spread is due to the host selection of the vector (McElhany *et al*., 1995). Chemical causal agents also occur in irregular patches (Grundon *et al.*, 1997; Couch, 2000). Clearly, the mode of spread of BGD and the identification of either a biological or other aetiological agent(s) need to be found before further conclusions can be made on the mechanisms of patch spread.

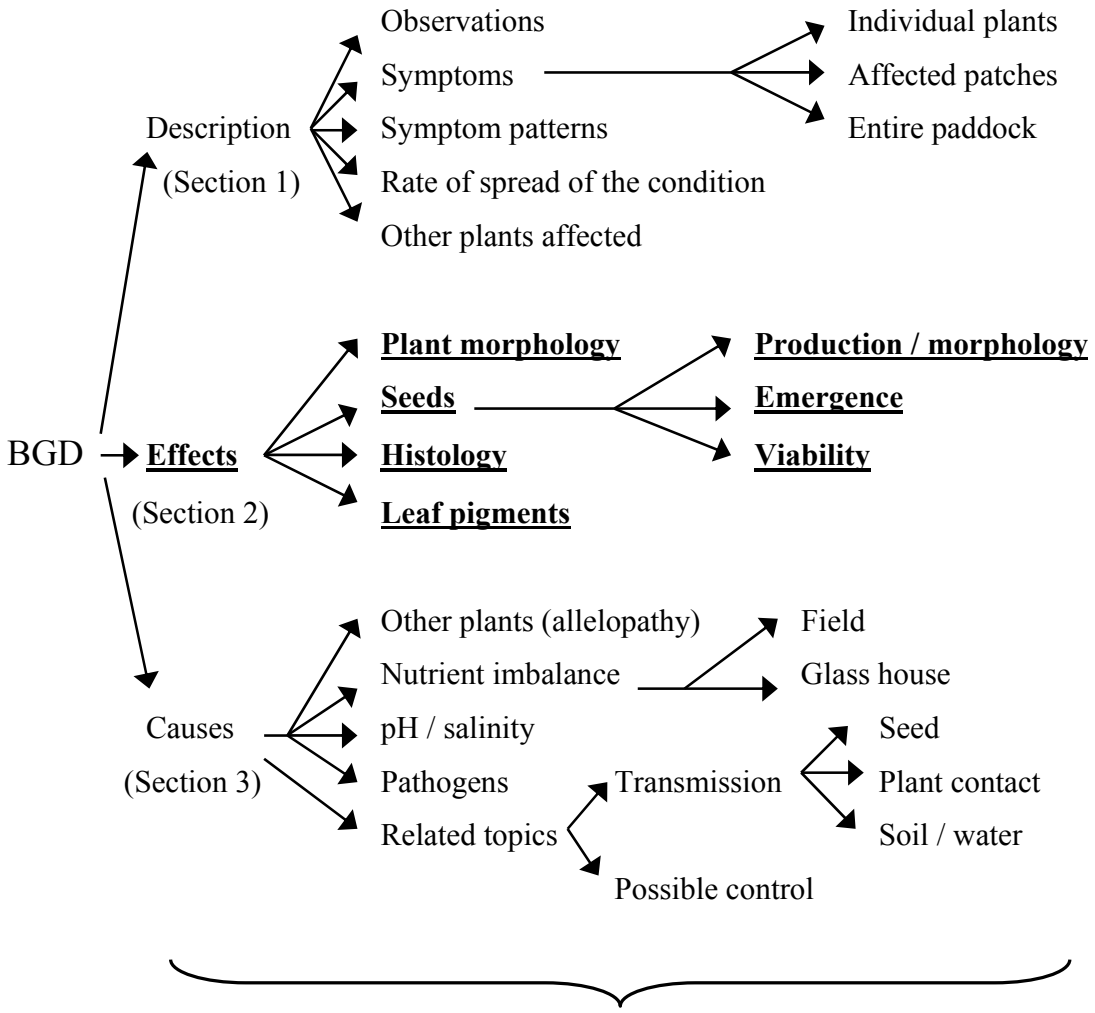
3.5 Concluding Statements

The above results clearly demonstrate how a paddock can be rapidly overrun by buffel grass dieback. Both radial spread and the emergence of new patches are responsible for the rapid loss of useful pasture. Since the rate of patch spread is variable and weather-dependent, it is impossible to estimate how long it would take for BGD to overrun a specific area. The closest approximation to this would be the observation that an 81 ha. paddock was overrun in 10 years.

Soil compaction and land slope do not appear to affect the patch spread of BGD. The cause of the uneven radial spread of patches is still unknown.

Section 2

Effects of Buffel Grass Dieback



Chapter 4

Effects of BGD on Plant Morphology and Seeds

4.1 Introduction

Many diseases and disorders of plants have adverse effects on plant growth and yield. For example, take-all of wheat, caused by the fungus *Gaeumannomyces graminis*, causes stunting of plant tops and a reduction of dry weight, as well as a severe decrease in grain yield (Manners and Myers, 1981). Similarly, a study by Schwartz and Safaya (1978) showed that a potassium deficiency in slender wheatgrass (*Agropyron trachycaulum*) caused extremely reduced shoot and root growth, and a reduction of dry matter. Symptoms such as these can often be used as an aid in identifying a particular cause. Therefore, it was considered necessary to perform a study on the morphological effects of BGD on buffel grass. Since BGD affected buffel grass plants produce seeds (Chapter 2), and, according to the property owner, primary producers often trade seed with each other and also sell seed to agents for commercial sale, seed yield and seed quality were also included in this study.

4.2 Morphological Differences Between Unaffected and BGD Affected Buffel Grass

4.2.1 Materials and Methods

Methodology

The results in Chapter 2 suggested that affected buffel grass plants were smaller and had a slower growth rate than unaffected buffel grass. If this were the case, then primary

producers would suffer a loss of productivity before the plants were dead. For this reason the morphological differences between unaffected and affected buffel grass were studied. These data would also provide further identifying features for this condition.

Method

Ten BGD affected plants and ten unaffected plants were collected with roots intact from the field. The BGD affected plants selected displayed typical red symptoms, similar to the completely affected plants pictured in Figure 5, p.44. Their roots displayed necrotic areas and lesions as described in Section 2.3.2, p.48, covering approximately 1% of the root surface. The plants were collected at random down to a soil depth of 70 cm. The plants were immediately measured and weighed. Plant height, longest leaf and longest internode were measured using a tape measure. Plant height was measured from the junction of the tillers and roots to the top of the plant in its natural habit. The longest leaf was measured from the tip to the ligule. The longest internode was measured from node to node. In addition, the numbers of non-senescent leaves and tillers were counted. Shoot and root fresh weight and dry weight were also obtained. To obtain dry weight, plants were put in a drying oven for five days at 70ºC. Percent dry matter of both roots and shoots were also calculated. To determine significant differences, *t*-tests were performed assuming unequal variance.

For clarification, Figure 23 is a diagrammatic description of the various plant parts which were measured. The propagules of buffel grass consist of one or more caryopses enclosed within paleas, lemmas and glumes, surrounded by a fascicle of bristles (Hacker and Ratcliff, 1989). The fascicle in Figure 23 is what is referred to as a ‘seed’ throughout the thesis.

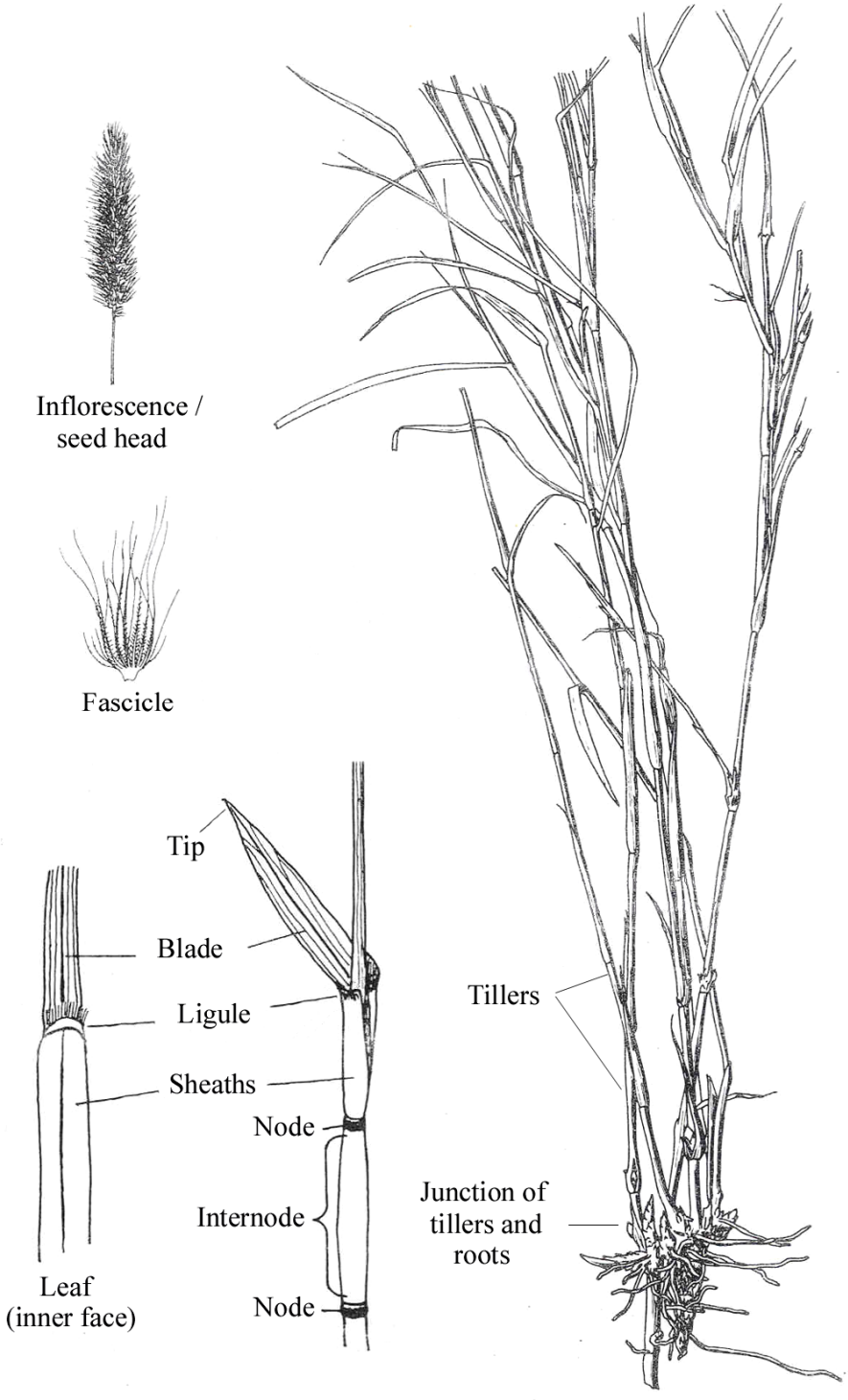


Figure 23 - Diagrammatic representation of the parts of the buffel grass (*Cenchrus ciliaris*) plants which were measured

(adapted from Lazarides (1970) and Tothill and Hacker (1983))

4.2.2 Results

Plant Attribute Measured	Healthy plants		Affected plants		Difference
	Mean	(± SE)	Mean	(± SE)	
Plant height (cm)	79.20	(2.61)	46.70	(2.12)	*P* <0.001
Longest leaf (cm)	37.05	(1.36)	25.61	(0.83)	*P* <0.001
Longest pseudostem (cm)	8.48	(0.29)	4.04	(0.28)	*P* <0.001
Number of tillers	93.20	(1.89)	81.70	(3.82)	*P* =0.01
Leaves per tiller	8.05	(0.35)	8.07	(0.38)	ns
Root fresh weight (g)	68.16	(3.33)	24.39	(3.05)	*P* <0.001
Root dry weight (g)	50.80	(3.56)	16.41	(2.01)	*P* <0.001
Shoot fresh weight (g)	95.74	(1.74)	31.87	(3.32)	*P* <0.001
Shoot dry weight (g)	59.61	(1.22)	21.85	(2.35)	*P* <0.001
Root % dry matter	73.83	(1.66)	67.88	(1.46)	*P* <0.01
Shoot % dry matter	62.26	(0.62)	68.36	(1.48)	*P* <0.01

Table 12 - Comparison of morphological attributes of unaffected buffel grass plants (*Cenchrus ciliaris*) and buffel grass plants affected by buffel grass dieback

Based on % dry matter, root:shoot ratios are:

Unaffected buffel grass: 1.19

BGD affected buffel grass: 0.99

BGD affected plants were significantly shorter in height, and had shorter leaves and internodes. They also had fewer tillers, but had the same number of leaves per tiller. Root and shoot fresh weight and dry weight were significantly smaller in BGD affected plants, as was percent dry matter of the roots. Conversely, percent dry matter of the shoots was larger in BGD affected plants.

4.2.3 Discussion

The BGD affected plants were noticeably shorter in height and had shorter leaves and internodes, and therefore would have less biomass for grazing. However, the number of leaves per tiller did not differ significantly. These attributes make affected plants look more 'bushy' in appearance. Plant fresh weight and dry weight supports that there is a large difference in biomass, with BGD affected buffel grass being on average two-thirds the weight of unaffected buffel grass.

The stunting of affected buffel grass plants could be due to water stress, a nutrient imbalance, and/or an alteration of the relative concentrations of plant growth regulators. Water stress can cause stunting, either through the reduced uptake of nutrients (Schoeneweiss, 1978), by causing a reduction in photosynthesis, and therefore reducing energy available for growth, or by altering the levels of cytokinins (Schoeneweiss, 1978), which promote and regulate cell division (Werner *et al.*, 2001). Ng *et al.* (1975) found that stem elongation in water stressed plants was greatly delayed, and plant dry weight and leaf area were reduced. Likewise, according to Ayres (1978), leaf expansion is one of the processes most sensitive to water stress. Both of these findings concur with the above results, suggesting that water stress is a factor in BGD. However, Ng *et al.* (1975) also reported that tiller number was not affected by water stress, contradicting the above results. While the research of Ng *et al.* (1975) involved *Panicum maximum*, not buffel grass, both grasses are from the same family and tribe. The difference in results could be attributed to the differences between the two grasses or it could indicate that water stress is not a factor in BGD. Unfortunately, at the time of this research, the equipment required to measure water stress in grasses (eg. a hygrometer) was not available, nor could it be borrowed.

Therefore, these data could not be collected. The topic of water stress is re-examined in Chapter 8.

Nutrient imbalances often cause stunting through a shortage of available building materials (nutrients) or by toxicity effects, which either damage cells or interfere with growth regulators (Salisbury and Ross, 1992; Grundon *et al.*, 1997). According to both Christie (1975) and Muller (2001), phosphorus and nitrogen are the main limiting nutrients in buffel grass. These are further investigated in Chapter 7.

Levels of plant growth regulators can also be altered or interfered with by pathogens, resulting in stunting. Often the alteration is made by the host plant as part of plant defence mechanisms (Salisbury and Ross, 1992). This may be triggered by the presence of a pathogen or as a result of toxins produced by the pathogen. These toxins may also interfere directly with plant growth regulators (Ayres, 1978). There are also numerous examples of microorganisms which produce plant growth regulators (Tamura *et al.*, 1974; Michniewicz, 1982; Costacurta and Vanderleyden, 1995), or insert into the plant genome a DNA fragment which alters plant hormones (Gaudin *et al.*, 1994), consequently influencing plant growth. In some cases the pathogen interferes with the water supply of the plant (Ayres, 1978), thereby indirectly causing stunting as outlined above.

The shoots of unaffected plants had lower percent dry matter than the roots, as supported by the root:shoot ratio data. This was expected as roots generally have a thicker cortex and less intercellular spaces, resulting in an increased dry matter content and, correspondingly, a decreased water content. However, the percent dry matter of affected plants was similar in both shoots and roots. This could be from malformed tissues, or from a disturbance in the water relations of the plant, possibly from cellular damage. Often

water stress or plant pathogens alter or disturb plant water relations (Ng *et al.*, 1975; Ayres, 1978; Durbin, 1978).

BGD affected roots had lower percent dry matter than unaffected roots, but affected shoots had a higher percent dry matter than unaffected shoots. However, on average, the percent dry matter of the entire BGD affected and unaffected plants were similar. This seems to indicate that the water in the BGD affected plants is being redistributed or partitioned to the roots rather than shoots, or that water is restricted from entering the shoots. Ayres (1978) reported that a decreased supply of water to the shoots may result from failure of the roots to take it up, or failure of the vascular system to conduct it. Since the average water contents of unaffected and BGD affected plants are similar, it can be concluded that the roots of BGD affected plants, however stunted, have no problem with taking up water. Therefore, the problem most probably lies with the vasculature which, as mentioned above, may stem from altered water relations including cellular damage. Further studies on vasculature are presented in Chapters 5 and 6.

From these results it appears that the morphological differences of BGD affected plants may in part be caused by some form of water stress and/or alteration in plant hormones. The reduced biomass and leaf material of affected plants decreases the amount of plant material available for grazing which, due to the loss of productivity, would be of great importance to primary producers.

4.3 Germination of Seeds From Various Sources

A note on the availability of seed

During what is considered a good growing season, that is, warm weather and adequate rainfall, *ca.*99% of unaffected buffel grass plants in the field would produce 3-4

seed heads each. In comparison, an estimated 5% of BGD affected plants produced seed, usually on only one seed head (Webb, pers.comm.). In addition, the seed heads of BGD affected plants had approximately 40% less seeds per head than that of unaffected plants (Section 4.4). Thus, obtaining sufficient seed from BGD affected plants proved difficult, and several experiments do not have the seed numbers usually required in such trials due to the paucity of seed.

4.3.1 Materials and Methods

Methodology

The International Seed Testing Association (ISTA) guidelines (1999) have several procedures used to determine the percentage germination of seed. While these procedures generally use specific substrates and controlled conditions, resulting in the potential percentage germination of a seed sample, the focus of this study is on what the primary producer would observe in the field: the actual emergence. Germination under controlled conditions does not guarantee that the seedlings would establish in the field (International Seed Testing Association, 1999), due to the large number of variables present. Therefore, to obtain a better approximation of the actual field germination, several modifications were made on the ISTA procedures. Firstly, a standard potting mix was used as the substrate. Soil or potting mix is not generally recommended for consecutive tests, due to the inconsistency of the medium (International Seed Testing Association, 1999). However, the guidelines state that it can be used for comparative or investigative purposes, providing that the soil is of a fine particulate nature and is not re-used (International Seed Testing Association, 1999). The experiment was conducted in a shade house instead of temperature and light controlled rooms, to more accurately reflect field conditions. Lastly, the

experiment was extended beyond the recommended 28 days for buffel grass (International Seed Testing Association, 1999), since there may be a delay in germination due to the less than optimum conditions. As a result of these modifications, the term 'emergence' will be used instead of 'germination', as seeds which have germinated but do not emerge as seedlings were not counted.

The minimum standard for commercially-sold buffel grass seed was 20% germination using standard ISTA methods (Cavaye, 1991). Therefore, commercial seeds were included in this study for comparison.

Method

Seeds were collected in the field during the summer growing season from both unaffected plants and plants affected with BGD. Commercial seeds were obtained from Queensland Independent Seeds, Rockhampton. Fifty seeds from unaffected plants, 50 seeds from plants affected with BGD, and 50 commercial seeds (all selected at random) were planted individually in pots containing a general purpose potting mix (consisting of 2 parts peat, 1 part top soil, 1 part sand, with pH ~ 6.5), and placed on a bench in a shade house in a completely random design. The bench had insecticidal strips on its legs to prevent insect interference. The pots were kept moist and were observed for emergence for a duration of ten weeks. A seedling was considered emerged when it formed a second leaf. At the end of the ten week period, the seeds which had not emerged were exhumed, to verify that the seeds had not been taken away by insects.

4.3.2 Results

Seed Source	Number Emerged (out of 50)	Percent Emergence
Commercial seed	16	32
Healthy plants	2	4
Plants affected with buffel grass dieback	0	0

Table 13 - Percent emergence of buffel grass (*Cenchrus ciliaris*) seeds from various sources

Due to the binomial nature of these data, and one of the values being '0' (hence having no variance), statistical analysis was not possible. This was therefore treated as a pilot study.

4.3.3 Discussion

The seeds from unaffected plants had an unexpected low emergence. Seeds from dieback affected plants failed to emerge, which may indicate high plant stress, or simply that the seed population size was insufficient. Perhaps one or more seeds may have emerged if more were used.

The low emergence of unaffected paddock plant seeds could stem from a variety of reasons; for example, pasture rundown and/or water stress. Another possibility is that the seeds were collected from plants that had the dieback condition but were not displaying typical symptoms. The very low emergence of seeds from dieback affected plants could also be from pasture rundown or water stress, since the numbers are very similar. Therefore, from these data, it cannot be concluded that the dieback condition affects emergence.

The relative age of the seeds should also be taken into account when exploring differences in emergence. According to Cavaye (1991), a high percentage of freshly-harvested seeds are often dormant, with this percentage reducing over time. Therefore, it is

possible that the field-harvested seeds had higher percentage dormancy than the commercial seed, which would probably have been in storage.

Due to the inconclusive nature of this study, it is important that this work be repeated, with particular attention given to the age of seeds, and the condition of the plants when harvested. Also, possible reasons for the lack of emergence, such as plant stress and seed viability, should be explored.

4.4 Morphology, Emergence After Treatment, and Viability of Seeds From Various Sources

4.4.1 Materials and Methods

Seed Collection

Harvesting the first seed crop after a rainfall event, when plants are still lush, would diminish the possibility of water stress influencing the parent plant, thereby influencing germination of the seeds. However, water stress may still be an issue. It was noticed in the field that buffel grass plants around a leaking water trough were noticeably healthier and larger than paddock plants, even after a moderate rainfall event. Including the seeds of these plants in seed trials would possibly eliminate the water stress factor.

Seeds were collected from the field several weeks after a rainfall event when the plants were still lush and producing new seeds. Previously BGD affected buffel grass was displaying symptoms. Intact, mature seed heads were collected from unaffected plants around a water trough, unaffected plants in the paddock, and from BGD affected plants. Fresh commercial seeds, reputed to be no more than one or two months old by the dealer, were also obtained. All of the following experiments used seeds obtained from this collection.

Seed Morphology – Methodology

Seed size and the number of seeds per head are important indicators of plant stress in both grasses and cereal crops, as in take-all of wheat (Manners and Myers, 1981). It was considered important to compare these attributes among the buffel grass seeds from various sources, since they might be linked to differences in emergence.

Seed Morphology – Method

Twenty seed heads were randomly selected from each source, with the exception of commercial seeds as these are already separated from the seed head. The number of seeds per head was counted.

Also, fifty seeds were randomly selected from each source, and the lengths were measured from the base to the tip of the awns with a millimetre rule and a dissecting microscope.

As there is only one water trough in the paddock, seeds from plants around the water trough could not be collected 'randomly'. As such, statistical analyses comparing the four seed sources were not possible. However, after excluding the 'trough' source, single factor ANOVAs were done comparing the other three seed sources, using LSDs at $P<0.05$ to determine significant differences. All four treatments are compared graphically with standard error bars.

Emergence After Treatment – Methodology

The previous seed emergence study exhibited very low emergence in some treatments, possibly due to seed dormancy. Therefore, it was considered necessary to attempt to break this dormancy in this study. Various treatments can be used to break the dormancy of seeds (International Seed Testing Association, 1999), mostly involving

temperature, chemical or light treatments. For example, Dr Ani Nkang, CQU (pers. comm., 2001), in an unpublished study, demonstrated that buffel grass seed dormancy can be broken by a concentrated sulfuric acid bath, followed by a 24 h period in water. For cultivation purposes, it is often recommended to plant buffel grass seeds in ash seed beds after burning off (Paull and Lee, 1978; Cavaye, 1991). This is probably due to the corrosive nature of the lye which leaches out with moisture. The lye would chemically scarify the seeds thereby increasing imbibition of water and consequently germination rate. This could be a useful method in shade house trials to diminish the effect of seed age and dormancy as a factor influencing germination, and would more closely resemble field conditions than other treatment methods. However, due to its fine particulate nature, ash is not a stable potting medium. Therefore, for experimental purposes, a mixture of ash and soil or potting mix would be more suitable, preferably with more ash than soil to simulate an ash seed bed in the field.

Emergence After Treatment – Method

One thousand seeds were selected randomly from each source. These were divided into 10 lots of 100 seeds for each source, and each 'lot' was planted in a seedling tray containing a 70:30 mixture of ash and potting mix respectively. This resulted in 40 trays, 10 for each seed source. These were placed randomly on a bench in a shade house. The bench had insecticidal strips on its legs to prevent insect interference. The trays were kept moist and carefully observed. The emergence in each tray was assessed after ten weeks. A seedling was considered emerged when it formed a second leaf. At the end of the ten week period, the seeds which had not emerged were exhumed, to verify that the seeds had not been taken away by insects. The experiment was started in early March.

The ‘trough’ seed source was once again excluded from statistical analyses. Percent emergence was analysed using a Generalised Linear Model (GLM) assuming a binomial error and a logit link function. The model simply included the effect of seed source. There was no evidence of overdispersion (mean deviance approx. unity). The procedure RPAIR was used to assess pairwise differences.

Number of Caryopses and Seed Viability – Methodology

Buffel grass seeds typically have 0 to 3 caryopses per seed, which varies between cultivars (Cavaye, 1991). Generally, a higher number of caryopses increases the chances of germination, assuming they are viable. Counts of caryopses from the various seed sources may explain differences in emergence.

The absence of caryopses or the presence of deformed caryopses is an obvious cause of the failure of a seed to germinate. Nevertheless, seemingly normal caryopses may not be viable due to biochemical problems. The presence of these problems is usually tested chemically. The 1,3,5 triphenyl tetrazolium chloride (TTC) test is the standard viability test recommended by the ISTA (International Seed Testing Association, 1999) and is widely used. It involves a seed treatment which stains metabolically active tissue that is present in a viable embryo. The viability of the seed is assessed by cutting the seed open to expose the embryo, and observing the areas of the seed which are deeply stained, partially stained, and not stained (Thompson *et al.*, 2001). The TTC test is a reliable indicator of biochemical problems in seeds, which may be the cause of variation in the emergence rate of commercial seeds and seeds from both unaffected and BGD affected plants.

Number of Caryopses and Seed Viability – Method

Fifty seeds were randomly selected from each source: commercial, unaffected plants around a water trough, unaffected plants in the paddock, and BGD affected plants, and were dissected. The number of caryopses per seed was assessed.

Fifty caryopses from seeds of each source were tested for viability using the TTC method (International Seed Testing Association, 1999; Thompson *et al.*, 2001). The procedure involved pre-soaking the caryopses in distilled water for 24 h, followed by immersing them in a 0.5% TTC solution. The immersed caryopses were covered and placed in a dark cupboard for 12-14 h with an ambient temperature of 20-25°C. The caryopses were then removed from the solution, rinsed with distilled water, cut longitudinally to expose the embryo, and photographed.

The colour of the embryo was used to assess viability (Figure 24). Red stained embryos were considered viable. Colours ranging from light red to pink were considered potentially viable. Embryos that did not stain (i.e. remained white) were considered non-viable (Thompson *et al.*, 2001). If the embryo stained red or pink, but the root tip did not stain (Figure 24, black arrow), the embryo was also considered non-viable (International Seed Testing Association, 1999).

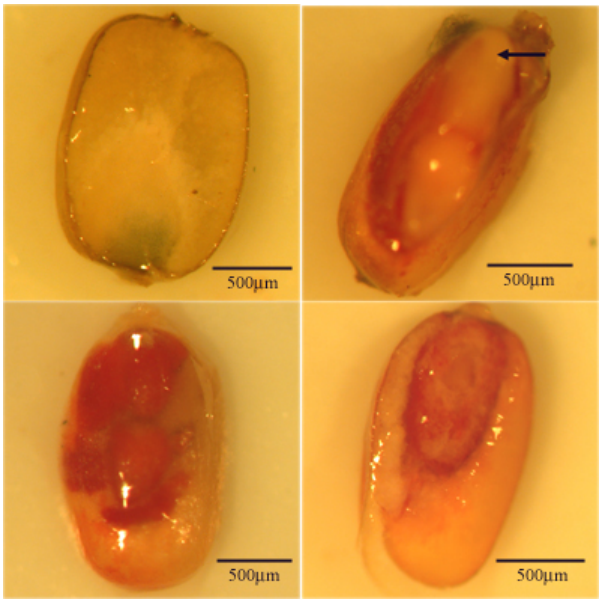


Figure 24 – Representative photographs of non-viable (top) and viable (bottom) seed embryos of buffel grass (*Cenchrus ciliaris*) tested by the TCC test

Note the white unstained root tip in the top right photograph (black arrow).

Due to the categorical nature of the data, a contingency table and chi-square analysis was done on both the number of caryopses and seed viability data. The Pearson chi-square statistic was used. Due to the low numbers of seed containing two or three caryopses, these data were combined.

4.4.2 Results

Seed Morphology

Figure 25 shows the average number of seeds per head of plants of the different types. There are no apparent differences between the plants around the trough and the otherwise unaffected plants. BGD affected plants (mean = 67.4) had significantly ($P < 0.001$) fewer seeds per head than unaffected plants (mean = 101.9).

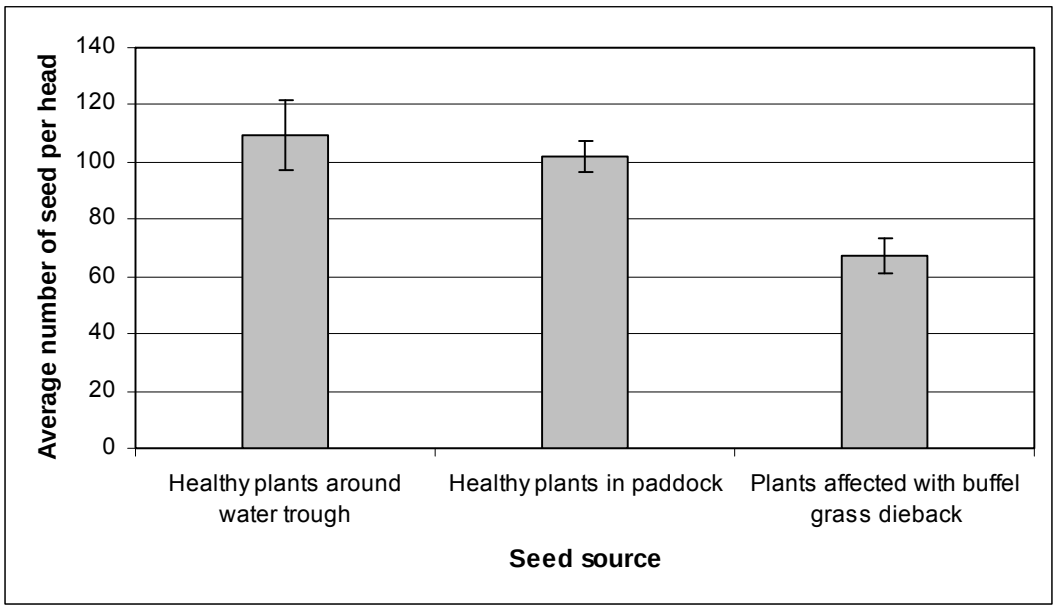


Figure 25 - Mean number of buffel grass (*Cenchrus ciliaris*) seeds per head from plants from various sources (± SE)

Figure 26 shows the average length of seed fascicles of the different plant types. There are no apparent difference between commercial seeds, seeds from plants around the trough and from unaffected plants. There was a significant difference ($P = 0.026$) between types, with the l.s.d. (1.15) showing that the difference was between seeds from BGD affected plants (mean = 9.0 mm) and the other two types. There was no significant difference between the commercial seed (mean = 10.5 mm) and the seeds from unaffected plants (mean = 10.3 mm).

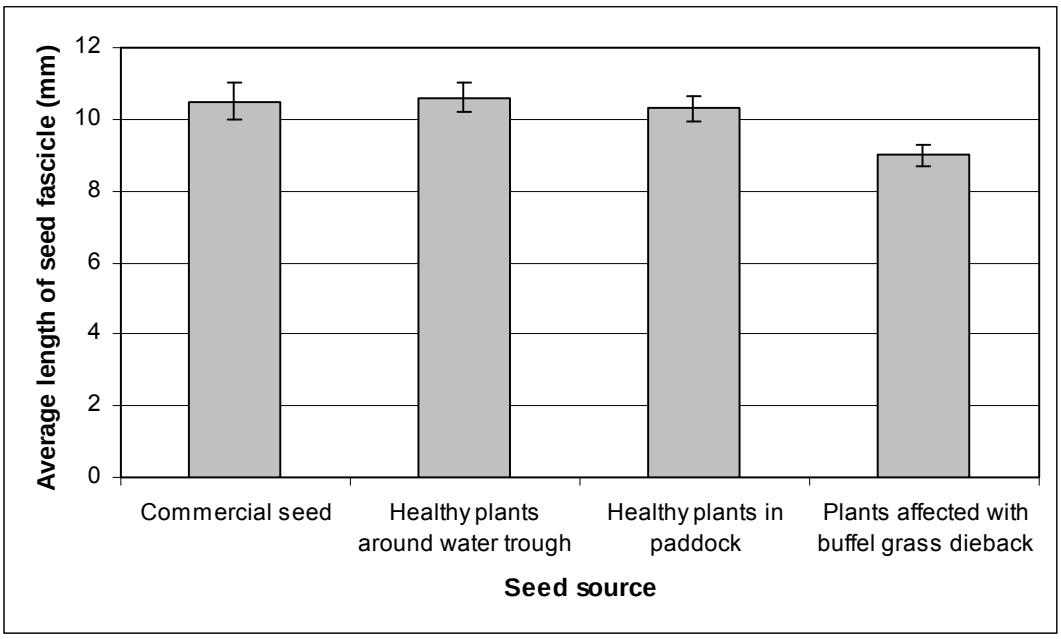


Figure 26 - Mean length of seed fascicle of buffel grass (*Cenchrus ciliaris*) seeds from various sources (± SE)

Emergence After Treatment

Commercial seed had the highest average emergence, followed by the healthy plants from around the trough (Figure 27). The healthy paddock plants had an average emergence which was approximately two-thirds that of commercial seed. The seed from BGD affected plants had the lowest average emergence (4%) (Figure 27).

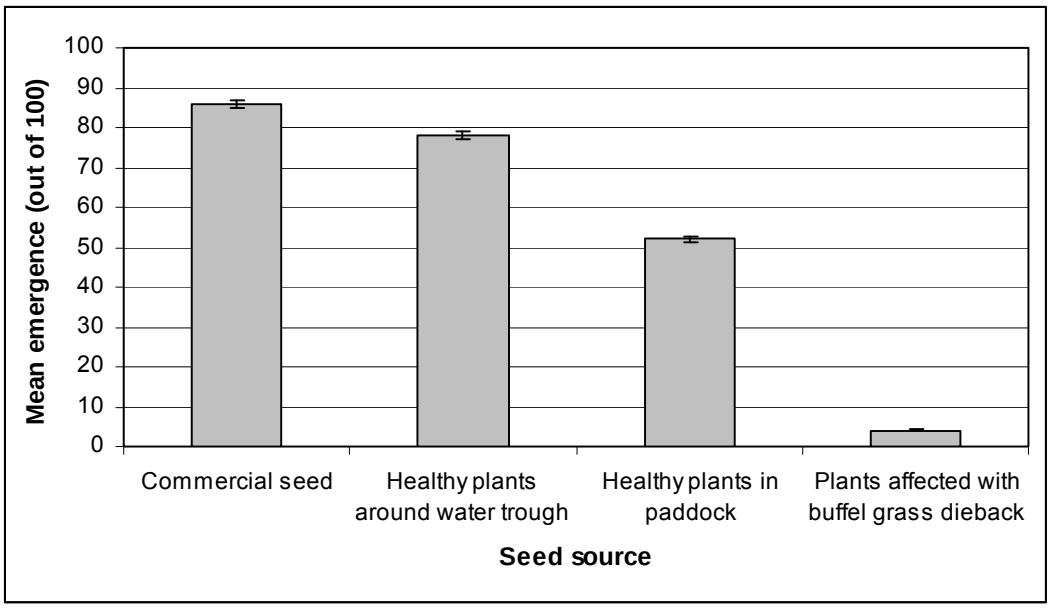


Figure 27 - Mean emergence in ash/soil mix of buffel grass (*Cenchrus ciliaris*) seeds from various sources (± SE)

Percent emergence differed overall ($P < 0.001$) among seed sources. Emergence for commercial seeds (86%) was greater than emergence for seeds from unaffected areas in the paddock (52%) ($P < 0.01$). Seeds from affected areas had lower emergence (4%) than all other sources ($P < 0.001$).

Number of Caryopses and Seed Viability

The commercial seed had the highest proportion of caryopses per seed, followed by the healthy plants from around the trough. Seed from the BGD affected plants had the lowest proportion of caryopses per seed (Table 14).

Seed Source	Number of Caryopses			
	0	1	2	3
Commercial seed	9	74	16	1
Healthy plants around water trough	28	68	4	0
Healthy plants in paddock	61	38	1	0
Plants affected with buffel grass dieback	75	24	1	0

Table 14 - Counts of buffel grass (*Cenchrus ciliaris*) seeds from various sources with differing numbers of caryopses

The proportion of caryopses was significantly influenced ($P < 0.001$) by seed source. The chi-square contributions table indicated that the greatest effect was the commercial seed, followed by the seed from BGD affected plants.

The commercial seed had the highest number of viable embryos (red and pink), closely followed by the healthy plants from around the trough. The seed from the BGD affected plants had the least number of viable embryos (Table 15).

Seed Source	Count with varying embryo colour			
	Red	Pink	White	Red w/ white root tip
Commercial seed	21	25	4	0
Healthy plants around water trough	26	18	4	2
Healthy plants in paddock	14	18	12	6
Plants affected with buffel grass dieback	4	10	23	13

Table 15 - Counts of varying colours of embryos of buffel grass (*Cenchrus ciliaris*) seeds following the TTC viability treatment

Seed viability was significantly influenced ($P < 0.001$) by seed source. The chi-square contributions table indicated that the greatest effect came from the seed from BGD affected plants, followed by commercial seed and seed from unaffected plants around a trough.

4.4.3 Discussion

BGD affected plants have significantly fewer seeds per head than unaffected plants from both in the paddock and around the trough. There does not appear to be any considerable difference between the unaffected plants of both sources, although the plants around the trough had a higher variability, the source of which is unknown. BGD affected plants also appear to have considerably shorter seed fascicles than unaffected plants from around the trough, although they may not be significantly shorter than seeds from

unaffected plants in the paddock and commercial seed. The commercial seed had a higher variability than the other sources, probably due to the wide range of plants and paddock conditions from which the seeds were sourced.

The result of the treated germination trial demonstrates that the seeds of BGD affected plants have a much lower emergence rate than seeds from other sources. This would severely reduce the chance of recolonisation of grazing land sown to buffel grass with self-sown seed. This would tend to favour recolonisation by other plant species, as was observed in Chapter 2.

The unaffected plants in the paddock also had a significantly lower emergence than the other unaffected seed sources. The cause of this may be linked to the number of caryopses. Seeds from BGD affected plants and unaffected plants in the paddock both had high proportions of nil or one caryopsis compared with seeds from other sources. The difference in emergence may also be attributed to seed viability, where once again these two seed sources have proportionately less viable seed.

The low emergence rate of BGD affected seed seems to be caused by biochemical problems more than by lack of caryopses, as evidenced by the high proportion of non-viable embryos. There was also a larger proportion of embryos with non-viable root tips, indicating that any future biochemical work should target this area.

In general, the number of caryopses and the seed viability test reflect the results of the emergence trial. The overall results suggest that the unaffected plants in the paddock are stressed, though not as much as the BGD affected plants. Perhaps the cause of this stress is what predisposes them to BGD.

4.5 Concluding Statements

Seed dormancy was apparent when comparing the results of treated and non-treated germination; a factor which should be taken into consideration in future trials.

The results suggest that the unaffected plants in the paddock are stressed, possibly from pasture rundown or lack of water, since the plants from around the trough yielded results comparable to those of commercial seed. It is also possible that this stress is what predisposes the plants to BGD.

Plants affected with BGD showed higher signs of stress in all aspects of seed production.

Chapter 5

Leaf Vasculature and Leaf Pigments

5.1 Introduction

Colour changes in plants are generally associated with developmental changes, such as growth and senescence, where nutrients and other cellular components are being translocated (Buchanan-Wollaston, 1997; Matile *et al.*, 1999; Close and Beadle, 2003). They can also be associated with plant stress, such as a nutrient imbalance, disease, or even insect or grazing injury (Smith, 1974; Salisbury and Ross, 1992; Close and Beadle, 2003). Often, a particular imbalance or disease will have a specific colour change associated with it, which varies between plant species. For example, leaves of *Eucalyptus stricta* turn a bright red colour when injured by the microscopic mite, *Eriophytes eucalypti* (Petrie, 1924). Boron deficiency in sunflower (*Helianthus annuus*) induces a purple pigmentation of the leaves (El-Shintinawy, 1999). Pathogens may also cause colour changes, as in *Rhizoctonia* bare patch ('purple patch') of wheat and oats, causing yellow and purple foliar symptoms (Hynes, 1933; Kirkegaard *et al.*, 1999; O'Brien, and Zamani, 2003). Many viruses produce characteristic lighter striations or mosaic foliar patterns (Teakle and Grylls, 1973; Greber, 1979; Hawkes and Jones, 1995). Because of the specificity of these changes, it is probable that the red colour change observed with BGD is specific to the causal agent(s). Investigating the nature and possible causes of the colour change may, therefore, provide clues as to the nature of the causal agent(s).

5.2 Damaged Leaves and Leaf Vasculature

5.2.1 Materials and Methods

Methodology

Preliminary observations (Chapter 2) revealed that on damaged leaves of BGD affected plants, the progression of red symptoms along the leaf was delayed at the site of damage; the damage in this case being a transverse fold of the leaf. Furthermore, it was noted on subsequent field trips that similarly damaged leaves from unaffected plants also occasionally turned red. It was considered necessary to validate these observations, as this information may confirm the involvement of the plant vascular system.

Method

In a BGD affected paddock, randomly selected field plants, 50 unaffected and 50 BGD affected, were tagged and one leaf from each of them was chosen. The chosen leaves were the first fully mature leaf from the top of the plant. These leaves were manually damaged by bending and by slightly creasing the blade about halfway down the leaf; similar to the damage caused in the field by passing cattle or vehicles. It was ensured that on the BGD affected plants the chosen leaf was at the stage of symptom emergence, such that the red symptom boundary was between the created crease and the leaf tip. An adjacent, undamaged leaf on the same plant was also tagged.

The plants were inspected on a fortnightly basis for 8 weeks. Any colour changes and delays in symptom progression (as compared with other leaves on the same plant) were recorded. This study was undertaken in March-April 2003, with average rainfall and temperatures for that time of year prevailing.

Due to the categorical nature of the data, a contingency table and chi-square analysis was done. The Pearson chi-square statistic was used.

5.2.2 Results

Of the unaffected damaged leaves, 15 displayed red symptoms though twice as many became necrotic. Only 5 were chlorotic. The BGD affected damaged leaves were almost equally divided between displaying red and necrotic symptoms (Table 16). All the tagged undamaged leaves remained green.

Plant type	Number of damaged leaves of varying colours		
	necrotic (brown)	red	chlorotic (yellow)
Unaffected	30	15	5
BGD affected	26	24	0

Table 16 - Frequency of colour symptoms of manually damaged leaves of both unaffected and BGD affected buffel grass (*Cenchrus ciliaris*)

The proportions of colour symptoms was not significantly influenced ($P = 0.451$) by plant type. The BGD unaffected leaves, though red, were not as dark as the BGD affected leaves (Figure 28). BGD affected leaves are commonly the shade of the RHSPPC red groups 46 A and B (The Royal Horticultural Society, 2001), whereas the unaffected leaves were approximated to the red group 51 A, tending more towards a dark pink or bright red. In both cases the site of leaf damage was conspicuous. Red symptoms stopped at the site of damage in BGD unaffected leaves, but progressed beyond the site of damage in BGD affected leaves. Many BGD affected leaves senesced and broke off at the tip, before the site of damage (Figure 28).

The progression of BGD symptoms on affected leaves was delayed by approximately 14 days compared with undamaged affected leaves on the same plant. The delay occurred not at the site of damage, but closer to the leaf tip.

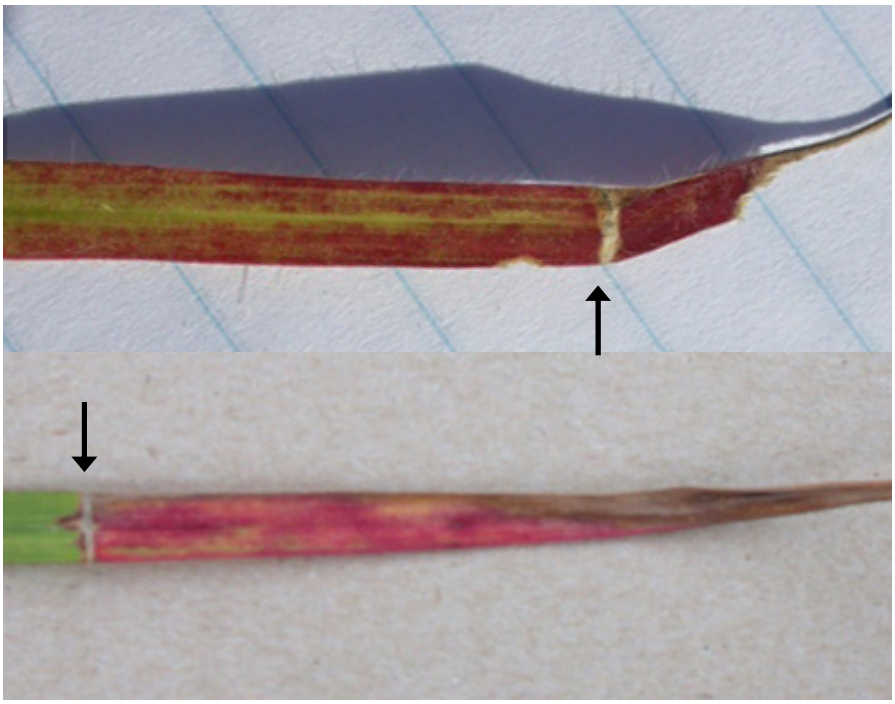


Figure 28 - Comparison of BGD affected (top) and unaffected (bottom) leaves of buffel grass (*Cenchrus ciliaris*) which were manually damaged (arrows)

5.2.3 Discussion

It was concluded that buffel grass leaves tend to turn red after damage, although not necessarily the deep red indicative of dieback symptoms. Damaging the vasculature had some effect on symptom progression. However, since the delay occurred before the site of damage, it is possible that the reddening from the delay point to the site of damage was caused by the damage itself. BGD symptoms may then have progressed from the site of damage. Any future attempts of this nature should observe the leaves more frequently, to determine if red symptoms still progress from tip to ligule, or if symptoms simply resume from the site of damage. Further histology work should be done to confirm vascular involvement (refer Chapter 6).

5.3 Preliminary Attempts to Study Red Foliar Pigments

While the red pigment in question is most likely an anthocyanin or related chemical, its identity may provide a clue as to the causal agent(s) of BGD. Therefore, several attempts were made to isolate and identify the pigment responsible for the red foliar BGD symptoms. However, in order to analytically identify the pigment, several milligrams of the dried pigment were needed. There were not enough affected plants in the vicinity of the laboratory (*i.e.* on campus) to obtain this amount of pigment.

In the interim, other tests on the pigment were performed from the few BGD affected plants which were maintained in the shade house. It was found that the pigment is soluble in methanol, slightly less soluble in acetone, and less again in water. Immersing fresh BGD affected red leaves in the solvent in a mortar, and grinding the leaves with a pestle until macerated extracted the pigment. The pigment could only be extracted from fresh leaves; it could not be extracted from dried leaf material or leaves on affected plants which had turned a bronze colour. This suggests that the pigment breaks down rapidly or is otherwise unstable, perhaps only existing in a stable state in living plant tissue. In support of this, extracted pigment left overnight turned a brown colour, similar to the bronze colour that older BGD affected leaves turn. This occurred with every solvent.

It was then attempted to bring back pigments from the field for analysis, as there were adequate BGD affected plants in the field to yield the required amount of pigment. Bright red sections of the leaves were crushed in a mortar and pestle in acetone, and the resulting red liquid was immediately poured into glass bottles and placed out of direct sunlight in an insulated container. Acetone was used instead of methanol due to its less hazardous nature. After the 2 h journey back to the laboratory, the liquid in the bottles had

turned bright green, similar to the colour resulting from chlorophyll extraction, suggesting that the breakdown of the pigment is accelerated by agitation, or by a time factor between extraction in the field and returning to the laboratory.

Unfortunately, a drought in late 2003 followed by low rainfall prevented this work from continuing further, as there was no longer a large supply of BGD affected plants in the field.

The unstable properties of the pigment is similar to the properties of anthocyanins, which break down under high temperatures and illumination, and are generally unstable *in vitro*, unless kept in acidic conditions or have added preservatives (Salisbury and Ross, 1992; Guo *et al.*, 2004). However, the red pigment intybin, reported by Winter (1958), also shares most of these properties. Intybin is light sensitive, heat sensitive, and is very unstable in solution, though slightly more stable in acidic conditions. The extracted colour fades over time, and changes to a yellow-brown when heated. Intybin is formed only by living plant tissue, specifically by damaged cell tissue, which should not extend to the whole of the plant tissue mass. It is formed not only in leaves but also in the stem and roots. The main differences between intybin and anthocyanins are that, firstly, the chance of intybin forming brown compounds by itself is slight; these are generally formed by heating or long storage. Secondly, intybin is not very soluble in acetone, though it is soluble in methanol and less so in water. Lastly, the two chemicals have possible differences in their absorption spectra. Intybin has peaks at 508 nm and 545 nm, whereas anthocyanins generally peak at approximately 529 nm, which can vary among this group of compounds (Sims and Gamon, 2002). Based on this comparison, it is possible that either both anthocyanins and intybin are responsible for the red BGD foliar symptoms; other

pigments may also be responsible. Any future work on this topic should take this information into consideration.

5.4 Foliar Pigments

5.4.1 Materials and Methods

Methodology

Foliar colour changes commonly involve the production, destruction and/or relocation of various plant pigments. For example, leaves of deciduous trees turn red in Autumn due to anthocyanin accumulation (Field *et al.*, 2001), and iron deficiency results in an 80% reduction in chlorophyll *a* in the leaves of apricot and pear trees (Val *et al.*, 1987). As mentioned in the introduction, these changes are often specific to the condition which causes them. Therefore, it was decided to carry out pigment analyses on BGD affected leaves and on other buffel grass leaves exhibiting similar colour symptoms. Since the red pigment could not be analysed and identified (part 5.3), it was thought that the pigment concentration could at least be compared with that of non-BGD affected red leaves, as could the concentration of other pigments. The resulting pigment profiles might disclose similarities between some treatments, providing an indication of likely contributors to the BGD condition.

As mentioned in part 5.2, leaf damage can cause red foliar symptoms in buffel grass, as can certain nutrient deficiencies, namely nitrogen, magnesium and potassium (refer Chapter 7). Therefore, both damaged leaves and leaves from nutrient deficient plants should be included in this analysis. In addition to the red leaves from these plant types, green leaves should also be included, as these may give an indication of whether the pigments are simply being translocated or are accumulated and/or destroyed. All leaves

used in this study must be of similar physiological age, as age affects the pigment concentration (Salisbury and Ross, 1992).

While there are many procedures describing the extraction and quantitative analysis of plant pigments, most of these are specific to only one pigment. Since the aim of this experiment was to compare several pigments, it was considered necessary to use a procedure which encompassed all of the desired pigments, enabling a comparative analysis which was not biased by procedure. For this reason, the analytical procedure in Sims and Gamon (2002) was used, since this paper presents a comparative analysis of plant pigments. As in this paper, analyses were only done for chlorophylls *a* and *b*, carotenoids, and anthocyanins.

Method

Leaf samples were taken from four types of buffel grass plants:

1. Control plants, unaffected green plants

2. Unaffected plants which were deficient in nitrogen, magnesium and potassium (refer Chapter 8), and were showing red deficiency symptoms

3. Unaffected plants which were manually damaged as in part 5.2.1, with the leaf section from the crease to the tip showing red colouring

4. BGD affected plants in the moderate stage of symptom progression.

The control plants, manually damaged plants and BGD affected plants were transplanted from intact soil cores (including plants) obtained from the field. The intact soil cores were to keep the BGD affected plants in a BGD affected state (refer to Chapter 10). The nutrient deficient plants were also obtained from the field in intact soil cores, but were washed of soil and transplanted into a mixture of vermiculite and sand. These were

watered with a nutrient solution (as described in Chapter 7) which generated the required nutrient deficiencies. All of the above plants were grown in a completely random design on a bench in a shade house, and were of similar physiological age.

Triplicate leaf samples were taken from 10 plants of each type, from both red and green areas of all plant types, with the exception of control plants which only had green areas. The samples were collected in the morning and were processed immediately.

The samples were processed and analysed according to Sims and Gamon (2002). The samples were ground in a solution, were centrifuged to remove particulates, and the supernatant analysed by absorption spectrophotometry. For quantification of chlorophylls and carotenoids, a cold acetone / tris buffer solution was used, whereas a cold methanol / HCl / water solution was used for quantification of anthocyanins. The only difference to the procedure used by Sims and Gamon (2002) was that rectangular, not circular templates were used to cut out leaf samples. Since buffel grass leaves are narrow, using a circular template would have included the midrib, which may have influenced the results since leaf pigments are found in lower concentrations in the vasculature (Mauseth, 1988). Additional triplicate samples of each leaf type were excised using the templates. These were weighed, dried in an oven at 70°C for 12 h, and re-weighed to obtain percentage dry matter, the values of which were later used to calculate pigment concentration per gram of dry weight. The samples were analysed at the wavelengths stipulated by Sims and Gamon (2002), as well as at wavelengths of 508 and 545 nm, to check for the presence of Intybin.

Statistical Analyses

An analysis of variance (ANOVA) was performed for each pigment to compare the 7 treatments. Furthermore, the treatment variation was partitioned into effects for the

control vs. the average of the other treatments (1 df) and the nested colour (red, green) x type (BGD affected, nutrient deficient, damaged) factorial (5 df). Pairwise comparisons of means was performed using the protected l.s.d. procedure at $P = 0.05$.

A canonical variate analysis (CVA) was also used to compare pigment concentration in the 7 treatments across the 4 variables (Chlorophylls *a* & *b*, carotenoids and anthocyanins).

5.4.2 Results

The spectral analysis revealed only trace amounts of absorbance at 508 and 545 nm, thereby confirming that anthocyanins, not intybin, is responsible for the red symptoms.

Compared with the control plants, green leaves from BGD affected plants had significantly different levels of all pigments (Figures 29 and 30). Red leaves from BGD affected plants had lower levels of chlorophylls, but elevated levels of carotenoids and anthocyanins. Green leaves from nutrient deficient plants had reduced levels of chlorophyll *a*, similar levels of chlorophyll *b*, and higher levels of carotenoids and anthocyanins. Red leaves from nutrient deficient plants had lower levels of chlorophylls but higher levels of carotenoids and anthocyanins. Green leaves from damaged plants had lower levels of all pigments. Red leaves from damaged plants had lower levels of chlorophylls but higher levels of carotenoids and anthocyanins. When comparing the relative proportions of the various pigments, damaged leaves, rather than the nutrient deficient leaves, more closely resembled the pattern of BGD affected leaves.

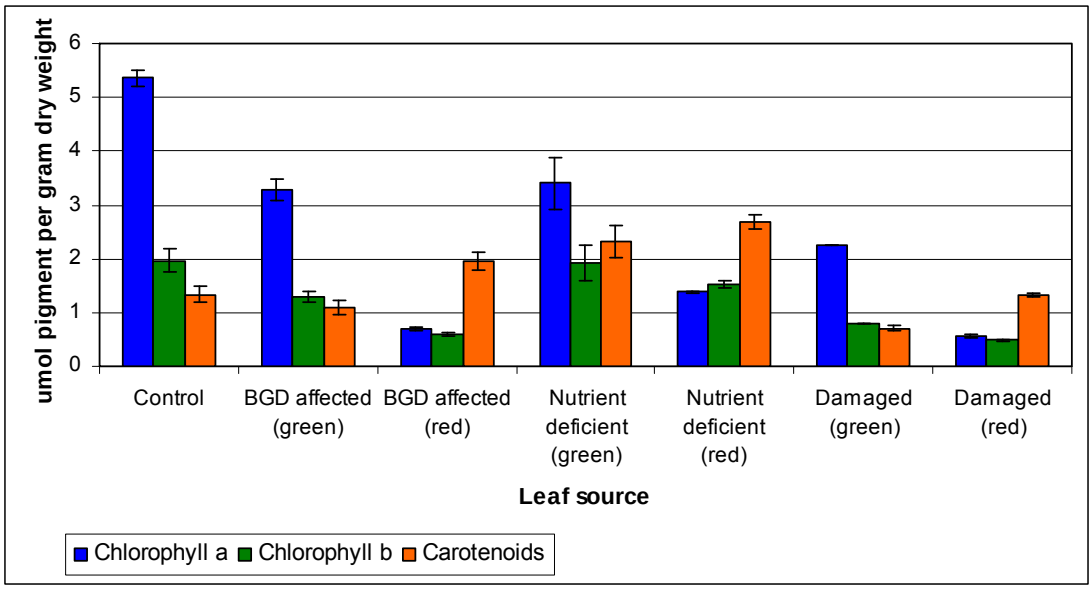


Figure 29 – Mean chlorophyll and carotenoid concentration of leaves of buffel grass (*Cenchrus ciliaris*) plants from various treatments (± SE)

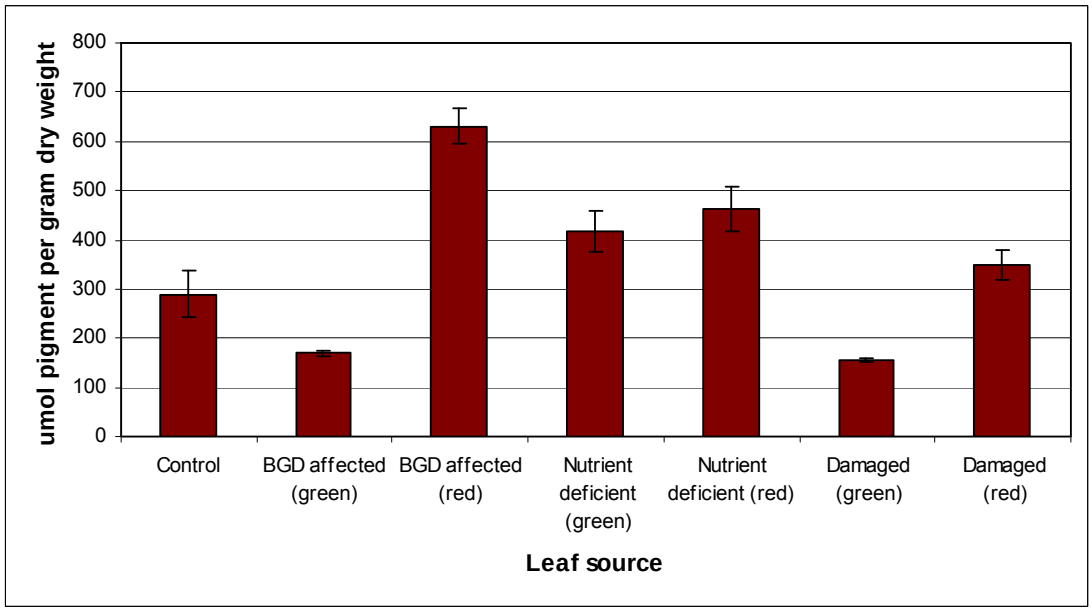


Figure 30 – Mean anthocyanin concentration of leaves of buffel grass (*Cenchrus ciliaris*) plants from various treatments (± SE)

For each pigment, there was a significant difference ($P < 0.001$) between the 7 treatments. The control was different ($P < 0.05$) from the average of the other treatments for chlorophylls *a* and *b* but was not significantly different for carotenoids ($P = 0.073$) and

anthocyanins ($P = 0.056$). There was no interaction ($P > 0.01$) between colour and type for all pigments except anthocyanins.

Chlorophyll *a* differed ($P < 0.001$) among type with damaged < BGD affected < nutrient deficient leaves (that is, damaged leaves had significantly less chlorophyll *a* than BGD affected leaves, *etc*.) and all were less than control leaves. There was also a significant main effect of colour (red and green leaves) ($P < 0.001$), with red < green (that is, red leaves had significantly less chlorophyll *a* than green leaves).

For chlorophyll *b*, there was a significant main effect of type with damaged < BGD affected < nutrient deficient. The amounts of chlorophyll *b* in control leaves and nutrient deficient leaves were not significantly different. There was also a significant main effect of colour ($P = 0.004$), with red < green.

There was a significant main effect of type ($P < 0.001$) for carotenoids damaged < BGD affected < nutrient deficient leaves, but neither damaged or BGD affected leaves were different from the control leaves. There was also a significant main effect of colour ($P < 0.001$), with green < red.

For anthocyanins, the interaction of colour and type was significant ($P < 0.001$) with a greater difference in anthocyanin levels between the green and red BGD affected leaves than damaged leaves, with levels always greater in red than green. However, there was no difference between red and green for nutrient deficient leaves, that is, the anthocyanin ratios between red and green leaves were different for each treatment type. Furthermore, levels in the control leaves were similar to the BGD affected and damaged leaves but less than the nutrient deficient leaves.

Two CVAs (1 and 2) were done explaining 95.0 and 2.7% of the variation respectively (*i.e.* total of 97.7%), meaning that canonical variate 1 is the most important

(Figure 31). Canonical variate 1 (CV1) is a contrast of chlorophylls *a* and *b*, while canonical variate 2 (CV2) is dominated by anthocyanins with some input from chlorophyll *a*. Figure 31 clearly shows how CV1 separates the treatments based on a contrast of chlorophylls *a* and *b*. CV2 does not separate the groups much but those to the right have higher anthocyanins (and maybe chlorophyll *a*) than those to the left.

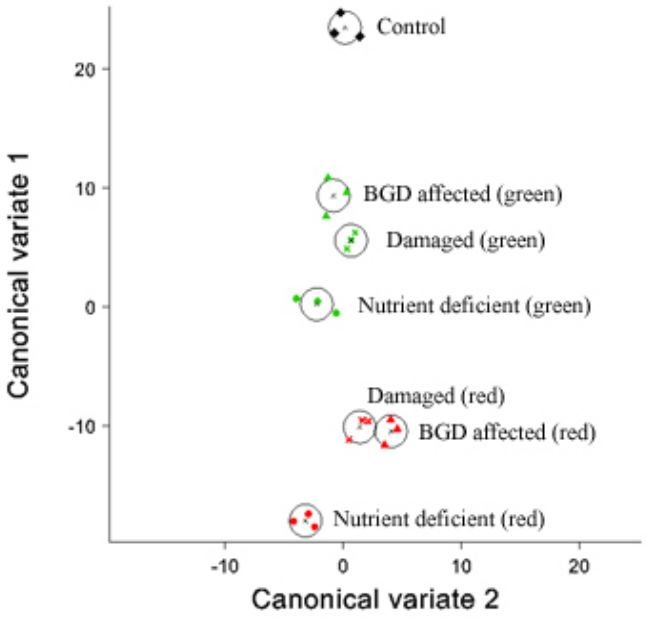


Figure 31 - Statistical distribution of pigment analysis of various buffel grass (*Cenchrus ciliaris*) samples

5.4.3 Discussion

Compared with the control plants, the other treatments had a decreased production or a degradation of chlorophyll *a*. Within the treatments, the red leaves had significantly less chlorophyll *a* than the green leaves. This result was expected, as according to Sims and Gamon (2002), chlorophylls tend to decline rapidly when plants are under stress. Chlorophyll *b* followed a similar pattern with the exception of nutrient deficient leaves, which overall had no significant difference to control plants, but still had a significant

difference between red and green leaves. This may suggest that the nutrient deficient plants were not as stressed as the plants in the other treatments.

Carotenoid concentrations were not very different between the control plants and the other treatments, but were significantly higher in the red leaves than the green leaves of each treatment, possibly contributing to the red colour. Anthocyanin concentrations were also not very different between the control plants and the other treatments, but this was expected as the anthocyanin data had a much larger range. Comparing the anthocyanin concentrations between red and green leaves of the various treatments, there was no difference in nutrient deficient leaves but a significant difference between the red and green leaves of damaged and BGD affected plants. This suggests that anthocyanins may be responsible for the colour change in damaged and BGD affected plants, but not nutrient deficient plants.

Comparing the red and green leaves of BGD affected plants, it appears that carotenoids and anthocyanins are accumulating or being produced in the red leaves, accounting for the red colour. Anthocyanins are generally produced in leaves prior to senescence (Field *et al.*, 2001). Moreover, chlorophylls seem to be accumulated in the green leaves, or perhaps they are simply destroyed or have limited production in the red leaves, also contributing to the colour change. In addition, it is possible that the red leaves are in the first stages of senescence, in which chlorophylls are broken down and translocated to other areas of the plant (Roberts and Whitehouse, 1976; Hopkins, 1995). The results show that these pigments are involved in the production of red BGD symptoms. The question is, what is the cause of the change in pigment concentration?

All of the treatments were significantly different, so nutrient deficiency or leaf damage are probably not the main cause of the red BGD symptoms. However, a visual

inspection of Figures 29 and 30 supported by the statistics may indicate which of nutrient imbalance or leaf damage may be the more likely contributors.

It appears that in the nutrient deficient plants, red symptoms are produced by a slight increase in carotenoid concentration and a decrease in chlorophylls. Conversely, in both the damaged and BGD affected leaves, it appears that red symptoms are produced by an increase in the concentration of carotenoids and anthocyanins and a decrease in chlorophylls. This may suggest that leaf damage is a contributing factor to BGD red symptoms. The anthocyanin concentration (Figure 30) appears to indicate otherwise, as the values for nutrient deficient leaves are not significantly different from that of BGD affected leaves (as opposed to damaged leaves). However, the significant type by colour interaction in the anthocyanin data shows that nutrient deficient and BGD affected leaves are significantly different in their red leaf to green leaf ratio. This can be seen in Figure 30, and is also supported by the statistics, which showed that nutrient deficient red and green leaves were not significantly different in their anthocyanin concentration. Furthermore, the CVA suggests that, overall, the pigment profile of damaged leaves is the closest to that of BGD affected leaves. Therefore, a plausible conclusion would be that a process similar to that caused by manual leaf damage is a likely contributor to the red BGD symptoms.

It is also possible that other plant pigments are involved, but these were not included in this work.

It is clear that anthocyanins may be excessively produced in BGD affected red leaves. Anthocyanins have antiviral and antimicrobial properties (Wrolstad, 2004), and are also thought to act as antioxidants in leaves (Gould *et al.*, 2002). Gould *et al*. (2002) found that, after foliar mechanical injury, accumulated anthocyanins scavenged the H_2O_2 produced by the oxidative burst. This not only supports the finding that anthocyanins are

accumulated in response to injury, but also suggests that the plants are using anthocyanins as a defence mechanism. This may be relevant when investigating the cause of BGD.

5.5 Concluding Statements

Compared with control plants and the green leaves of BGD affected plants, red leaves of BGD affected plants had lower concentrations of chlorophylls *a* and *b*, and higher concentrations of carotenoids and anthocyanins. Whether the pigments are destroyed or excessively produced still remains to be determined. The increased anthocyanins may be a plant defence response. A comparison of the relative proportions of the various pigments showed that the red symptoms of BGD are most closely related to those from leaf damage, suggesting that leaf damage in some form may be a contributor to the red symptoms of BGD.

Chapter 6

Histological Studies

6.1 Introduction

The observable symptoms of BGD and the morphological effects of BGD on the host plant have already been detailed in Chapters 2 and 4 respectively. However, whether or not BGD affects the plants at the cellular level is unknown.

Many plant disorders have histological symptoms which are visible microscopically. These symptoms include the physical presence of pathogens, cellular damage and cellular modifications. For example, *Agrobacterium tumefaciens* causes hyperplasia and hypertrophy resulting in galls or tumours on roots and other organs of many fruit crops (Narayanasamy, 2001). Other histological symptoms include inclusion bodies and tyloses. Inclusion bodies are intracellular inclusions, often found in the vacuole, which generally consist of a local accumulation of polyphenols (Musetti *et al.*, 2000). They can be crystalline or amorphous in structure and are usually associated with pathogens, whether bacterial, fungal or viral (Musetti *et al.*, 2000). Tyloses are an outgrowth of parenchyma cells into the vessel cavity of the xylem of plants (Zimmermann, 1983). They are produced as a result of wounding, injury, or certain pathogens, and provide the plant with a mechanism to seal off functional tissue from dead or injured parts (Zimmermann, 1983). As an example of tylosis formation, wilt pathogens such as *Fusarium oxysporum* can colonise the vascular tissue of many plants, creating both a physical blockage and wounding the plant. This induces the plants to produce further blockage in the form of

tyloses, thereby hindering water movement and producing the characteristic wilt symptoms (Parry, 1990).

As many histological symptoms are characteristic of a particular condition, it was considered essential to conduct an investigation into the effect of BGD at the cellular level for two reasons, firstly, to develop a cellular symptom profile of the condition, and secondly, to investigate possible causal agent(s) of this condition.

This chapter deals exclusively with histological studies. Isolations and pathological studies are dealt with in Chapter 9.

6.2 Materials and Methods

Methodology

There are many methods available for histological studies, many having been developed for a special purpose. However, since this study is primarily on cellular changes and anatomical features, a simple approach was considered adequate. Either whole tissue or sections of the organs of interest can be examined. Inspecting the whole tissue is usually accomplished by tissue clearing. This is especially advantageous when investigating potential plant-microbe interactions (Liberato *et al.*, 2005; Massicotte *et al.*, 2005). Closer examination of these tissues requires embedding specimens and sectioning them.

Specimens for histological work using light microscopy are generally embedded in paraffin wax or resin. Resin embedding has been shown to provide greatly improved cellular definition (ProSciTech, accessed 2003) and is, therefore, more commonly used. There are many different types of resin available: some for specialised purposes and others for more general use. One commonly used general purpose resin is L.R. White. This resin is hydrophilic, allowing stains to easily penetrate, and shows minimal non-specific staining.

The low viscosity of the resin also allows for easier infiltration of plant tissue (ProSciTech, accessed 2003).

L.R. White resin has guidelines for the preparation of material prior to sectioning including fixation, dehydration and infiltration. To produce optimal results, these guidelines were followed closely, except for the dehydration series. The recommended guidelines (ProSciTech, accessed 2003) state that the dehydration series should consist of two changes of 70% ethanol of 30 min each, followed by two changes of absolute ethanol, also of 30min each. However, O'Brien and McCully (1981) stated that for minimal damage and distortion to occur, there should be a gradual change in the water content of the specimen from fixation to final dehydration. Neutral buffered formalin, the recommended fixative when using L.R. White resin, is approximately a 4% solution. While healthy specimens may withstand the large gradient between a 4% and a 70% solution without ill effects, more fragile tissue, such as possibly diseased, BGD affected tissue, may result in unwanted artefacts. Therefore, for the purpose of this study, a more gradual dehydration series was used; one starting at 10% ethanol with 10% ethanol increments.

Most stains give good results with tissues imbedded in L.R. White resin (ProSciTech, accessed 2003). The metachromatic stain Toluidine Blue was chosen for this study as it is both simple and quick to use and results in contrasting colours. It is widely used in the study of plant tissues, both for differential staining (Sakai, 1973) and for the detection of plant pathogens (Meenakshi *et al.*, 1972).

Since the aetiology of BGD is unknown, the tissues displaying symptoms were the logical choice when deciding on which tissues to examine: that is, the leaves and roots (refer Chapter 2). Given that red leaves become necrotic from the tip to the ligule, the red segments of leaf chosen for this study were those adjacent to the red/green boundary, where

there is a minimal chance of the cellular morphology being affected by necrosis. Apart from general stunting, typical root symptoms included dark necrotic depressions (Figure 11, p.49). Therefore, segments with these symptoms were chosen for investigation.

This study only included BGD affected and unaffected plants. While the results in Chapter 5 suggested that the plant vasculature was involved with the BGD condition, the inclusion of mechanically damaged leaves in this study was not considered appropriate. The leaves used in the Chapter 5 study were mechanically damaged to produce red symptoms, possibly causing a physical break in the vasculature, and as such would not provide a true comparison.

Method

Leaves and roots from BGD affected plants (30 plants) and unaffected plants (30 plants) were collected from the field. Of the BGD affected plants, leaves which displayed red symptoms were selected, as were roots with necrotic areas and/or lesions. Corresponding parts of the unaffected plants were also selected. The samples were washed free of dirt and debris, and cut into segments 2 cm long. The leaf samples were cleared and stained as per Liberato *et al.* (2005), technique 3. Leaf pieces were immersed in a 1:1 mixture of 100% acetic acid and absolute ethanol for 48 h, were rapidly washed in distilled water and mounted on slides in 85% lactic acid with 1 $g.L^{-1}$ aniline blue. The root samples were cleared and stained as per Massicotte *et al.* (2005). Root pieces were placed in 10% KOH in an oven at 60°C for 20 h, were rinsed in distilled water for 30 min, and stained with trypan blue. All were observed under light microscopy for evidence of pathogen involvement. The slides were viewed with a Leica DMLB microscope and photographed using a Color View 2 – Soft Imaging System camera.

In addition, leaves and roots from both BGD affected and unaffected plants (a further 30 plants of each) were collected from the field and were briefly washed in water to remove any dirt. These were immediately cut with a scalpel into small segments (*ca.* 5 mm long) and the desired pieces were placed into McCartney bottles containing neutral buffered formalin, as per ProSciTech (accessed 2003). For the unaffected plants, the desired segments were any which did not have blemishes. For the BGD affected leaves, the desired segments were those which were on the red/green boundary or those immediately on the red side of the boundary, which also did not have additional blemishes. The desired BGD affected root segments were those from roots which displayed the typical damaged or necrotic symptoms (refer Chapter 2). The segments were kept in formalin and refrigerated for at least 48 h or until needed.

All segments were dehydrated using a graded ethanol series. This series progressed from 10% ethanol to absolute ethanol using 10% ethanol increments, with two changes of absolute ethanol. Each change was for 15 min. The segments were then infiltrated with L.R. White resin for two changes, one for 2 h and the other overnight. All segments became translucent and sank to the bottom of the container, indicating that they were properly infiltrated (ProSciTech, accessed 2003).

The segments were placed in resin molds (with wells 10 x 7 x 7 mm deep) and were orientated to produce both transverse and longitudinal sections. L.R. White resin (with accelerator added in the prescribed amount) was then poured into the molds and allowed to set, making sure that the segments remained in their correct orientation. Plastic stubs were mounted onto the hardened resin blocks using Leica historesin.

The sections were cut from the blocks using a Leitz 1512 rotary microtome. The sections from unaffected leaves and roots were cut 8 µm thick. Those from BGD affected

leaves and roots were cut between 8 and 10 μm thick. The BGD affected specimen appeared more fragile and tore more easily. Only transverse sections were cut; the longitudinal sections continuously tore no matter the thickness. Roots also seemed to be more fragile than leaves, with the cortex very easily separating from the endodermis.

The sections were mounted eight to a slide and stained with Toluidine Blue according to O'Brien and McCully (1981). They were then mounted in the mounting medium DPX (consisting of Dibutyl phthalate, Polystyrene resin and Xylene) and allowed to set. The slides were viewed with a Leica DMLB microscope and photographed using a Leica DC 100 camera.

6.3 Results

6.3.1 Tissue Clearing

Leaves

Leaves of both BGD affected and unaffected buffel grass were covered with spores (Figure 32), the vast majority of which had not germinated. An estimated <1% of spores had germinated, but only two instances of hyphal growth were seen. Some spores were identified as *Helminthosporium* sp., *Dreschlera* sp. and *Alternaria* sp.. Most spores (one and two celled) could not be identified. Few bacteria were seen.

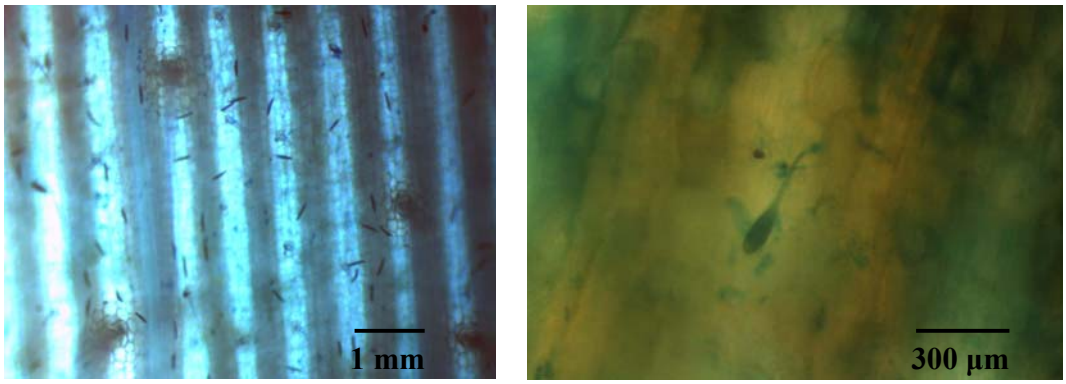


Figure 32 – Cleared leaves of BGD affected buffel grass (*Cenchrus ciliaris*) showing large numbers of spores (left) and a germinated spore (right)

Roots

The roots of unaffected plants had very few hyphae. Conversely, the roots of BGD affected plants had hyphal masses on the surface, mostly in the observed necrotic areas (Figure 33). Hyphae were not seen to penetrate the roots. Most of the hyphal masses did not have reproductive structures. However, in four instances structures were seen which were identified as spores, leading to the tentative identification of those hyphal masses as *Pythium* sp.. Some bacteria were also seen at the site of root necrosis.

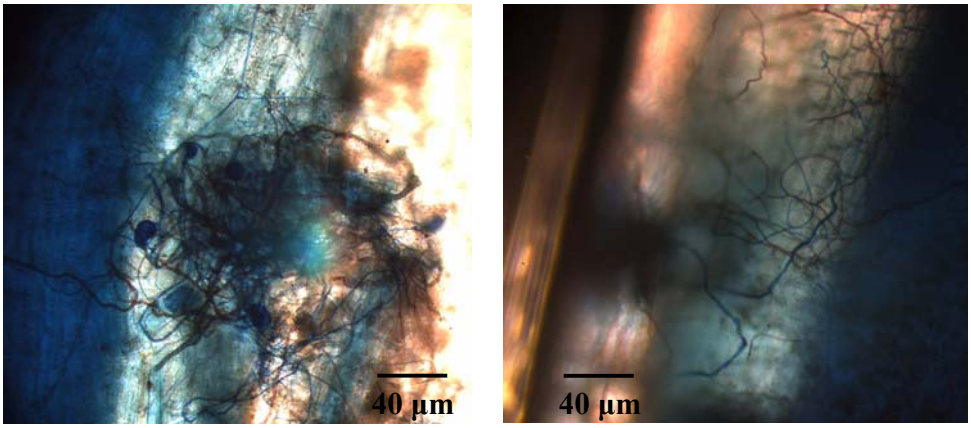


Figure 33 – Cleared roots of BGD affected buffel grass (*Cenchrus ciliaris*) showing hyphal mass and spores (left) and a hyphal mass on the root surface (right)

6.3.2 Sectioned Specimen

The healthy unaffected specimen displayed typical anatomical features of a C_4 monocotyledon (Figures 34 and 35), including undifferentiated mesophyll cells and parallel veins in leaf tissue, and alternating phloem and xylem bundles around a central pith in root tissue. There was no apparent accumulation of colour at the red/green boundary.

Green leaves from BGD affected plants were similar to leaves from unaffected plants, in that there were no vascular occlusions or evidence of cellular damage.

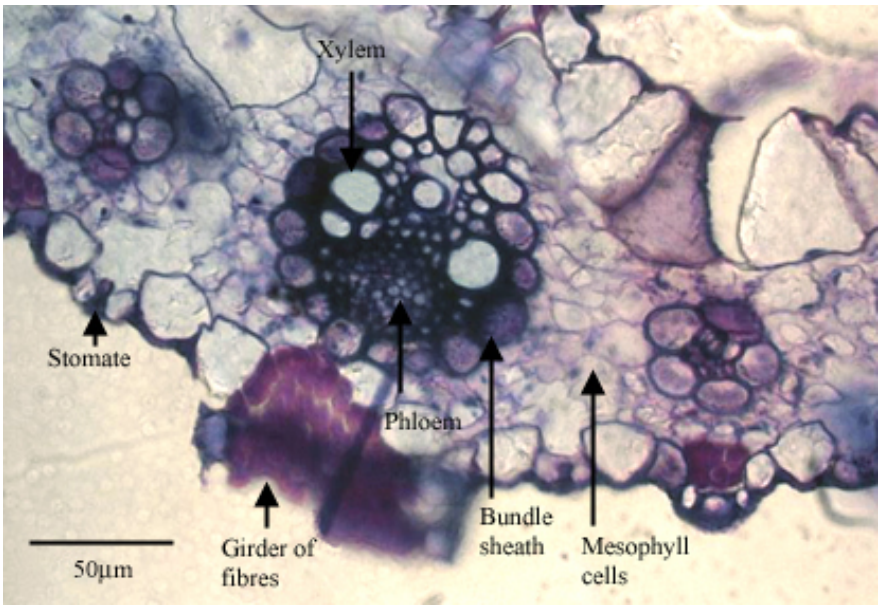


Figure 34 – Transverse section of a healthy leaf of buffel grass (*Cenchrus ciliaris*)

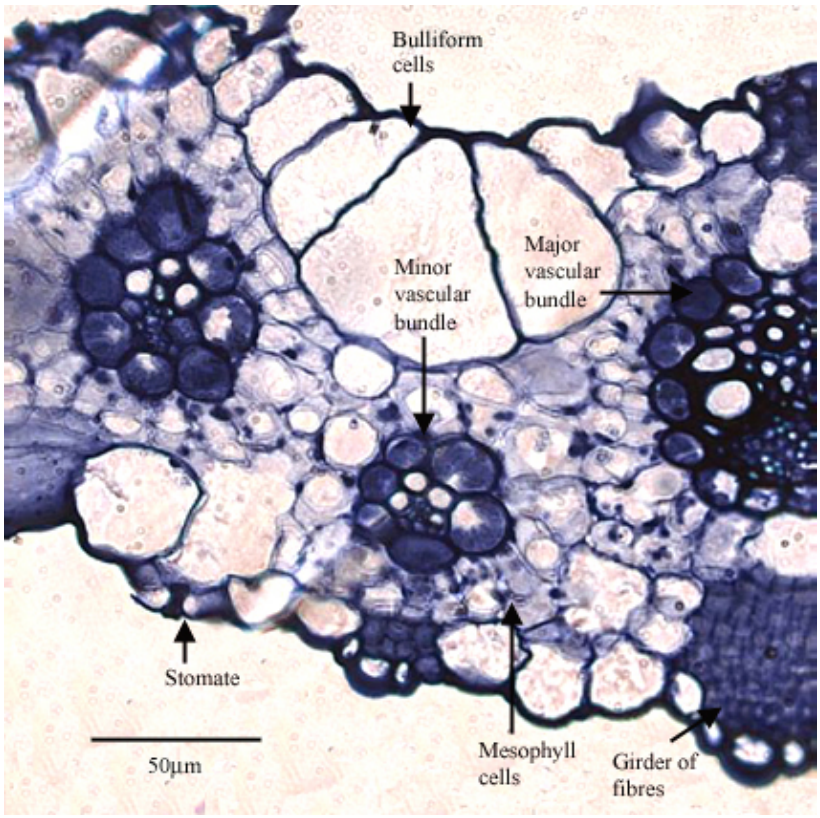


Figure 35 - Transverse section of a healthy leaf of buffel grass (*Cenchrus ciliaris*) showing minor vascular bundle and bulliform cells

Leaves

The cells were approximately the same size in both BGD affected and unaffected leaves. The cells of BGD affected leaves seemed slightly irregular in shape, especially the bulliform and mesophyll cells (Figures 36 and 38).

The xylem vessels of BGD affected leaves were partially occluded by tyloses (Figure 37). There were also green-staining inclusion bodies; mainly in the mesophyll cells (Figure 38). The chloroplasts were not easily discernible in the affected leaves and were not in their usual alignment (Figures 36 and 38).

There were no fungal hyphae or bacterial colonies visible.

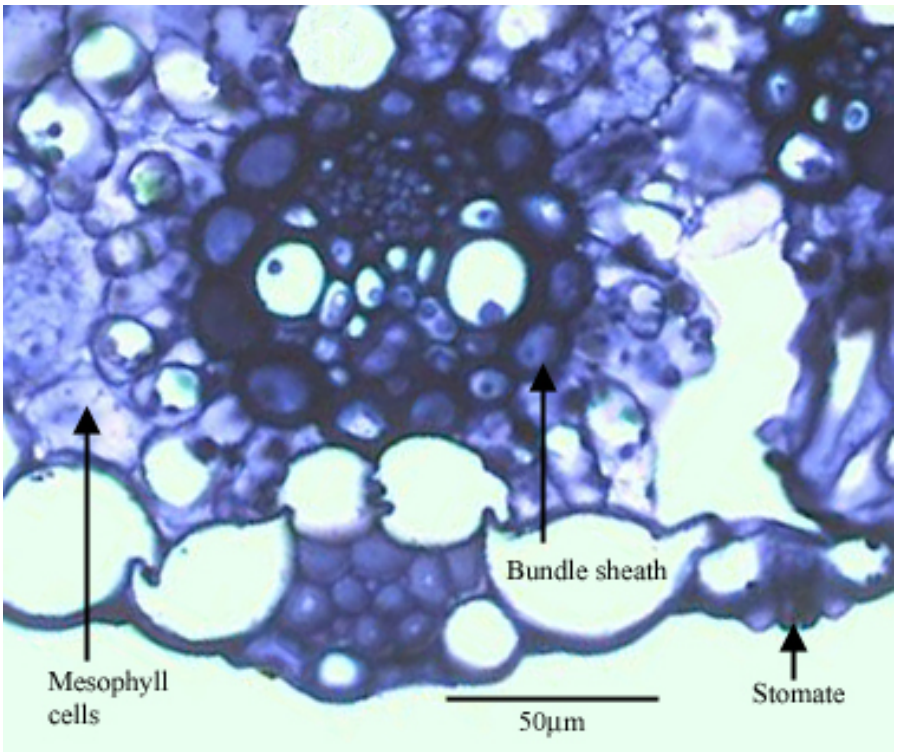


Figure 36 - Transverse section of a BGD affected leaf of buffel grass (*Cenchrus ciliaris*) showing the disruption to the bundle sheath and mesophyll cells

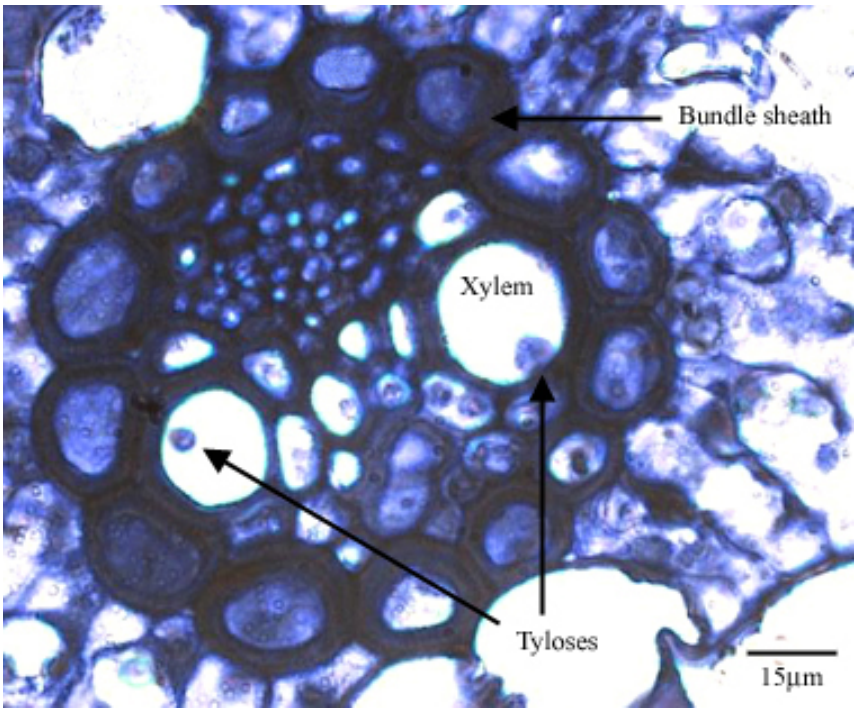


Figure 37 - Transverse section of a BGD affected leaf of buffel grass (*Cenchrus ciliaris*) showing tyloses in the xylem vessels

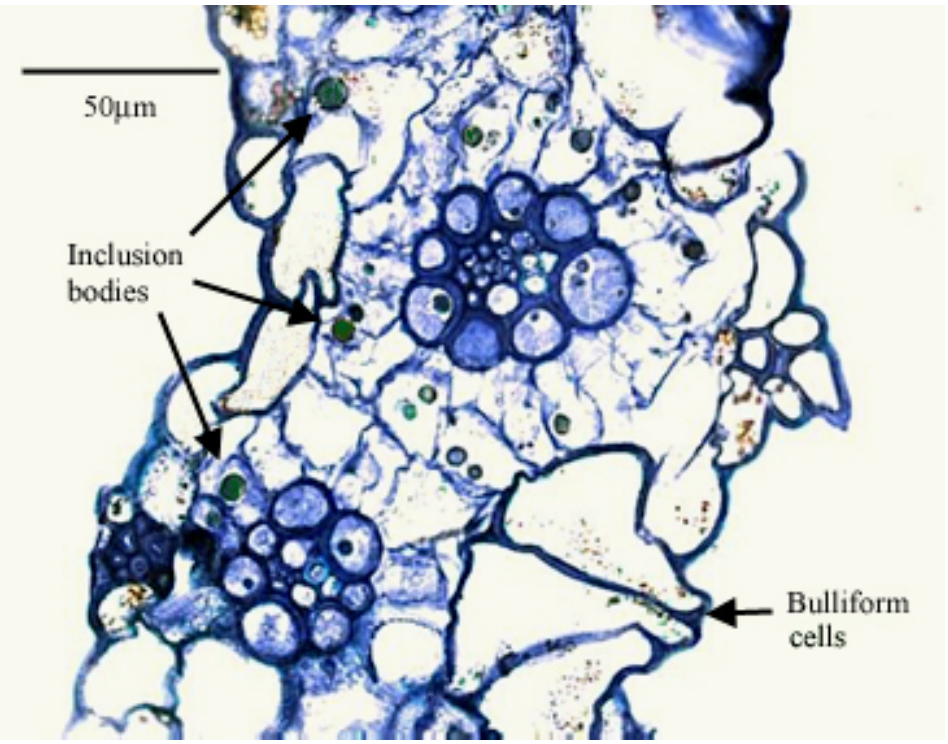


Figure 38 - Transverse section of a BGD affected leaf of buffel grass (*Cenchrus ciliaris*) showing inclusion bodies and irregularly shaped cells

Roots

The cells were approximately the same size in both BGD affected and unaffected roots (Figures 39 and 40). The mesophyll cells appeared damaged or disrupted in BGD affected roots (Figure 40).

The root tissue seemed more fragile than the leaves, with the cortex often separated and missing from around the endodermis. The pith was often torn (Figure 40). Both of these observations were more prevalent in BGD affected roots compared with unaffected roots.

The main xylem vessels of BGD affected roots contained possible tyloses or foreign material (Figure 40). On occasion, the root occlusions completely blocked the vessel.

There were no fungal hyphae or bacterial colonies visible.

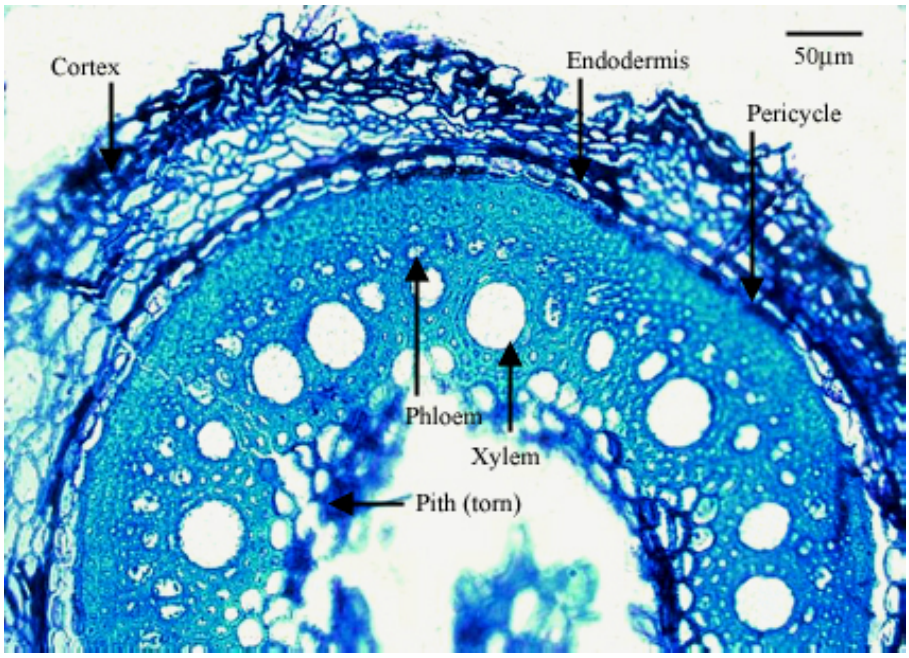


Figure 39 - Transverse section of a healthy root of buffel grass (*Cenchrus ciliaris*)

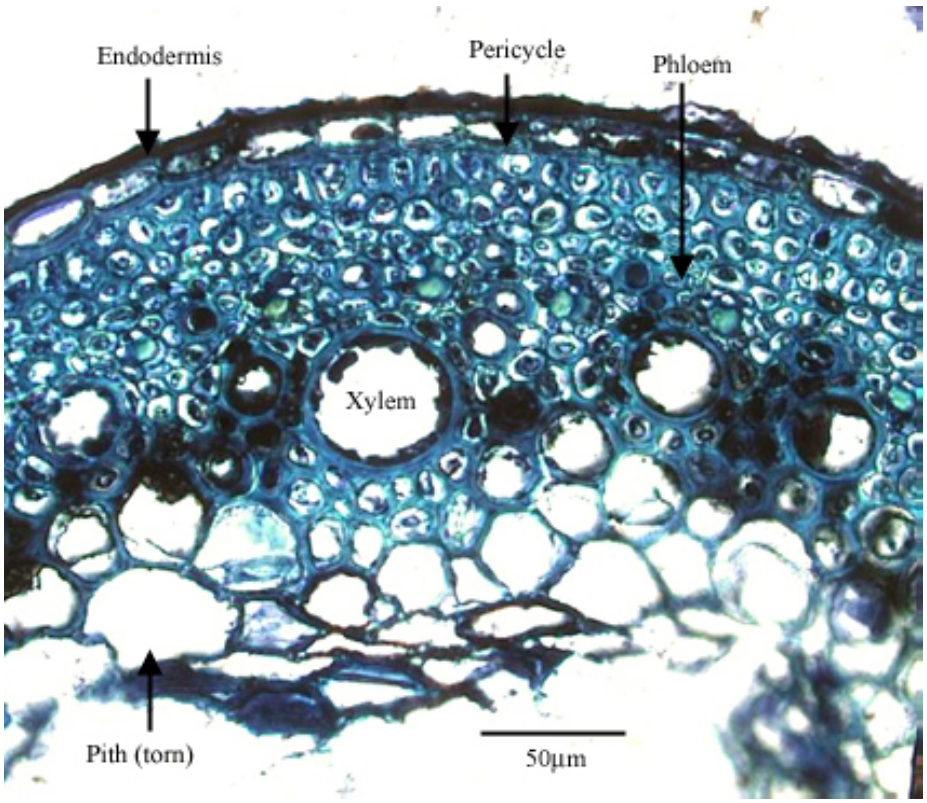


Figure 40 - Transverse section of a BGD affected root of buffel grass (*Cenchrus ciliaris*) showing cellular damage and xylem occlusions. The cortex was missing, as was typical of these root sections

6.4 Discussion

There was no hyphal penetration of either leaf or roots of BGD affected plants, indicating that either the causal agent(s), if a pathogen, is an ectoparasite or simply was not seen inside the plant at this time. The large numbers of ungerminated spores on leaves of both BGD affected and unaffected plants suggest that this was the result of high numbers of spores in the air rather than the spread of a causal agent. The identified spores all belong to species of which some are recognised as plant pathogens. However, as most were ungerminated, it can be suggested that these spores may not be responsible for symptoms of BGD. Those spores which did germinate require further investigation. *Pythium* sp. can also be pathogenic, infecting both roots and leaves. Certain species produce patch diseases of turfgrass (Couch, 2000), and damping-off and root rot of many plant species (Boehm and Hoitink, 1992; Craft and Nelson, 1996). Though hyphae were not seen to penetrate, it is possible that *Pythium* sp. may have some involvement in the BGD condition.

Though BGD affected plants are generally stunted (Chapter 4), there was no obvious difference in the size of the cells and vasculature between BGD affected and unaffected plants. This may eliminate some possible causes of BGD, as selected pathogens, such as certain viruses, can cause phloem degeneration (Narayanasamy, 2001).

The cells of the BGD affected leaves appeared to be more irregular in shape than those of unaffected leaves. This occurred mainly in the bulliform and mesophyll cells; the shape of the vascular tissue did not seem to be affected. Whether these symptoms are a result of cell collapse due to structural damage or whether the cells are simply crenated is unknown.

Generally, the photosynthetic bundle sheath cells of BGD affected leaves appeared disrupted when compared with the unaffected leaves. The chloroplasts in the bundle sheath cells were not in their usual alignment (towards the outside of the sheath) and in some cases were not distinguishable from the cell contents. This may suggest that there is a breakdown of the chloroplasts in BGD affected leaves. This supports the findings of Chapter 5, which found that BGD affected leaves have a lower concentration of chlorophylls *a* and *b*. The disruption of chloroplasts also occurred to a lesser extent in the mesophyll cells of BGD affected and unaffected leaves. Chloroplast disruption has been reported in relation to the presence of fungal proteins (Keates *et al.*, 2003), suggesting that there may be fungal involvement in BGD.

The roots of BGD affected plants appeared more damaged compared with unaffected plants, especially regarding the cortex. In most sections of BGD affected roots, the cortex was missing, supposedly having been sloughed off in the specimen preparation process. While the cortex was damaged and/or separated in unaffected root sections, it was never entirely missing. This suggests that BGD affected roots are more fragile and possibly damaged. This is supported by the fact that sections were taken from areas on the roots which displayed the typical damaged/necrotic symptoms. The mesophyll cells of BGD affected roots appeared disrupted compared with unaffected roots. The reason for this is unknown.

There were no discernible differences between the phloem vessels of the BGD affected and unaffected leaves and roots. However, there were differences in the xylem vessels. The xylem vessels of the BGD affected leaves were often partially occluded by structures which could be identified as tyloses. Similar structures also occurred in the roots, but were stained a darker colour. It is possible that these dark structures consisted of

an accumulation of phenols or polyphenols. The rapid synthesis and local accumulation of phenols is generally associated with the plant's first line of defence against pathogen invasion (Matern and Kneusel, 1988). These compounds are implicated as having antimicrobial properties, are involved in cell wall reinforcement, seal off infection sites or injury, induce further plant responses (Clerivet *et al.*, 1996; Beckman, 2000), and are also associated with the formation of tyloses; that is, as tyloses form, there is an accumulation of these compounds in the surrounding cells (Del Rio *et al.*, 2001). Another possibility is that the roots were in the first stages of necrosis, in which case the cell content including the tyloses may have been partially decayed. This hypothesis may also explain the missing cortex of the roots and the disrupted mesophyll cells.

Though some roots were completely occluded, the tyloses of both the leaves and roots probably rarely occlude the majority of the vasculature as the symptoms of BGD do not include wilting (refer Chapter 2). However, these tyloses may be partially responsible for the sporadically seen uneven spread of red symptoms down a leaf. They may even be partially responsible for the stunting of the plants, as there would be reduced water and minerals travelling to the leaves.

In the mesophyll cells of the leaves, structures which could be described as intracellular inclusions were found. These assumed the characteristic metachromatic green colour of inclusion bodies stained with toluidine blue (Musetti *et al.*, 2000). Inclusion bodies are generally symptoms of pathogen infection, and generally consist of proteins (Narayanasamy, 2001). In relation to the presence of pathogens or pathogen involvement, there were no hyphae or bacterial colonies found in any of the root or leaf sections. There were no aecia, pycnia, or telia (as found with rust fungi), no pycnidia or acervuli (as in the

coelomycetes) or any other sporing structures, either in the sub-stomatal cavity or otherwise. In fact, there did not seem to be any direct involvement of pathogens.

Pathogens cause many different types of symptoms, though some are similar throughout the different groups. Bacteria generally live in intercellular spaces. They produce enzymes which may cause loss of turgor, cell collapse, and cell death. Bacterial ooze may also appear on the leaf surface and cavities may be formed in affected tissues (Narayanasamy, 2001).

Some bacteria and fungi invade the vasculature, where they are transported to organs. There they produce toxins, causing a breakdown of cells, the production of tyloses and an accumulation of cellular material. The clogging of the vasculature may produce stunting, wilting and death (Narayanasamy, 2001).

Fungal facultative parasites produce many kinds of biologically active substances (enzymes, toxins, growth regulators, polysaccharides, antibiotics) which may affect cells directly or cause structural changes (Narayanasamy, 2001). Pathogens that cause damping off and root rots produce macerating enzymes that produce extensive structural breakdown in affected tissue (Narayanasamy, 2001). Virus infection can cause starch accumulation, inhibition of plastid development and the destruction of chloroplasts (Narayanasamy, 2001).

Many of these symptoms were present, suggesting that the main causal agent of BGD is a pathogen. However, the physical presence of a pathogen was not seen. Several possibilities arise. First, the pathogen was present in the plant, but in low numbers or in different parts of the plant, so that they were not recovered as part of the sample for sectioning. Second, the main causal agent is viral, and would therefore not be visible under light microscopy. Future work with a scanning or transmission electron microscope may

reveal more detail in this matter, though this option was unavailable in this study. Third, the primary causal agent resides in the roots or simply parasitises the roots, producing a toxin which travels throughout the plant (using the vasculature) and produces tyloses, etc. This possibility may also explain the damaged nature of the roots. Last, the primary causal agent is made up of several different pathogens as well as other factors. Further investigation will be required to determine the answer.

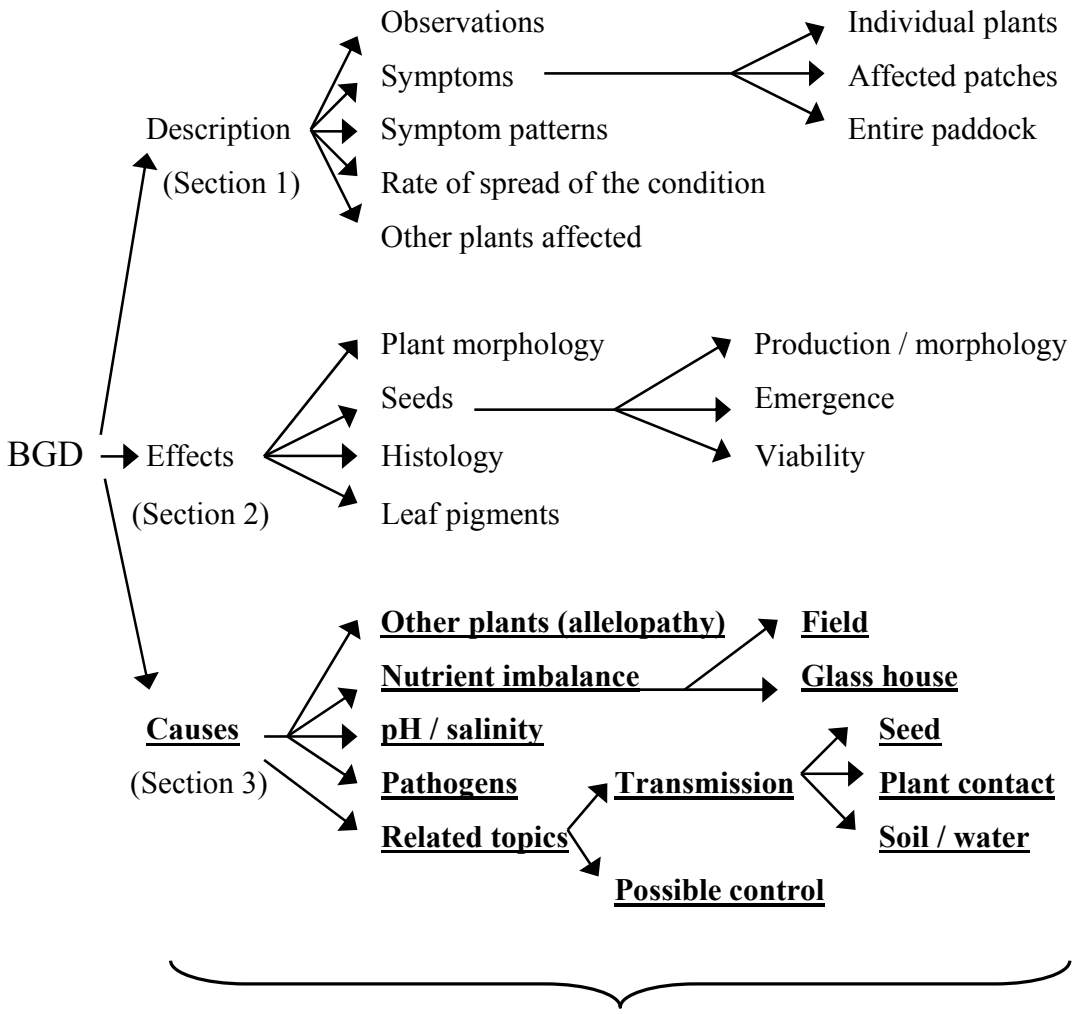
6.5 Concluding Statements

There was no discernible difference in the size of the cells of BGD affected plants compared with unaffected plants. Bulliform and mesophyll cells of BGD affected plants appeared damaged, especially in the roots. The xylem of BGD affected plants was often partially occluded by tyloses, and inclusion bodies were found in the mesophyll cells. There was also a possible accumulation of phenols in the roots.

The histology work suggests that there is pathogen involvement, though not directly within the plant, with the possible exception of viruses. The causal agent(s) seems to be soilborne, and possibly infects or parasitises the roots and produces a toxin which initiates the other symptoms.

Section 3

Causes of Buffel Grass Dieback



Chapter 7

Nutrient Deficiencies in Buffel Grass

7.1 Introduction

Early in this research (2001-2002), when little was known of the nature of BGD, it was decided to investigate potential chemical causes of the condition. Previous work and observations had suggested that BGD is seemingly primarily caused by a disease causing organism, and that some underlying environmental parameter was stressing the plants thereby making them more susceptible to disease. This environmental parameter could have included climate, water availability or a nutrient imbalance. It was improbable that climate was the cause, as BGD outbreaks have occurred in every season (refer Figure 4, Chapter 2, p. 43). Water stress could have been a contributor, but it was improbable that it was the main stress, as symptoms were merely delayed with lush growth, not abated (refer Chapter 2). It was therefore likely that the stress was a nutrient imbalance or some other factor.

Nutrient deficiencies and/or toxicities often result in visual, usually foliar symptoms that are unique in their appearance, relating to colour and/or morphology, the biochemical degradation of some plant part, or disease infestation (Salisbury and Ross, 1992; Grundon *et al.*, 1997; Pais and Benton Jones Jr., 1997). As an example of the latter, a deficiency of P, K and Mg will increase disease severity in take-all of wheat, caused by *Gaeumannomyces graminis* (Reis *et al.*, 1982). Therefore, in relation to BGD, it is possible that either a nutrient imbalance is one of the causes of the condition, or is weakening the

plants making them more susceptible to disease. While the results of Chapter 6 (leaf pigments) indicated that leaf damage was a more likely contributor to the red BGD symptoms than nutrient deficiency, a nutrient imbalance can also manifest itself as growth changes. These results could be compared with those of Chapter 4, and possibly indicate which nutrient imbalance(s) is/are the likely contributors. For these reasons, a study of nutrient imbalances in buffel grass was considered essential.

7.2 Materials and Methods

Methodology

While nutrient imbalances generally have characteristic growth and/or colour symptoms, these vary slightly between plant species (Salisbury and Ross, 1992). There has been some research on nutrient disorders of buffel grass (Smith, 1974), but this was only on the Gayndah cultivar. The differences, if any, between the American and Gayndah cultivars are unknown. Also, Smith's (1974) work did not include morphological data such as plant height or seed yield, which are important symptoms of BGD (refer Chapter 4). Smith's (1974) work was also done using young plants; the trial did not encompass plant maturity and seed production.

It is becoming more common to identify nutrient deficiencies and/or toxicities by plant chemical analyses. While this method is useful, it does not convey colour symptoms and morphological changes. Comparing the colour and morphological symptoms of various nutrient deficiencies to that of BGD affected plants may give clues as to which, if any, nutrient imbalance is contributing to the BGD condition.

A commonly used method of determining the colour and morphological symptoms of a nutrient imbalance is growing plants hydroponically, as was done with slender

wheatgrass (Schwartz and Safaya, 1978), green panic (Smith, 1972), and buffel grass (Smith, 1974; Christie, 1975). Unlike in a soil medium, where the nutrient concentration is variable, the various nutrient concentrations in a hydroponic solution can be accurately altered to suit the purpose. Therefore, both nutrient deficiency and toxicity trials can be done using hydroponics. While both of these can provide valuable information, the current study concentrated on nutrient deficiencies. There are two main reasons for this. Firstly, nutrient toxicities are generally not as commonly found in Australian soils as nutrient deficiencies, with the exception of sodium, chloride and in some areas boron (Naidu and Rengasamy, 1993). Secondly, previous surveys of the chemical composition of the soil at the Baralaba field site showed no toxicities. This applies to surveys done before (Webb *et al.*, 1982) and after (Graham and Conway, 2000) the emergence of BGD.

There are two main types of nutrient deficiency trials: nutrient reduction and nutrient omission. In nutrient reduction trials, the concentration of one or more nutrients is reduced, often in increments over several treatments (Salisbury and Ross, 1992). This is often used to determine the amount of a nutrient that a plant requires or the concentrations of that nutrient at which the plant exhibits various deficiency symptoms. In nutrient omission trials, one or more nutrients are excluded from the nutrient solution. This is often used to determine limiting nutrients, as with phosphorus in buffel grass (Christie, 1975), or to determine the effect of a nutrient on plant morphology and foliar symptoms, as was done by Schwartz and Safaya (1978) with slender wheatgrass. Since the aim of this study was to determine the foliar and morphological symptoms of nutrient deficiency, it was considered that an omission trial was more appropriate.

Several methods are available for hydroponic solutions and omission trials. The method chosen for this study was from Roberts and Whitehouse (1976). This method

originated at the Long Ashton Research Station, UK, and has been widely used in Australia on crops and pasture grasses (Wallace, 1947; 1961). The method was altered in the current study to include omission trials for additional nutrients (refer Table 17 in Methods for complete list), so that the experiment included all nutrients which are generally accepted as being the essential elements (Salisbury and Ross, 1992).

The method used in this experiment was a modified version of traditional hydroponic culture, in that the plants were in pots containing an inert medium, which were then suspended in the solution. The medium used, vermiculite, is inert, has excellent water holding capabilities, and provides aeration (Martinez and Barbosa, 1999), negating the need for an aerator in the solution. Unlike other plants commonly used in hydroponics, buffel grass forms 'clumps', that is, one plant forms many tillers, generally from the base. As such, wider than usual pots were used to accommodate the plants and not hinder growth. Since one of the aims of this study was to observe morphological changes, the plants were grown to maturity. Therefore, the nutrient solutions were regularly replaced to prevent any secondary nutrient deficiencies. The pH of the solutions was maintained at 6.5, as this is the level at which the majority of elements are plant-available (Salisbury and Ross, 1992).

This experiment was done as a large exploratory pilot study, and as such was not replicated. This was mostly due to the lack of available space and resources. Therefore, it was decided to set up a large pilot study with all nutrients rather than concentrate on a few. In this manner, the results would give an indication of the nutrients involved, which could then be expanded upon in future replicated trials. Results were, therefore, treated as indicative rather than definitive. Given that the aim of this study was to compare the observed symptoms with that of BGD affected plants, the same measurements were taken as in Chapter 4.

Method

Hydroponic solutions were made according to Table 7 in Roberts and Whitehouse (1976), with the following modifications:

1. Six different micronutrient stock solutions were made. One was made according to Roberts and Whitehouse (1976), the other five each had one component omitted, to be used in deficiency treatments for Mn, Zn, Cu, Mo, and B.
2. To create a chloride deficient treatment, a seventh micronutrient stock solution was made, substituting chloride salts with sulfate salts.

In all modified treatment solutions care was taken to ensure that the concentrations of the various nutrients (except the one or more omitted) were the same as in the original unmodified treatment solutions.

The treatments were as follows:

Treatment	Code
complete nutrients	complete
no phosphorus	-P
no potassium	-K
no calcium	-Ca
no nitrogen	-N
no magnesium	-Mg
no sulfur	-S
no iron	-Fe
no manganese	-Mn
no copper	-Cu
no zinc	-Zn
no boron	-B
no molybdenum	-Mo
no chlorine	-Cl
no nitrogen, phosphorus, potassium	-NPK
no Ca, Mg, S, Fe, Mn, Cu, Zn, B, Mo, Cl (micronutrients)	-micro

Table 17 - Treatment descriptions and codes for a nutrient omission trial on buffel grass (*Cenchrus ciliaris*)

While it is recognised that not all nutrients listed in the micronutrients treatment are, in fact, micronutrients, for the purpose of the current study that group of plant nutrients will be referred to as 'micronutrients'.

Sixteen previously unused styrofoam boxes (39 x 58 x 25 cm deep) were lined with black plastic bags, and eight holes (7 cm diameter) were cut into each lid. The boxes were placed in a glass house in a 4 x 4 block, with enough space between boxes to allow access and to minimise shading effects. Treatment solutions prepared with reverse osmosis (RO) water were poured into each box in the amounts prescribed by Roberts and Whitehouse (1976), and were made up to 40 L with RO water. This resulted in 16 boxes, each with a different treatment. pH was adjusted to 6.5, making sure the acids and bases used for each particular treatment did not contain the element(s) that were omitted in that treatment. An electrical conductivity (EC) reading was also taken for each treatment at the start of the trial and on a weekly basis, using a combination pH/EC meter.

One hundred and twenty-eight previously unused pots (7 cm diameter x 12 cm deep), each with a mesh lining, were filled with vermiculite and placed in the holes in the lids of the styrofoam boxes. Commercial buffel grass seeds were obtained from Queensland Independent Seeds in Rockhampton. These were the seeds also used in the study in Chapter 4. Four commercial seeds were placed into each pot, and observed for emergence. Only one seedling was kept per pot if more than one emerged. Once emerged, black plastic granules were placed in a 0.5 cm thick layer around the seedling on top of the vermiculite to minimise evaporation and algal growth (Figure 41). The boxes with solutions and plants are hitherto referred to as treatment units.

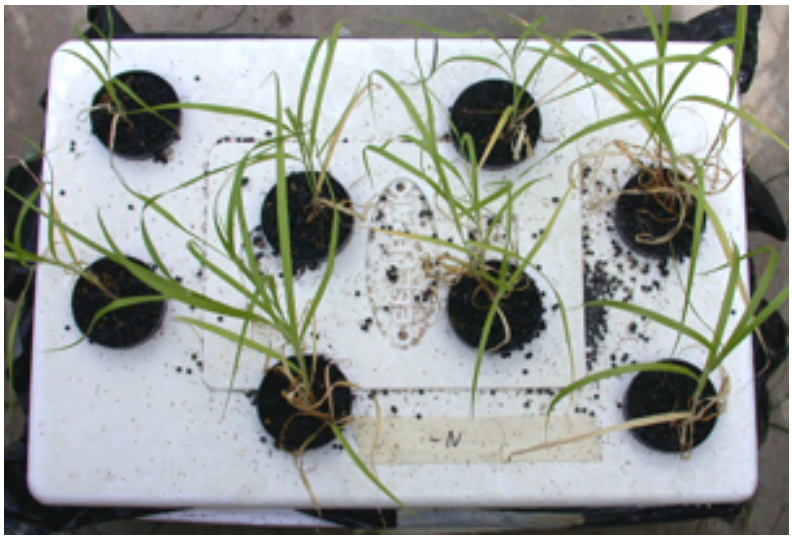


Figure 41 - Experimental set up of a treatment unit in a hydroponics trial of buffel grass (*Cenchrus ciliaris*)

The experiment was run for 13 weeks from emergence. Each week, pH was measured and adjusted to 6.5 if necessary. EC was also measured weekly as an approximate indication of the ratio of nutrients to water being used. Based on the EC reading, water and/or nutrients were added up to the 40 L capacity. The solutions were replaced every two weeks.

The following data were measured on a weekly basis:

- Plant height, from the junction of the tillers and roots to the top of the plant in its natural habit
- Longest leaf, from the ligule to the tip
- Longest internode, from node to node
- Number of primary and secondary tillers
- Number of non-senescent leaves.

For a diagrammatical description of these measurements, refer to Figure 23, Chapter 4, p.90.

Measurements were collected within 3 hours. All of these were measured for each individual plant. Seed heads were collected when mature. Also throughout the experiment, foliar colour symptoms were recorded and photographed.

In the fourteenth week, plants were individually harvested by being cut at the junction of the tillers and roots, and the shoot fresh weight was immediately measured. The roots had grown together as one mass, so these were weighed as one mass and the measurement divided by the eight plants. The harvest and fresh weight measurements were done in the morning to minimise weight loss through transpiration. Shoots and roots were then placed in a drying oven for 3 days at 70°C and dry weight was measured at the end of this period.

Once dry weight was measured, foliar samples for each treatment and nutrient solution samples for each treatment were sent to the CSBP (Cumming Smith British Petroleum) laboratory (Bibra Lake, Western Australia) for analysis, to verify that the plants were nutrient deficient. The foliar samples consisted of leaves of the same developmental stage.

For each treatment which produced seed, the total number of seed heads produced per plant was counted. Ten seed heads from each treatment were selected at random and the length of each seed head was measured. The number of seeds on each of these seed heads was also counted. Twenty seeds were selected at random from each treatment, and the length of the seed fascicle was measured to the nearest 0.5 mm. For a diagrammatical description of these measurements, refer to Figure 13, Chapter 4. Fifty seeds from each treatment, also selected at random, were planted out individually in pots in a completely random design. The pots contained a 70:30 mixture of ash and potting mix (as done in Chapter 4). These were kept moist and observed for emergence over ten weeks. The data

were modelled by a generalised linear model (GLM) assuming a binomial error and a logit link function. The model simply included the effect of nutrient treatment. There was slight evidence of underdispersion (mean deviance 0.72) and hence a quasi-likelihood was fitted in which the dispersion parameter was estimated. The procedure RPAIR was used to assess pairwise differences. Another fifty seeds from each treatment, again selected at random, were dissected and the number of caryopses per seed counted. Due to the categorical nature of the data, a contingency table and chi-square analysis was done. The Pearson chi-square statistic was used. Unfortunately there were not enough seeds produced to perform seed viability tests as in Chapter 4.

7.3 Results

7.3.1 Foliar Colour Symptoms

The following table gives descriptions, images and RHSPPC codes (The Royal Horticultural Society, 2001) for the various treatments.

Boron deficient plants exhibited white interveinal areas. These were also apparent in the chlorine and magnesium deficient plants, though these also exhibited red leaf margins and tip necrosis. The manganese, zinc, sulfur, nitrogen, phosphorus and NPK deficient plants all displayed red foliar symptoms, tip necrosis and small brown necrotic areas. These brown necrotic areas were the prevalent symptom of the potassium deficient plants. The molybdenum and copper deficient plants predominantly displayed chlorotic leaf tips. The iron deficient plants exhibited marked interveinal chlorosis. The leaves of calcium deficient plants had numerous transverse breaks. The micronutrient deficient plants all exhibited all of the above symptoms.

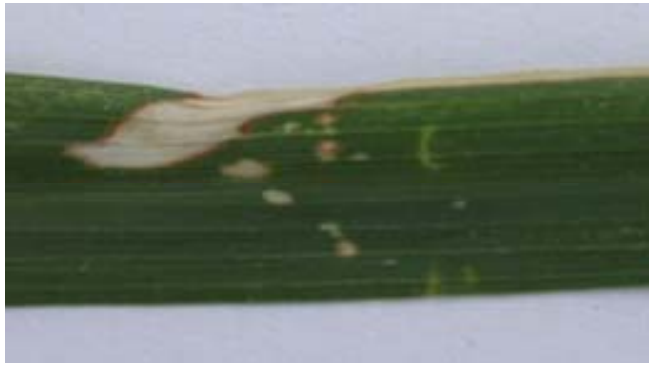
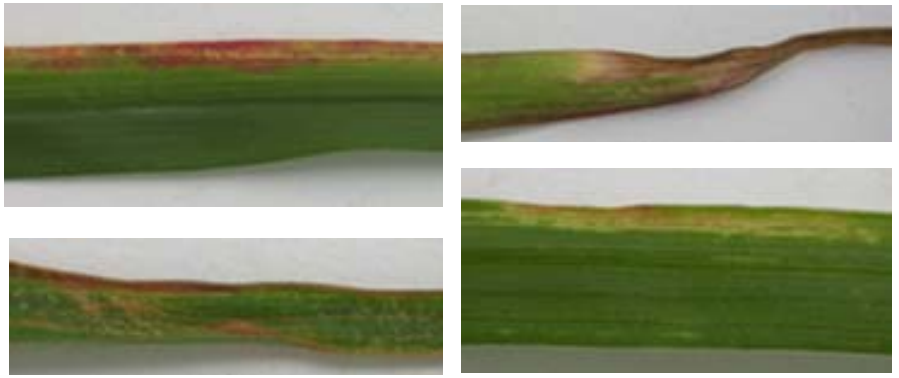
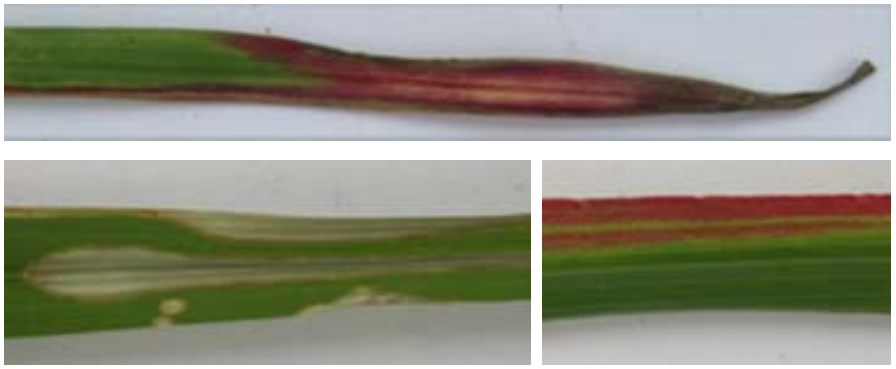
Nutrient omitted	Description	RHS colour chart	Images
none (complete nutrients)	Leaves green No blemishes or discolouration	Green group: 137 A, B, C 138 A 143 A, B	 
Boron	Leaves dark green Emerging leaves failed to unroll Tip necrosis White areas (5 - 25 mm) developed in the interveinal areas, then spread	Green group: 143 A, C 144 A Greyed-white group: 156 B	 

Table 18 - Foliar symptoms of nutrient deficiency in buffel grass (*Cenchrus ciliaris*)

Nutrient omitted	Description	RHS colour chart	Images
Chlorine	Withered, necrotic tips, turning bronze Occasional red leaf margins Some white areas as in -boron	Green group: 143 A, C 144 A, B Red group: 46 B Greyed-orange group: 165 B Greyed-white group: 156 B	
Manganese	Leaf tips tending to curl Brown or red margins Leaves drooping Some chlorotic areas on leaf margin Necrotic tips and some small necrotic areas 0.5 - 3 mm diameter	Green group: 143 A, B Yellow-green group: 145 B Red group: 46 A, B Greyed-orange group: 165 B	

(Table 18 continued)

Nutrient omitted	Description	RHS colour chart	Images
Potassium	Tips of older leaves chlorotic Chlorotic areas progressed from tip to culm Grey/brown necrotic areas 0.5 - 2 mm diameter	Green group: 137 A, B, C 143 A, B Yellow-green group: 151 C, D Red group: 46 A, B	
Magnesium	Red tips on older leaves, not progressing towards culm Red margins on some leaves Tip necrosis White areas progressing longitudinally	Green group: 143 A, B 144 A, B Red group: 46 A, B Greyed-white group: 156 C	

(Table 18 continued)

Nutrient omitted	Description	RHS colour chart	Images
Iron	Marked interveinal chlorosis, starting on youngest leaves In advanced stages, entire leaf chlorotic	Green group: 144 A, B 137 A Yellow-green group: 154 C, D	
Molybdenum	Chlorotic leaf tips Some red tips Above tips became necrotic Leaf tips tending to curl Some red leaf margins	Green group: 143 A, B 144 A Red group: 46 B Yellow-green group: 150 C Greyed-orange group: 165 B	

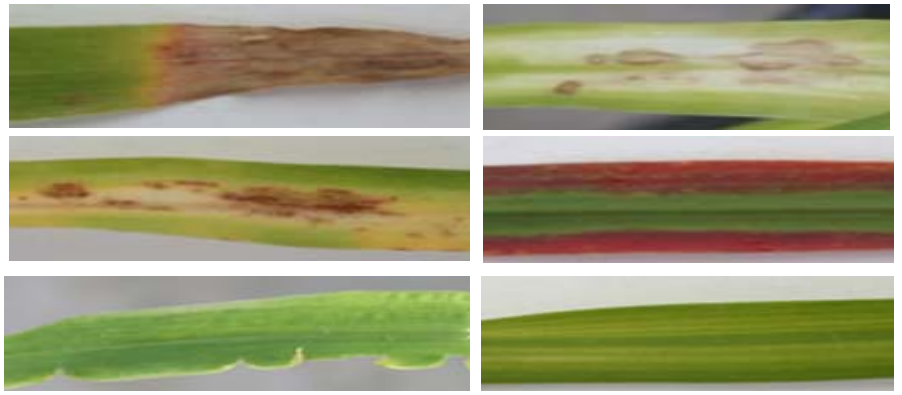
(Table 18 continued)

Nutrient omitted	Description	RHS colour chart	Images
Copper	Withered necrotic tips Tips tending to curl or droop	Green group: 137 A, B 143 A, B, C Greyed-orange group: 165 B 164 A	 
Zinc	Red margin along leaf blade Brown necrotic areas, 0.5 - 2 mm long, developed in interveinal areas, then coalesced into large areas Some tip necrosis	Green group: 143 A, B, C 144 A Red group: 46 A, B Greyed-white group: 156 B Greyed-orange group: 165 B	  

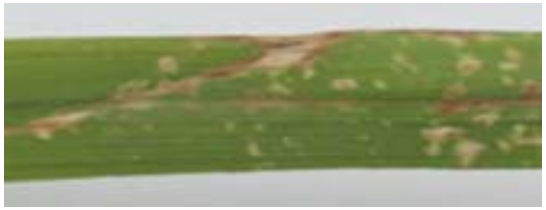
(Table 18 continued)

Nutrient omitted	Description	RHS colour chart	Images
Sulfur	Leaves pale green Leaves brittle Tips tending to curl Some red/brown margins Chlorosis of older leaves Some red leaf tips	Green group: 143 A, B 137 D Red group: 46 A, B Yellow-green group: 144 C	
Calcium	Failure of emerging leaves to fully unroll resulting in tip necrosis Leaves had numerous transverse breaks Leaves and stems brittle Stems bent at internodes	Green group: 137 A, B 143 A, B, C 144 B	

(Table 18 continued)

Nutrient omitted	Description	RHS colour chart	Images
Micronutrients (as listed in Table 17)	A mixture of other symptoms Red tips and/or margins, necrotic areas 0.5 - 2 mm diameter, white areas, interveinal chlorosis, necrotic tips	Green group: 143 B, C; 144 A Red group: 46 B Yellow-green group: 154 C, D Greyed-white group: 156 B Greyed-orange group: 165 B	
Nitrogen	Leaves pale green Thin red margins on older leaves Brown necrotic areas 0.5 - 1.5 mm long in interveinal areas Leaves turning red in a diffuse pattern	Green group: 144 B, C Greyed-orange group: 164 B Greyed-red group: 179 B Red group: 46 B	

(Table 18 continued)

Nutrient omitted	Description	RHS colour chart	Images
Nitrogen, Phosphorus, Potassium	Some red leaf tips Chlorotic margins and tips Grey/brown necrotic areas 0.5 - 2 mm diameter	Green group: 144 A, B, C Yellow-green group: 150 C Red group: 46 A, B Greyed-orange group: 165 B	  
Phosphorus	Leaves tending to curl Red leaf margins Some red tips Tip necrosis	Green group: 144 A, B Red group: 46 B Greyed-orange group: 165 B	  

(Table 18 continued)

7.3.2 Plant Biomass and Morphology

For consistency, the following data are presented with the treatments listed in the same order as with shoot dry weight, except that the complete nutrient treatment is always listed first for comparison. The exception to this is the seed emergence data. Standard error bars are shown in graphs where an estimate of variation around a mean was possible.

Plant Biomass

Compared with the complete nutrient plants, average shoot dry weight was similar in the boron and chlorine deficient plants. It was lower in the manganese, potassium, magnesium, iron, molybdenum and copper deficient plants, which were all similar. It was even lower in the zinc, sulfur, calcium and micronutrient deficient plants, which were also similar to each other. It was lowest in the nitrogen (0.55 g), NPK (0.15 g) and phosphorus (0.04 g) deficient plants (Figure 42).

Average shoot fresh weight followed a similar pattern, with several differences. Compared with the complete nutrient plants, fresh weight was similar in the boron, chlorine and manganese deficient plants. It was lower in the magnesium, iron, molybdenum and copper deficient plants, which were all similar. It was lower still in the potassium deficient plants, which for fresh weight did not appear to be similar to any other treatment. The zinc and sulfur deficient plants had lower fresh weight, though similar to each other, as did the calcium and micronutrient deficient plants, which were lower again. The lowest average plant fresh weight was in the nitrogen (1.27 g), NPK (0.56 g) and phosphorus (0.10 g) deficiency treatments (Figure 42).

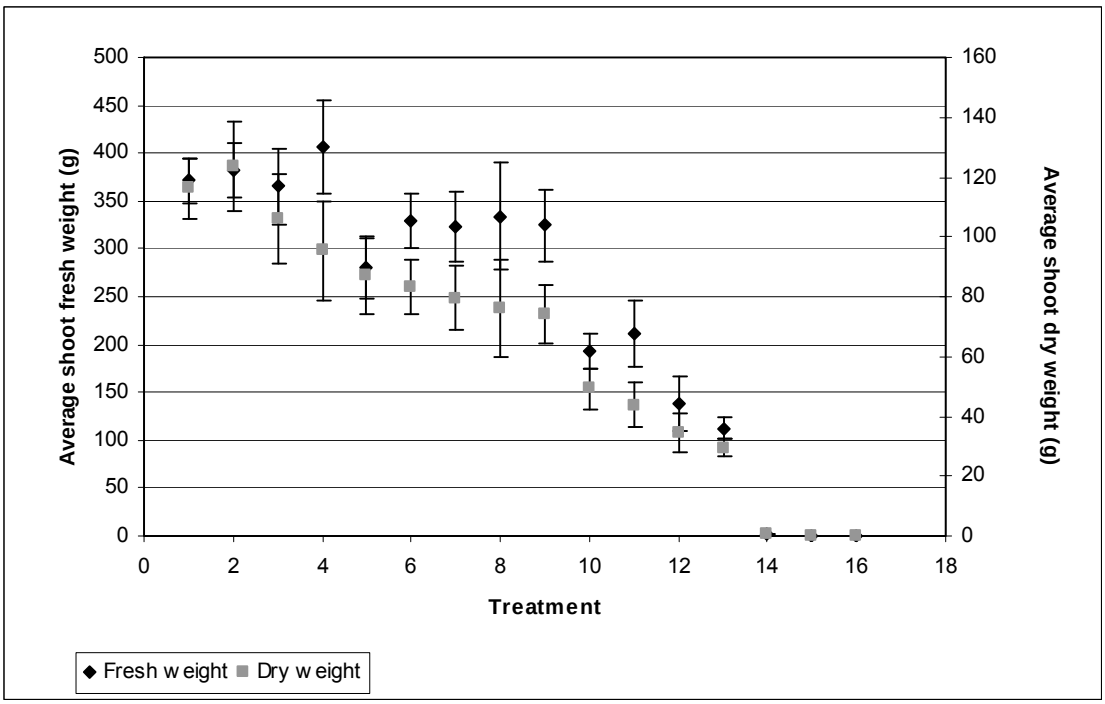


Figure 42 - Average shoot fresh weight and dry weight of buffel grass (*Cenchrus ciliaris*) with various nutrient deficiencies (± SE)

Roots had grown together as one mass in each polystyrene box and could not be separated on a per plant basis. Therefore, average root weight per plant was calculated by dividing the total root weight by eight (the number of plants). Consequently standard errors could not be calculated, and estimates of sampling error were not possible.

Average root dry weight was highest in the complete nutrient plants, followed by the boron, iron, chlorine and potassium deficient plants respectively. The manganese, magnesium, molybdenum and copper deficient plants had approximately half the average root dry weight of the complete nutrient plants. Lower still were the average root dry weights of the zinc, sulfur, calcium and micronutrient deficient treatments. The lowest average root dry weights were that of the nitrogen (0.54 g), NPK (0.14 g) and phosphorus (0.06 g) deficient plants (Figure 43).

Average root fresh weight followed a similar pattern to that of root dry weight, with the exception that, compared with the complete nutrient plants, the fresh weight of the other treatments were proportionately higher (Figure 43).

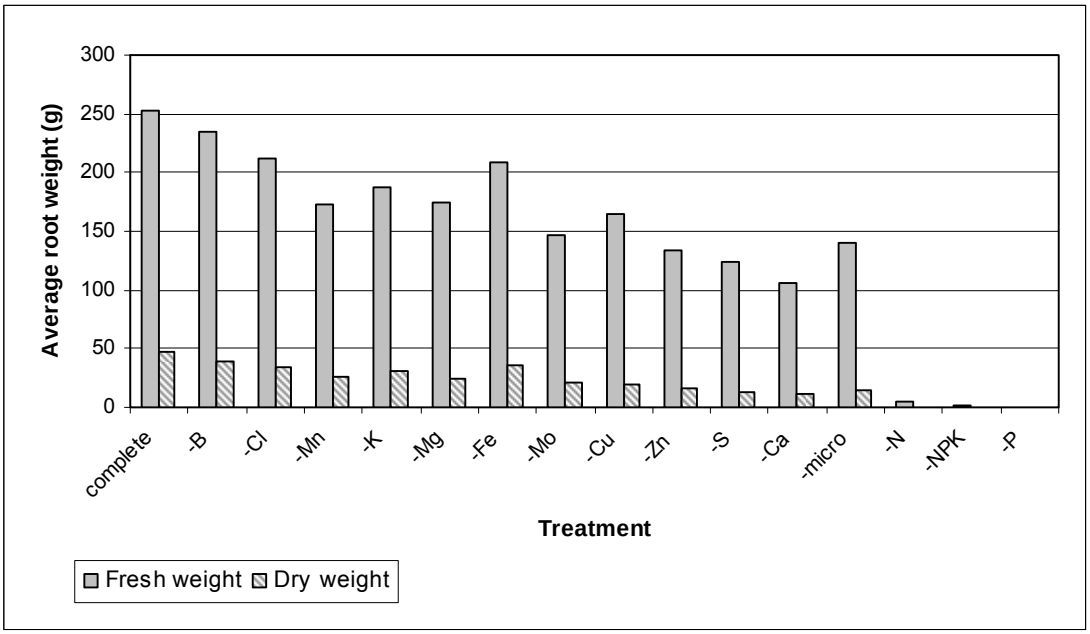


Figure 43 - Average root fresh weight and dry weight of buffel grass (*Cenchrus ciliaris*) with various nutrient deficiencies

Percent shoot dry matter was markedly higher in the nitrogen deficient plants, followed by the phosphorus deficient plants. The complete nutrient plants and the boron and potassium deficient plants followed, and themselves were followed by the chlorine, micronutrients and NPK deficient plants. The magnesium, zinc and calcium deficient plants had the next lowest % shoot dry matter, followed by the iron deficient plants. The % shoot dry matter of the manganese, molybdenum and copper deficient plants were slightly higher than that of the sulfur deficient plants, which had the lowest % shoot dry matter (Figure 44).

Percent root dry matter was highest in the complete nutrient plants. This was followed by the iron, potassium, boron and chlorine deficient plants respectively. The next

lowest were the manganese, molybdenum and magnesium deficient plants. These were followed by the copper, zinc and phosphorus deficient plants, which were similar to each other. The calcium deficient plants had the next lowest % root dry matter, followed by the sulfur, micronutrients and nitrogen deficient plants, which were also all similar. The lowest % root dry matter at 6% was that of the NPK deficient plants (Figure 44).

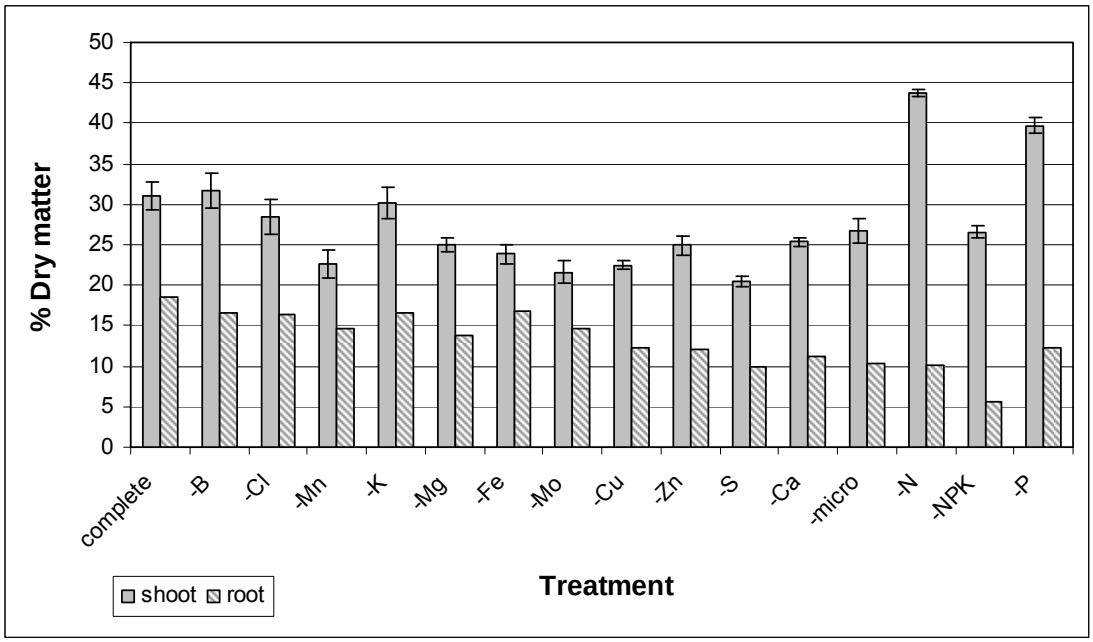


Figure 44 - Average percent dry matter of buffel grass (*Cenchrus ciliaris*) grown with various nutrient deficiencies (± SE)

Continuous Growth Measurements

Some of the following attributes have results presented as two graphs for ease of observation. In these attributes, several treatments followed a similar trend. Therefore, in the first graph, the results of these 'similar' treatments are shown along with an additional line which is an average of these treatments. The second graph shows the aforementioned additional line and those treatments which showed marked differences. In the interests of consistency, the additional line was formulated using the same treatments for each attribute.

In most of the treatments, plants increased in height until 9 weeks after emergence, and then slightly decreased in height. This was due to the topmost leaves further elongating and bending. Compared with the complete nutrient plants, most of the treatments did not differ in average plant height (Figure 45). The molybdenum deficient plants were slightly lower. The micronutrient deficient plants grew more slowly but eventually reached a similar height to that of the complete nutrient plants (Figure 46). The calcium deficient plants were similar in height to the complete nutrient plants until 8 weeks after emergence, when they started decreasing in height. This was due to the transverse leaf breaks (Table 18), which increased in severity resulting in sections of leaves falling off. The nitrogen deficient plants increased in height until 5 weeks after emergence, then maintained the same height. The NPK and phosphorus deficient treatments hardly increased in height (Figure 46).

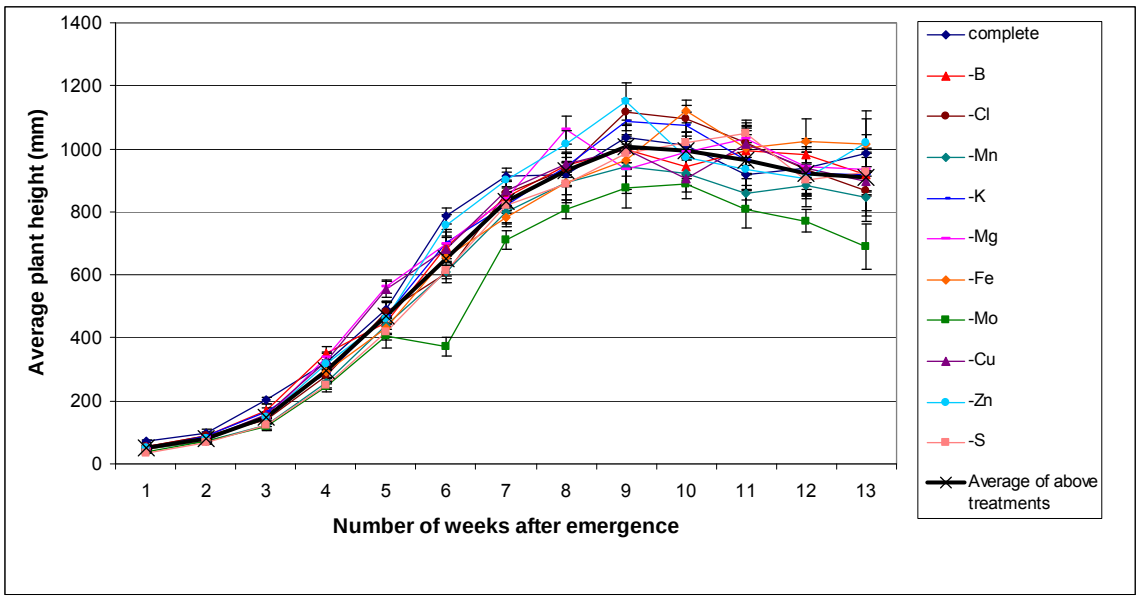


Figure 45 - Average plant height of buffel grass (*Cenchrus ciliaris*) grown with various nutrient deficiencies – similar treatments (± SE)

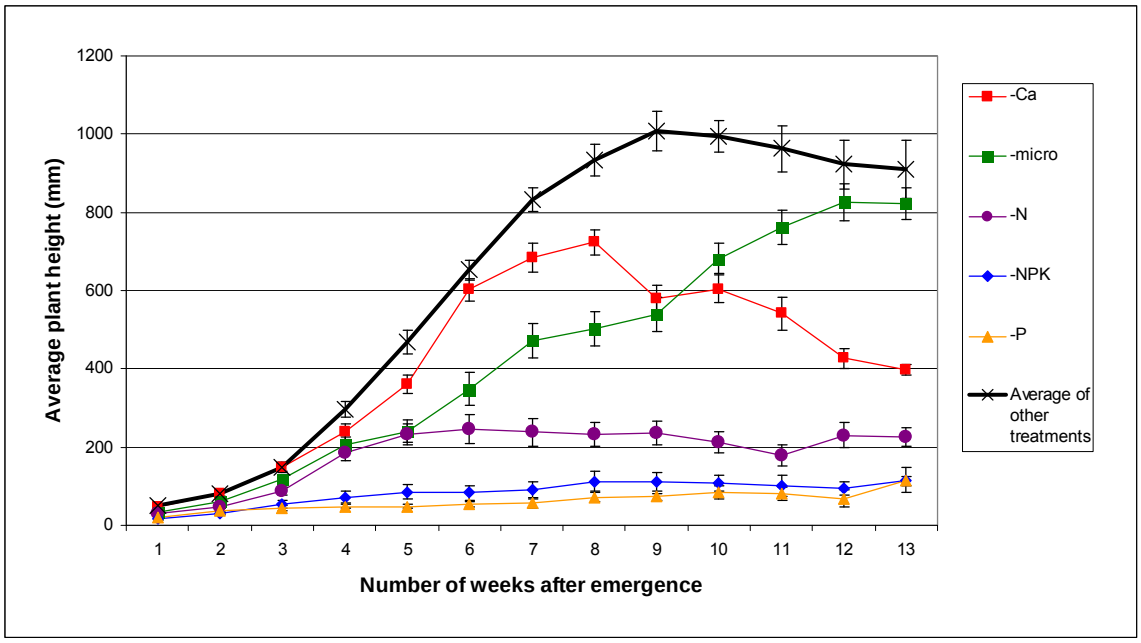


Figure 46 - Average plant height of buffel grass (*Cenchrus ciliaris*) grown with various nutrient deficiencies – dissimilar treatments (± SE)

In most of the treatments, leaves reached their maximum length 9 weeks after emergence (Figure 47). The average lengths of the longest leaves then slightly decreased. The plants in two thirds of the treatments reached similar longest leaf lengths to that of the complete nutrient plants (Figure 47). The calcium deficient plants had shorter leaves, mostly due to the transverse leaf breaks which caused sections of the leaves to fall off (Figure 48). The micronutrient deficient plants had similar leaf lengths to the calcium deficient plants, except for a period between 7 to 9 weeks, where several older leaves had died and new leaves were emerging. The nitrogen deficient plants showed leaf elongation until 4 weeks after emergence and then had no further increases in leaf length. The NPK and phosphorus deficient plants had the lowest average longest leaf length, which slowly increased over the 13 week duration (Figure 48).

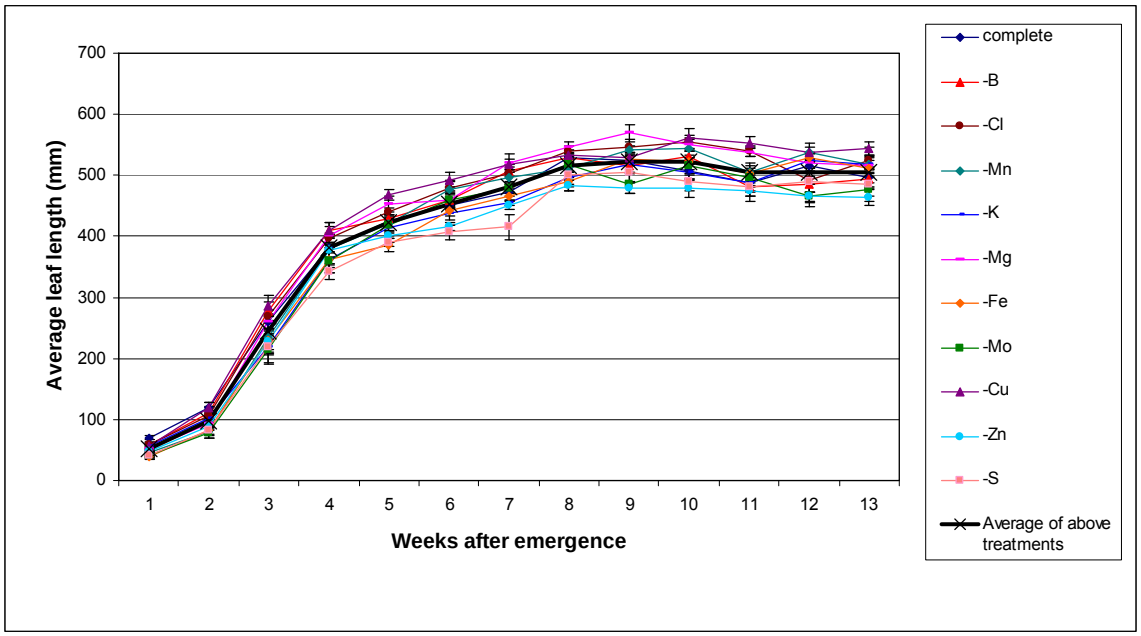


Figure 47 - Average length of longest leaf of buffel grass (*Cenchrus ciliaris*) grown with various nutrient deficiencies – similar treatments (± SE)

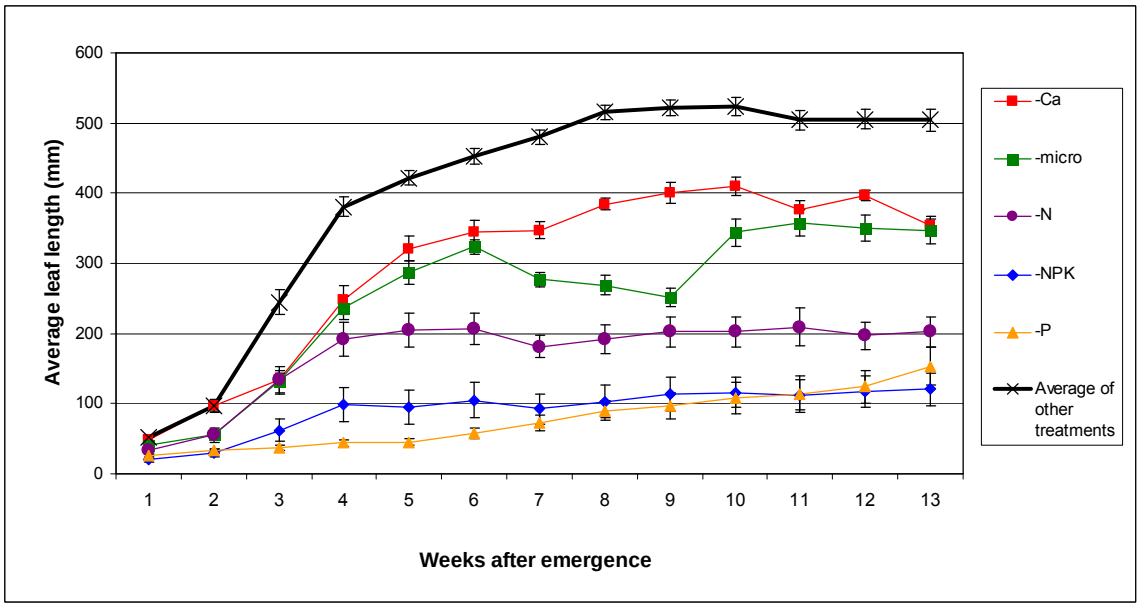


Figure 48 - Average length of longest leaf of buffel grass (*Cenchrus ciliaris*) grown with various nutrient deficiencies – dissimilar treatments (± SE)

In most of the treatments, the longest internode elongated until 6 weeks after emergence, after which there was no further elongation for 2 weeks (Figure 49). There was a further rapid increase in internode length after 8 weeks, with the rate of increase

subsequently slowing. The calcium deficient plants followed the same pattern, but had shorter internodes (Figure 50). The micronutrient deficient plants also followed a similar pattern, but had even shorter internodes. The nitrogen, NPK and phosphorus deficient treatments also increased in internode length until 6 weeks after emergence, but to a lesser extent compared with the other treatments. They all had a decrease in average longest internode length between 6 to 8 weeks after emergence, and an increase after or at 8 weeks. After 8 weeks from emergence, the nitrogen and NPK deficient plants had no further increases, whereas the phosphorus deficient plants had a slight increase in average longest internode length (Figure 50).

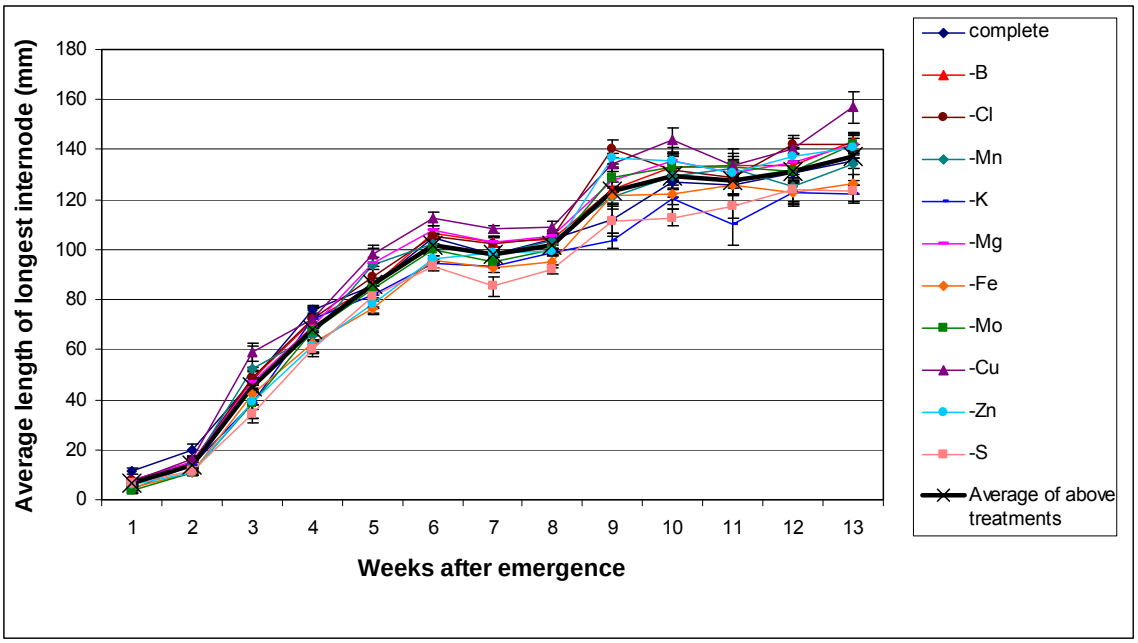


Figure 49 - Average length of longest internode of buffel grass (*Cenchrus ciliaris*) grown with various nutrient deficiencies – similar treatments (± SE)

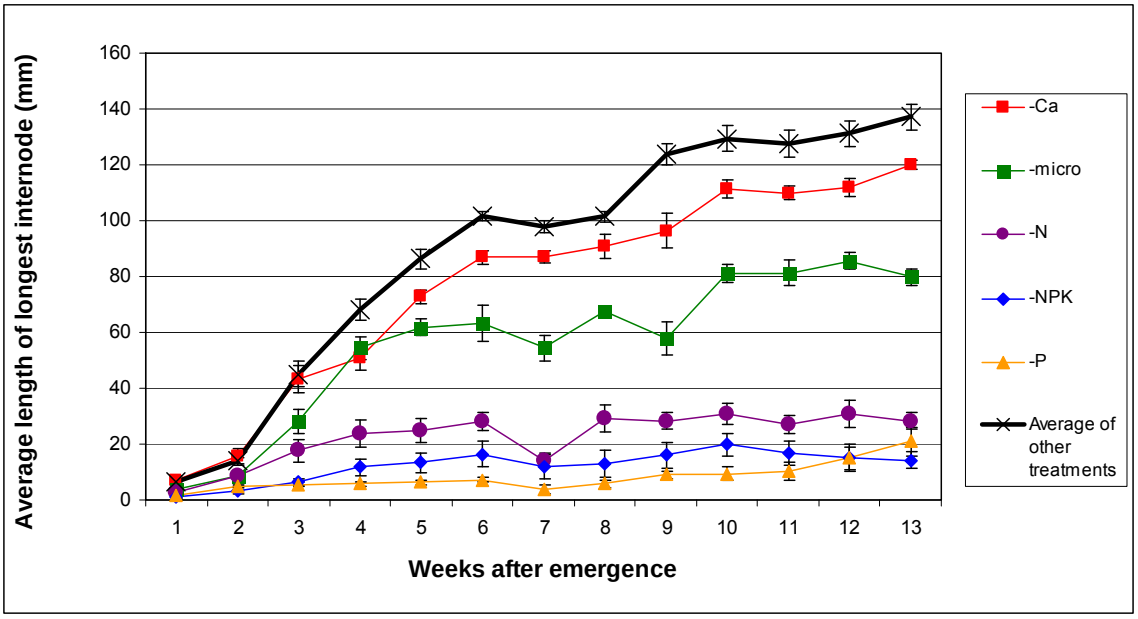


Figure 50 - Average length of longest internode of buffel grass (*Cenchrus ciliaris*) grown with various nutrient deficiencies – dissimilar treatments (± SE)

In most treatments, there was an increase in the number of non-senescent leaves per tiller until 6 weeks after emergence. The exception was the complete nutrient plants, in which the number of leaves per tiller decreased at 2 weeks after emergence, and then followed the same pattern as the other treatments (Figure 51). There was no increase in the number of non-senescent leaves per tiller between 6 to 7 weeks after emergence. From 7 weeks after emergence, there was a decrease in the number of non-senescent leaves per tiller (Figure 51), possibly due to the increase in the number of tillers (refer next part, Figure 53). The micronutrient deficient plants followed the same pattern as the majority of treatments, with the exception that the decrease in the number of leaves per tiller occurred from 8 rather than 7 weeks after emergence (Figure 52). The calcium deficient plants followed a similar pattern to the micronutrient deficient plants, but they had a lower overall number of leaves per tiller. The nitrogen and NPK deficient plants showed an increase in the number of leaves per tiller until 5 weeks after emergence, and then a decrease in

number and remained steady between 6 and 8 weeks after emergence. Both had a sudden increase in the number of leaves per tiller 9 weeks after emergence, which corresponded with an increase in the number of leaves (refer Figure 54). Both then decreased and numbers remained steady due to the death of leaves. The phosphorus deficient plants followed a similar pattern to that of the nitrogen and NPK deficient plants, but had fewer overall leaves per tiller and no increase 9 weeks after emergence (Figure 52).

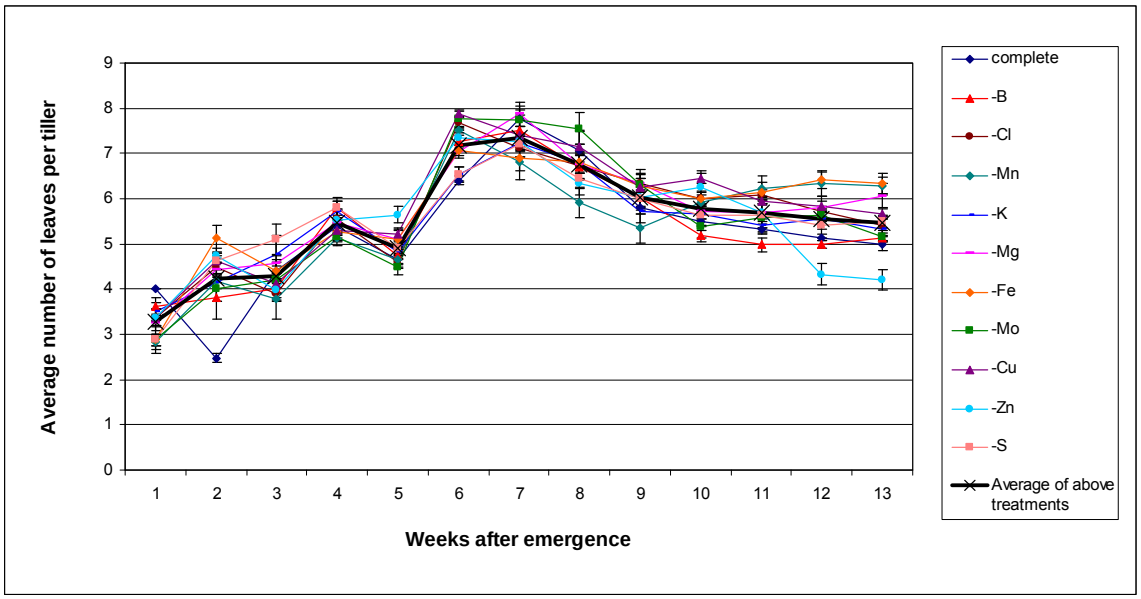


Figure 51 - Average number of non-senescent leaves per tiller of buffel grass (*Cenchrus ciliaris*) grown with various nutrient deficiencies – similar treatments (± SE)

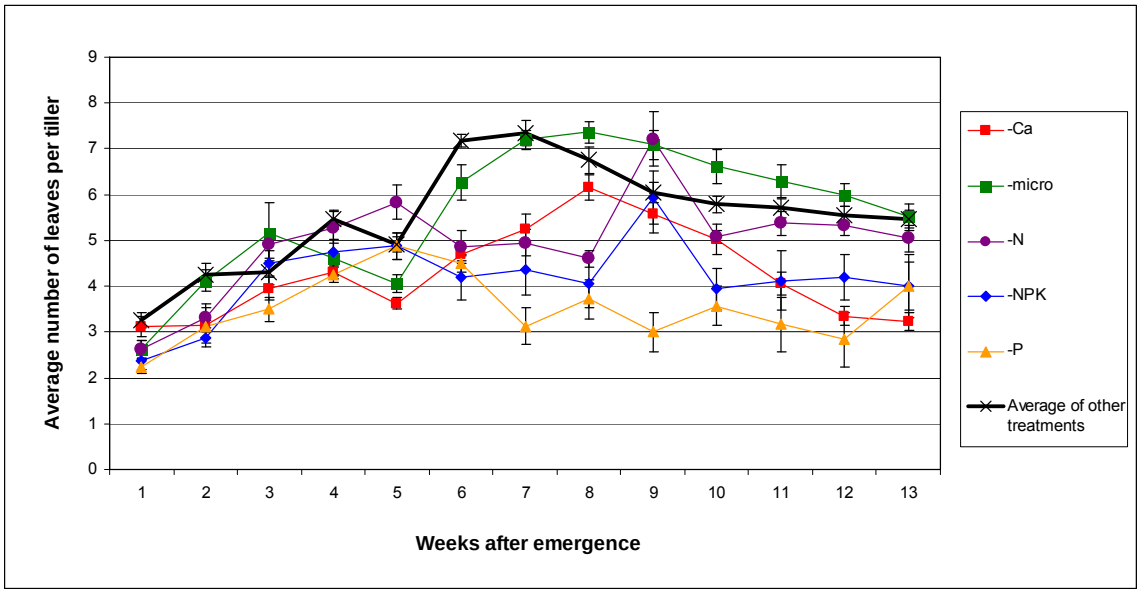


Figure 52 - Average number of non-senescent leaves per tiller of buffel grass (*Cenchrus ciliaris*) grown with various nutrient deficiencies – dissimilar treatments (± SE)

For the number of tillers and leaves per plant there were few similarities between treatments, so each parameter is shown on one graph.

Most of the treatments had a general increase in the number of tillers over time (Figure 53), though the different treatments produced tillers at different rates. The nitrogen, NPK and phosphorus deficient plants had very little increase in the number of tillers, with numbers remaining steady for the entire duration of the experiment (1-3 tillers).

Similarly, most treatments had a general increase in the number of leaves (Figure 54), again producing leaves at different rates. The nitrogen, NPK and phosphorus deficient plants showed very little change in the number of leaves throughout the experiment, although the nitrogen and NPK deficient plants had an increase in the number of leaves at 9 weeks after emergence (not obvious on the graph). This was an increase from 8 to 13 leaves in the nitrogen deficient plants and an increase from 4 to 6 leaves in the NPK deficient plants (Figure 54).

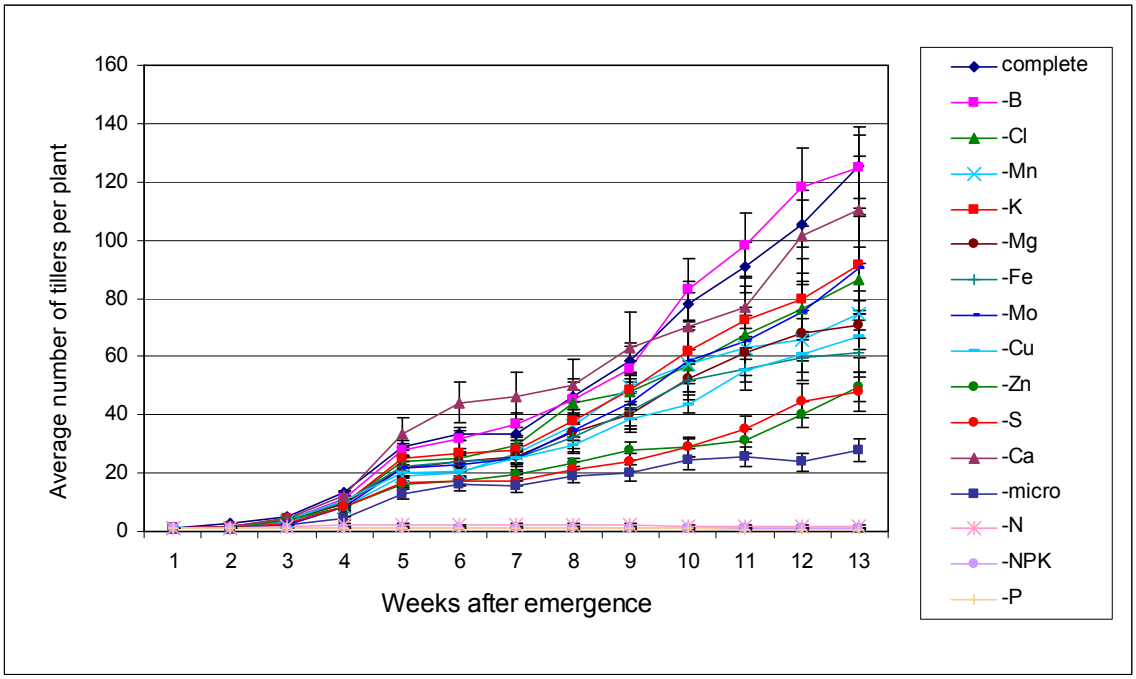


Figure 53 - Average number of tillers per plant of buffel grass (*Cenchrus ciliaris*) grown with various nutrient deficiencies (± SE)

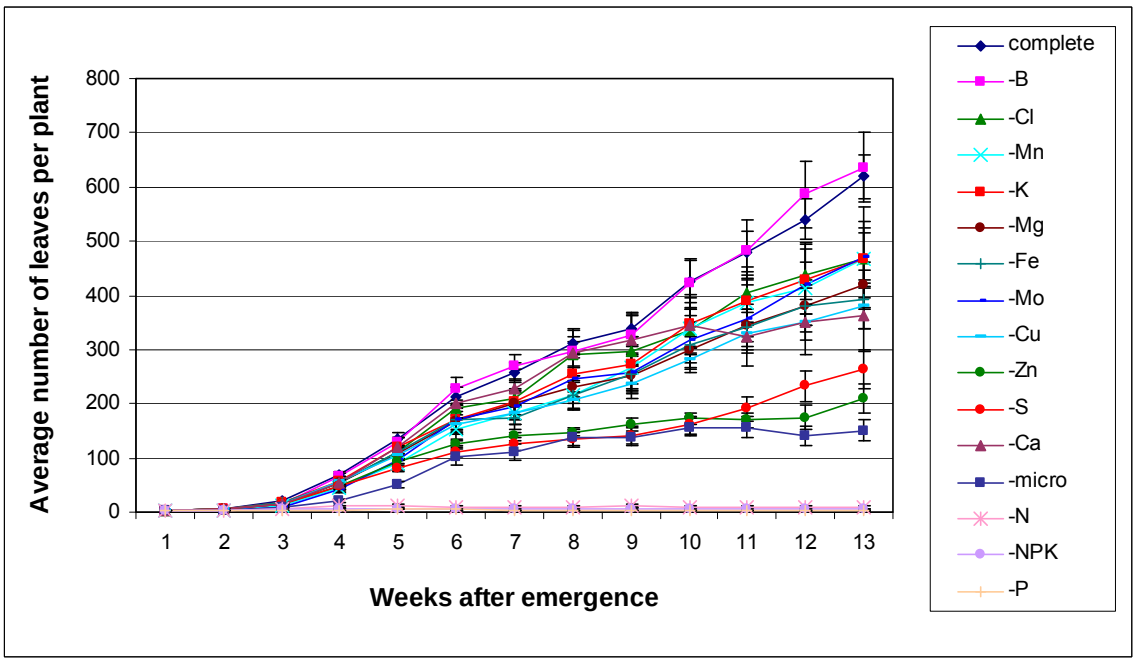


Figure 54 - Average number of non-senescent leaves per plant of buffel grass (*Cenchrus ciliaris*) grown with various nutrient deficiencies (± SE)

Seed Production, Morphology, and Emergence

Three of the treatments, no phosphorus, no nitrogen, and no NPK, did not produce seed, and are therefore not included in the data presentation.

Plants which were boron deficient produced, on average, the same number of seed heads as plants with complete nutrient (Figure 55). The potassium deficient plants produced the next highest number of seed heads, followed by the chlorine deficient plants and the micronutrient deficient plants respectively. The next lowest producers of seed heads were the magnesium, molybdenum and zinc deficient plants, which were all similar to each other. These were followed by the sulfur deficient plants. The manganese, iron and copper deficient plants produced slightly fewer seed heads than the sulfur deficient plants, and were all similar to each other. The calcium deficient plants produced the least number of seed heads amongst those that did produce seed heads (Figure 55).

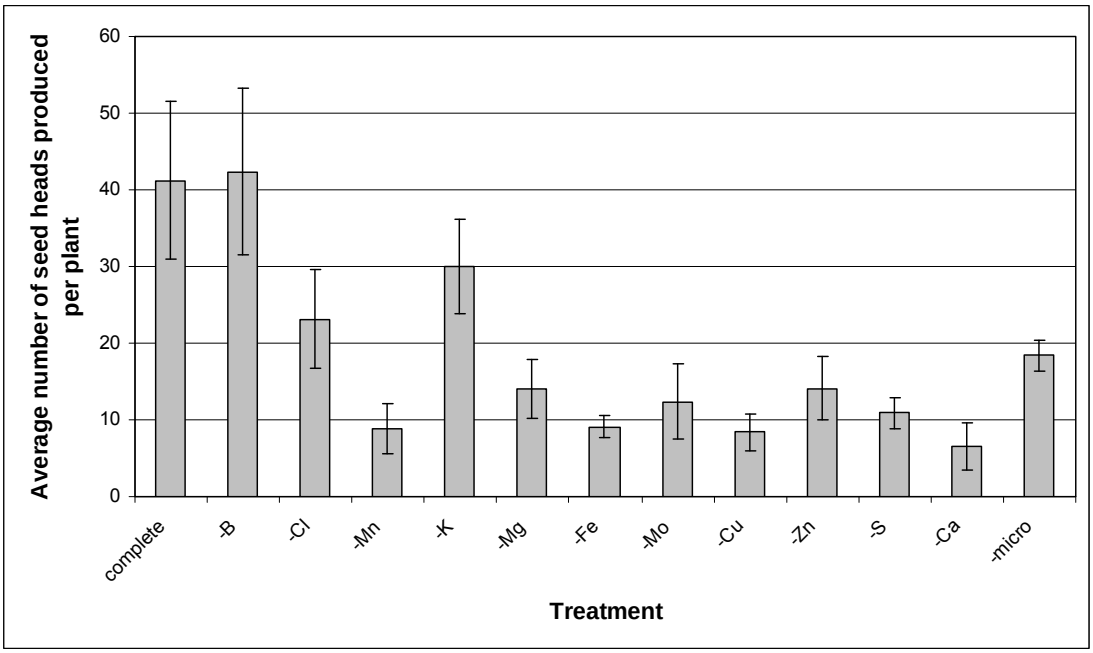


Figure 55 - Seed heads produced per plant of buffel grass (*Cenchrus ciliaris*) grown with various nutrient deficiencies (± SE)

The iron deficient plants produced the highest average number of seed per head, followed by the complete nutrient plants (Figure 56). The chlorine, molybdenum and copper deficient plants followed, and were all similar to each other. The zinc, boron, manganese and magnesium deficient plants were the next lowest, but were similar to the previous treatments. The plants which produced the lowest average number of seed per head were from the potassium, sulfur, calcium and micronutrient deficient treatments, and were all similar to each other (Figure 56).

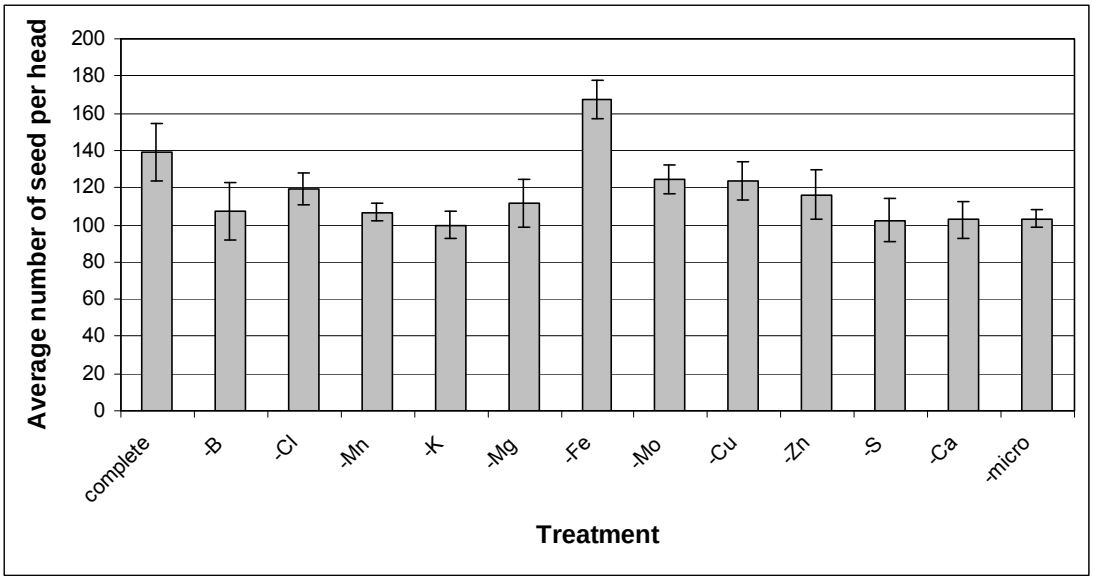


Figure 56 - Number of seeds per head of buffel grass (*Cenchrus ciliaris*) grown with various nutrient deficiencies (± SE)

The chlorine deficient plants on average produced the longest seed heads, followed by the complete nutrient and iron deficient plants, which were similar to each other (Figure 57). The next longest seed heads were produced by the potassium, boron, magnesium and copper deficient plants respectively. These were followed by the molybdenum and manganese deficient plants respectively. The lengths of seed heads of the zinc and sulfur deficient plants were similar to each other, and were the next lowest. The calcium and micronutrient deficient plants produced the shortest seed heads (Figure 57).

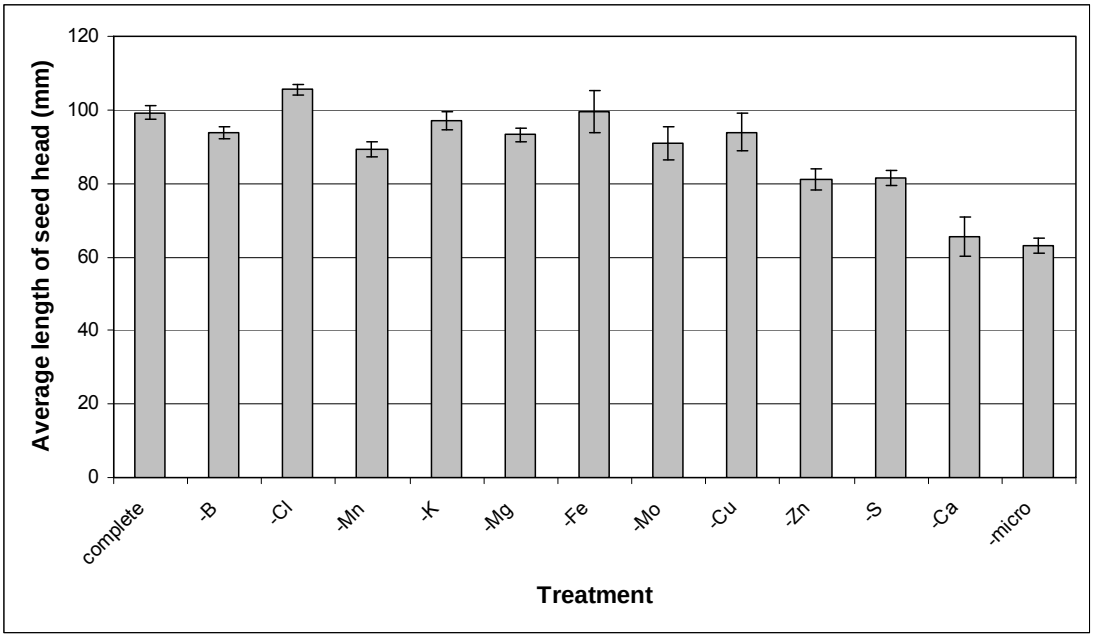


Figure 57 - Length of seed head of buffel grass (*Cenchrus ciliaris*) grown with various nutrient deficiencies (± SE)

The complete nutrient plants produced seeds with the longest seed fascicle, followed by the boron deficient plants (Figure 58). The chlorine, manganese, potassium, magnesium, iron, sulfur, calcium and micronutrient deficient plants produced the next shortest seed fascicles, and were all similar to each other. These were followed by the zinc and copper deficient plants. The molybdenum deficient plants produced the shortest seed fascicles (Figure 58).

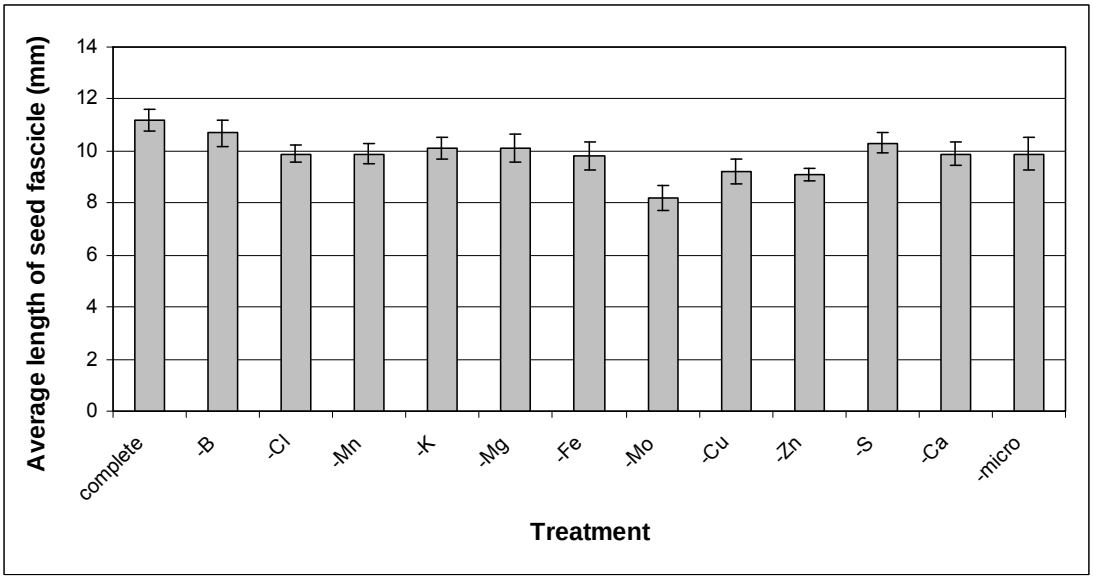


Figure 58 - Length of seed fascicle of buffel grass (*Cenchrus ciliaris*) grown with various nutrient deficiencies (± SE)

Figure 59 shows the percent emergence of the seeds produced by the various nutrient treatments. Since these seeds were collected at random and in large numbers, a generalised linear model was possible, and significant differences between treatments calculated.

Percent emergence differed ($P < 0.001$) among nutrient treatments with greatest emergence for the complete solution (46%). Boron did not appear to be a limiting factor with similar emergence (32%). There was some reduction when molybdenum was excluded but the major effect on emergence occurred when calcium, potassium, sulfur, copper, or micro nutrients were excluded.

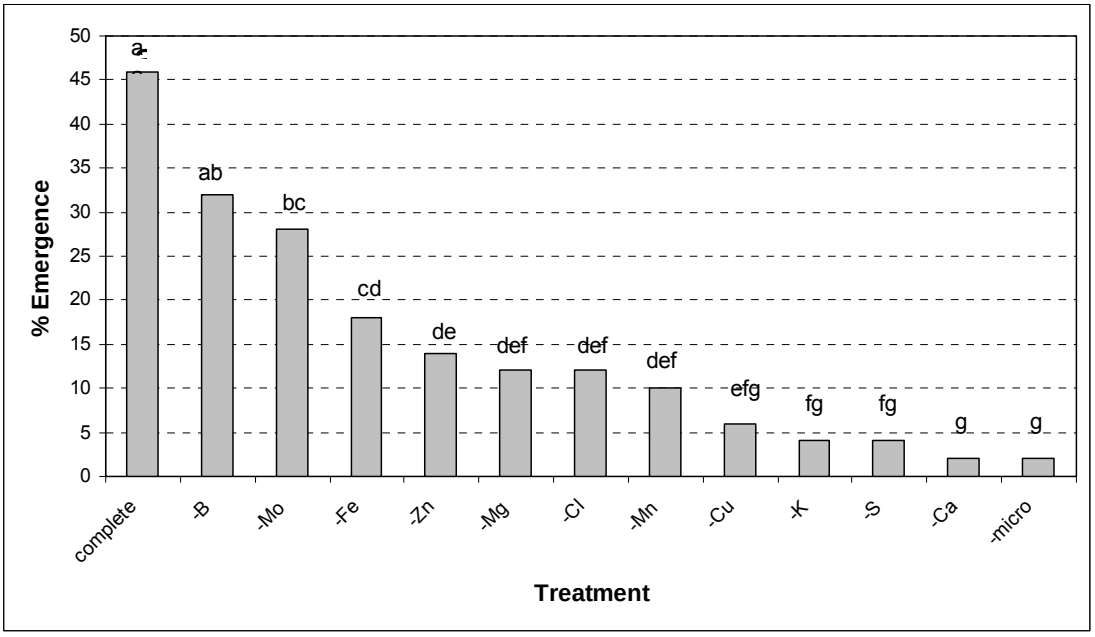


Figure 59 - Percent emergence of seeds from buffel grass (*Cenchrus ciliaris*) grown with various nutrient deficiencies

Note: The letters above the columns signify statistical significance, that is, if two treatments have different letters then they are significantly different.

Table 19 displays the number of caryopses in the seeds of the plants of the various nutrient regimes. The iron, molybdenum, boron and sulfur deficient plants had more caryopses per seed than the complete nutrient plants. The calcium deficient plants had the least number of caryopses per seed.

The proportion of caryopses was significantly influenced ($P < 0.001$) by seed source. The chi-square contributions table (data not presented) indicated that the greatest effect was the iron deficient plants, followed by the calcium and boron deficient plants.

Treatment	Number of caryopses		
	0	1	2
complete	18	30	2
-B	10	30	10
-Cl	23	27	0
-Mn	30	20	0
-K	27	18	5
-Mg	28	22	0
-Fe	5	45	0
-Mo	8	40	2
-Cu	23	27	0
-Zn	25	23	2
-S	15	35	0
-Ca	35	15	0
-micro	20	30	0

Table 19 - Number of caryopses in seeds of buffel grass (*Cenchrus ciliaris*) grown with various nutrient deficiencies

Due to the small amounts of seeds produced by some treatments, a well replicated seed viability trial (as in Chapter 4) was not possible as there were not enough seeds.

Overall, while many of the nutrient treatments produced similar-sized seed, similar numbers of seed per head and similarly-sized seed heads, they had greatly reduced reproductive capabilities compare to the complete nutrient plants due to the larger number of seed heads produced by this treatment. Even though several of the treatments had more caryopses per seed than the complete nutrient plants, the emergence rates showed that the seed viability was low in comparison. The only treatment which did not appear to greatly affect seed production and quality was boron deficiency.

The plant chemical tests (data not shown) confirmed that the plants were deficient in the nutrient(s) omitted, with no apparent interactions between deficiency treatments.

7.4 Discussion

The trial showed that there were differences between treatment units. Presuming that the commercial seeds obtained were a homogeneous mixture and that possible glasshouse variations were minimised, it was reasonable to assume that any differences between treatment units were due to the treatment, with the plant responses reflecting the treatments applied. Nevertheless, as an unreplicated pilot study, the results were treated as indicative rather than definitive.

7.4.1 Foliar Colour Symptoms

There were many different colour symptoms but none that exactly matched dieback symptoms. Many treatments, for example zinc, magnesium and phosphorus, produced red foliar symptoms, but these were mostly on the edge of the leaves. Some leaves, in plants deficient in sulfur and phosphorus, had red tips, but the colour did not spread towards the ligule, nor did it always emerge first in the older leaves. Other treatments had leaf tip necrosis, but again none that matched the BGD pattern. This suggests that none of the nutrient deficiencies were solely responsible for the colour symptoms of BGD. This suggestion supports the findings in Chapter 5 that presented data on the comparison of foliar pigments between BGD affected plants and nutrient deficient plants.

The observed foliar deficiency symptoms were mostly in accordance with those in Smith's (1974) work, although there were some symptoms which were not reported by Smith, and others which were reported but not seen in this study. It is possible that certain symptoms appear at a certain developmental stage, and since Smith (1974) did not grow the plants to maturity these may not yet have developed upon completion of that work. Also, Smith (1974) did not replace the solutions, as was done in this study, therefore it is possible

that secondary deficiency symptoms may have developed in his work. The disparity observed may, however, be due to the different physiology of different cultivars, or to some other factor, such as differences in the specific nutrient regimes used.

7.4.2 The Effects of the Various Treatments

The following observations are presented and discussed as comparisons to the complete nutrient treatment.

Boron Deficiency

Boron deficiency seemed to have the least effect of all the treatments. The plants had smaller seed heads, and fewer seeds per head. The plants also had a slightly higher proportion of seeds with more than one caryopsis. Unlike in Smith's (1974) work, the plants were not stunted. This could be due to the difference in cultivars or the fact that Smith did not grow the plants to maturity.

In plants with growing points, boron deficiency is often associated with abnormal growth (Dell and Huang, 1997; Pais and Benton Jones Jr., 1997), resulting in necrosis and brittle leaves. These symptoms were observed as a leaf tip necrosis. Boron also has a function in pollen germination and growth (Grundon *et al.*, 1997), which may explain the decrease in the number of seeds per head.

Chlorine Deficiency

The chlorine deficient plants had fewer leaves and tillers. Seed heads were longer, but seed fascicles were shorter. There was a significant decrease in seed emergence.

Chlorine is involved in photosynthesis and cell division, as well as maintaining internal turgor (Terry, 1977; Salisbury and Ross, 1992; Grundon *et al.*, 1997; Pais and Benton Jones Jr., 1997). A decrease in photosynthesis may account for the fewer leaves

and tillers, and possibly the decrease in emergence, since fewer resources would be partitioned into seed production. The reason for the longer seed heads is unknown.

Manganese Deficiency

The manganese deficient plants had a decreased root dry weight and fresh weight, and a decreased shoot and root percent dry matter. There were fewer leaves and tillers. The seed heads and seed fascicles were smaller, and there were fewer seed heads produced per plant. There were possibly fewer seeds per head. There was a significant decrease in seed emergence.

In the same manner as zinc and magnesium, manganese activates numerous enzymes involved in photosynthesis and growth, including electron transport (Grundon *et al.*, 1997). This may account for the reduced biomass and reproductive capacity.

Potassium Deficiency

The potassium deficient plants had a decreased shoot and root dry weight and fresh weight. There were fewer leaves and tillers. The seed fascicles were somewhat smaller, and there were fewer seeds per head. There was a significant decrease in seed emergence.

Potassium activates many enzymes essential for photosynthesis and respiration, and is also a major contributor to the osmotic potential of the cells, and therefore turgor pressure including stomatal opening. It also regulates cellular pH and the cation-anion balance (Salisbury and Ross, 1992; Grundon *et al.*, 1997). As with many of the other treatments, a decreased enzymatic activity would result in decreased biomass production. A loss in cell turgor pressure would account for the observation of drooping leaves (refer Table 18).

Magnesium Deficiency

Magnesium deficient plants had a decreased shoot dry weight, and decreased root dry weight and fresh weight. They also exhibited a decrease in shoot and root percent dry matter. There were fewer leaves and tillers. Seed heads and seed fascicles appear to be smaller, and there were fewer seed heads produced per plant. There was a significant decrease in seed emergence.

Magnesium activates many enzymes needed in photosynthesis and respiration, including CO_2 assimilation, as well as being the central atom in chlorophyll (Salisbury and Ross, 1992; Grundon *et al.*, 1997; Shaul, 2002). This includes those processes in which zinc is also necessary, which is probably why the magnesium and zinc results are similar. A decreased enzymatic and photosynthetic activity would decrease growth and reproduction.

Iron Deficiency

Iron deficient plants exhibited less shoot dry weight, and possibly decreased root weight. They also had less shoot dry matter, and fewer tillers and leaves. Seed fascicles were shorter, but there was an increase in the number of seeds per head. There was, however, a marked decrease in the number of seed heads produced per plant. Seed emergence was significantly lower, but there were more seeds containing one caryopsis, indicating that some other factor was causing the low emergence.

Iron is an important component in many plant enzyme systems, including those for photosynthesis and electron transport (Salisbury and Ross, 1992; Grundon *et al.*, 1997; Pais and Benton Jones Jr., 1997). This may explain the above results, in that the plants had less energy for growth and reproduction.

Molybdenum Deficiency

The molybdenum deficient plants had a slightly lower shoot dry weight, a lower root weight, and a decreased percent dry matter of both shoots and roots. Plant height was lower, and there were slightly fewer tillers and leaves. Seed fascicles were smaller, as were the seed heads. There was a marked decrease in seed heads per plant, and a significantly lower seed emergence.

Molybdenum is a component of two major enzyme systems, namely nitrogenase and nitrate reductase. The former is involved in the conversion of the nitrate anion to the ammonium cation (Grundon *et al.*, 1997; Pais and Benton Jones Jr., 1997). Molybdenum influences the level of nitrates in non-legume species primarily because it is required for nitrate reduction (Johansen, 1978). Therefore, a molybdenum deficiency often resembles a nitrogen deficiency, which explains the above results.

Copper Deficiency

Copper deficient plants had a decreased shoot dry weight, a decreased root dry weight and fresh weight, and a decreased shoot and root percent dry matter. There were fewer leaves and tillers. Seed fascicles were shorter, and there were fewer seed heads produced per plant. There was a significant decrease in seed emergence.

Copper is a constituent of the chloroplast protein plastocyanin, and is a part of the electron transport system linking photosystems 1 and 2. It also has a role in pollen formation and fertilisation (Grundon *et al.*, 1997; Pais and Benton Jones Jr., 1997). These may explain the reduced biomass, since less energy would be available for these purposes, and the decreased reproduction.

Zinc Deficiency

Zinc deficient plants had decreased biomass in general, including shoot and root dry weight, fresh weight and percent dry matter. The plants had fewer leaves per tiller towards the end of the trial, and had fewer leaves and tillers overall. Seed heads and seed fascicles were smaller, and there were fewer seed heads produced per plant. There was a significant decrease in seed emergence.

Zinc is involved in many enzymatic functions, many of which are related to plant growth, including nucleotide synthesis and membrane integrity (Grundon *et al.*, 1997; Pais and Benton Jones Jr., 1997) and photosynthesis (Salisbury and Ross, 1992), which may explain the above mentioned decrease in production. Zinc often causes internodal shortening, resulting in stunted plants. This was observed by Smith (1974), but not in the above results. The treatment produced foliar symptoms of zinc deficiency but not all growth symptoms.

Sulfur Deficiency

Sulfur deficient plants had an overall decrease in biomass in both shoots and roots. Initially there were fewer leaves per tiller and there were fewer leaves and tillers overall. Seed heads were smaller, as were seed fascicles. There were fewer seed heads produced per plant, and fewer seeds per head. There was a significant decrease in seed emergence.

Sulfur is mostly present in plants in the form of proteins, specifically in the amino acids cysteine and methionine, and has a role in protein synthesis and function (Salisbury and Ross, 1992; Grundon *et al.*, 1997; Hawkesford, 2000; Bloem *et al.*, 2005). Limiting these amino acids may account for the decreased biomass and seed production.

Calcium Deficiency

Calcium deficient plants had an overall decrease in biomass in both shoots and roots. The plants were shorter and had shorter leaves and internodes. There were fewer leaves per tiller, fewer leaves, but only slightly fewer tillers. Seed heads and seed fascicles were shorter, and there were fewer seed heads produced per plant, as well as fewer seeds per head. There was a significant decrease in seed emergence, and a higher proportion of seeds with no caryopses.

Calcium is required when cell division occurs to form a new middle lamella in the cell plate that arises between daughter cells (Salisbury and Ross, 1992), and is also essential for membrane function, osmoregulation and the cation-anion balance (Grundon *et al.*, 1997; White and Broadley, 2003). Calcium deficiency produces stunted growth and brittle plant tissues, and also suppresses seed development (Hewitt and Smith, 1975). This accounts for the observed results.

Micronutrient Deficiency (no Ca, Mg, S, Fe, Mn, Cu, Zn, B, Mo, Cl)

The micronutrient deficient plants had an overall decrease in biomass in both shoots and roots. Plant height was initially shorter but eventually reached the height of the control plants (complete nutrient). Leaves and internodes were shorter. The number of leaves per tiller was similar to the control plants; however, overall there were fewer leaves and tillers. Seed heads were smaller, as were seed fascicles. There were fewer seed heads produced per plant, and fewer seeds per head. There was a significant decrease in seed emergence.

The micronutrient deficient plants exhibited a combination of foliar symptoms, so it was reasonable to assume that the biomass, morphological and seed symptoms were also a combination of the symptoms of the omitted nutrients. The above results concur with this.

Nitrogen Deficiency

Nitrogen deficient plants showed a marked decreased in the shoot and root dry weight and fresh weight. Percent dry matter of the shoots was higher than the control plants, whereas root dry matter was lower. Similarly to the NPK deficient plants, plant height, and leaf and internode length were considerably smaller. The number of leaves per tiller did not differ greatly from the control, but this was possibly due to the small number of tillers. The numbers of leaves and tillers were substantially decreased. There were no seeds produced.

Nitrogen is present in many essential compounds and has a role in many physiological processes (Salisbury and Ross, 1992; Grundon *et al.*, 1997). Nitrogen deficiency results in a decrease in most plant functions (Hewitt and Smith, 1975), which accounts for the above results. One consequence of nitrogen deficiency is the accumulation of starch in the chloroplasts (Hewitt and Smith, 1975), which may explain the increase in shoot percent dry matter.

NPK Deficiency

The NPK deficient plants had a substantial overall decrease in biomass in both shoots and roots. Plant height and leaf and internode length were considerably smaller. The number of leaves per tiller did not differ greatly from the control, but this is possibly due to the small number of tillers. The numbers of leaves and tillers were substantially decreased. There were no seeds produced.

As with the micronutrient deficient treatment, it was assumed that these symptoms were a combination of the symptoms of the omitted nutrients.

Phosphorus Deficiency

In parallel with the nitrogen deficient plants, phosphorus deficient plants showed a decrease in shoot and root dry weight and fresh weight. Percent dry matter of the shoots was higher than the control plants, whereas root dry matter was lower. Plant height and leaf and internode length were considerably smaller. The numbers of leaves per tiller, as well as the overall numbers of leaves and tillers, were considerably smaller. This is probably due to the fact that each plant only produced one tiller. No seeds were produced.

Phosphorus is an essential part of many sugar phosphates, most of which are involved in photosynthesis, respiration, and other metabolic processes such as energy storage and transfer (Salisbury and Ross, 1992; Grundon *et al.*, 1997). It is also a part of DNA, RNA, and lipid membranes. The many roles of phosphorus explain the recorded results. A phosphorus deficiency causes the leaf accumulation of sucrose, reducing sugars, and sometimes starch (Hewitt and Smith, 1975), which may account for the increased percent dry matter of the shoots.

7.4.3 Comparison With BGD Affected Plants

Due to the difference in growth conditions and media, a direct comparison between the results reported in this chapter and those of Chapter 4 (Effects of BGD on plant morphology and seeds) is not possible. However, results from this chapter may give an indication as to which (if any) nutrient(s) is/are causing or contributing to the dieback condition.

In the field, BGD affected plants showed a reddening of the leaves from the tip to the ligule, which originated on the older leaves and progressed to the younger leaves. BGD affected plants exhibited a decreased shoot and root dry weight and fresh weight, a

decreased percent dry matter in the shoots, and an increased percent dry matter in the roots. Plant height and length of leaves and internodes were shorter. Tillers were fewer, but the leaves per tiller were the same as for unaffected plants. BGD affected plants had shorter seed heads and seed fascicles, and produced fewer seed heads per plant. They also had fewer seeds per head. Seed emergence was considerably reduced, and there was a larger proportion of seeds with no caryopses.

As mentioned above, none of the treatments in this trial reproduced the foliar colour symptoms of BGD. In addition, none of the treatments produced the exact morphological symptoms of BGD affected plants. However, there were some treatments which had most of the characteristic symptoms of BGD. The calcium, nitrogen and phosphorus deficiency treatments (including no NPK) are the three treatments which most closely reproduce BGD symptoms. These are followed by the micronutrient, sulfur and zinc deficiency treatments. It is also possible that a nutrient imbalance associated with BGD is a combination of these, or another nutrient imbalance not included in this study.

7.5 Concluding Statements

It can be assumed that the primary cause of BGD is not one of the nutrient deficiencies examined in this study. This does not eliminate a nutrient imbalance as a possible contributing factor, since this study did not take into account all possible nutrient disorders or combinations thereof. The above results, however, indicate that shortage of calcium, nitrogen, phosphorus, sulfur, zinc, or a combination of these, may be a contributor to the syndrome. Particular attention should be given to these nutrients in any plant chemical tests undertaken on BGD affected plants (Chapter 8).

Chapter 8

Investigations into Chemical Causes of BGD

8.1 Introduction

Many plant disorders are caused by a chemical imbalance in the soil. These imbalances may include pH, salinity, or nutrient toxicity or deficiency. While soil chemical imbalances do not generally occur in circular patches, it is possible that an imbalance is weakening the buffel grass plants, thereby making them more susceptible to the BGD condition. It is also possible that an imbalance is a contributor to BGD symptoms. The results of Chapter 7 suggested that certain nutrient deficiencies produced similar symptoms (both growth habit and colour) to that of BGD. Therefore, field chemical tests were necessary to determine whether any of those nutrients were contributing factors, or whether another chemical imbalance is present.

Previous work on soil chemistry by Graham and Conway (1998; 2000) had varied results. The first paper suggested that salt was the causal agent (Graham and Conway, 1998) due to elevated sodium concentrations found in BGD affected buffel grass compared with unaffected buffel grass. The second paper (Graham and Conway, 2000) reported elevated concentrations of nitrogen and lower concentrations of phosphorus and potassium in BGD affected plants, with the sodium and chloride data being inconclusive. Therefore, it was considered necessary to repeat this work.

Since soil pH has known implications in nutrient uptake in plants, it was included in this study. Salinity was also measured since previous work identified salt as a possible problem. Both soil and plant chemical compositions were also investigated.

8.2 Soil pH and Salinity Pilot Study

8.2.1 Materials and Methods

Methodology

Before undertaking a comprehensive study, a small pilot study was done to determine if there were differences in soil pH and salinity between BGD affected patches and BGD unaffected paddocks. Sampling from a separate unaffected paddock was considered necessary due to the high BGD patch density in the affected paddock, which may have compromised the accuracy of the results. Due to high soil variability the two paddocks chosen were adjacent to each other, and samples were taken within a 100 m radius. Neither of the two paddocks had held cattle for at least 6 months prior to this trial, as according to Dantzman *et al.* (1983), the presence of cattle may cause variations in the results.

Method

Twenty soil samples from five BGD affected patches of buffel grass (24º 21.20' S; 194º 52.28' E), and twenty soil samples from an adjacent BGD unaffected paddock (24º 19.43' S; 149º 51.05' E) were collected in brown paper bags. The samples were collected from the top 20 cm of soil and were collected a few weeks after a rainfall event, when BGD symptoms were prevalent. The samples were brought back to the laboratory where they were oven-dried at 70ºC for three days, then ground in a mortar and pestle to a particle size

of < 2 mm. The mortar and pestle were thoroughly cleaned between samples. Three of the unaffected samples were lost in storage. A 3 g aliquot of each sample was mixed with 15 mL of reverse osmosis water, producing a soil:water ratio of 1:5. The pH and salinity of each sample were measured according to Rayment and Higginson (1992), using a TPS WP-81 pH and salinity meter.

8.2.2 Results

The data were analysed using two-sample *t*-tests assuming unequal variance. There was a significant difference ($P = 0.05$) in pH between BGD affected and unaffected paddocks, that is, the mean pH was significantly higher in BGD affected patches than in the unaffected paddock (Table 20). There was no difference in salinity ($P = 0.22$) (Table 21).

pH	BGD Affected	Unaffected
Mean	6.645	6.405
SE	0.078	0.089

Table 20 – Mean pH of soils from BGD affected patches and a BGD unaffected paddock

Salinity (ppm)	BGD Affected	Unaffected
Mean	45.250	39.471
SE	3.740	2.740

Table 21 – Mean salinity of soils from BGD affected patches and a BGD unaffected paddock

8.2.3 Discussion

There was a difference in pH but no difference in salinity, suggesting that salt is not the main causal agent of BGD. However, while these samples were taken in close proximity to each other, the results may still reflect paddock variability. Further studies should consist of samples taken closer to each other, for example, within a BGD affected

patch and at several small distances from the patch. Further work should also incorporate samples taken from different depths of soil. The pH and salinity results may be different down the soil profile.

8.3 Soil pH and Salinity – Incremental Radial Measurements

8.3.1 Materials and Methods

Methodology

As the above pilot study suggested, samples should be taken in close proximity to each other due to paddock variability in soils. Therefore, in this study, samples were taken at intervals along transects from within BGD affected patches to 6 m outside the patch boundary. Samples were also taken from incremental depths down to 1 m, to investigate any differences down the soil profile encompassing the majority of the root system.

While this study may provide more comprehensive pH and salinity results than the pilot study, it may also serve a second purpose. The boundary of a BGD affected patch is assumed to be the boundary between plants displaying red BGD symptoms and asymptomatic plants. However, the causal agent(s) of this condition may be further than the visible boundary, affecting plants which do not yet show symptoms. Taking samples in set increments away from the visible patch boundary may reveal the true patch boundary. This information, combined with the data in Chapter 3 (rate of patch spread) may provide the time period between when the plant is first affected and the appearance of symptoms.

Method

Soil cores were collected from a BGD affected paddock (24º 20.08' S; 149º 51.67' E) several weeks after a rainfall event, when BGD symptoms were prevalent and the

symptomatic patch boundary was easily discernible. The cores were taken to a depth of 1 m using a hydraulic soil sampling and coring machine mounted on a four-wheel drive vehicle. This equipment was borrowed from the Queensland Department of Primary Industries, Emerald. The cores (32 mm diameter) were obtained from five BGD affected patches, each of approximately 3 m in diameter, which were relatively isolated from other BGD affected patches, that is, there were no other BGD affected patches within a 15 m radius. The cores were taken along transects from within the patch in an outward direction, and were always taken within 30 cm of a buffel plant. The transect distances were:

- Within the patch, near the BGD affected buffel plant which was closest to the patch centre (inside)
- Just inside the patch boundary, again near a BGD affected buffel plant (edge)
- 50 cm from the patch boundary (50 cm)
- 1 m from the patch boundary (1 m)
- 3 m from the patch boundary (3 m)
- 6 m from the patch boundary (6 m).

There were two transects of cores taken from each of the five patches. Each core was immediately cut into the following segments:

- 0 –10 cm
- 10 – 20 cm
- 20 – 30 cm
- 30 – 40 cm
- 40 – 60 cm
- 60 – 80 cm

- 80 – 100 cm.

The segments were placed in pre-labelled plastic containers and brought back to the university where they were oven-dried at 60°C for five days. Each segment was then ground to a particle size of < 2 mm using a Lab Mill (Christy and Norris Ltd. 8 inch Lab Mill, 8000 rpm), which was thoroughly cleaned between samples. At this point the corresponding samples for each distance and depth interval from the two transects of each patch were bulked into one sample. The samples were then placed into cool, dry storage.

A 3 g aliquot was taken from each sample and mixed with 15 mL reverse osmosis water, producing a soil:water ratio of 1:5. The pH and salinity of each sample were measured according to Rayment and Higginson (1992), using a TPS WP-81 pH and salinity meter.

Both pH and salinity were analysed as repeated measures data due to the expected correlation structure among depths. Profile plots (both comparing patches within each treatment and average treatment (distance) profiles) were produced. Data were analysed by residual maximum likelihood (REML) and modelling the variance-covariance structure of the data.

8.3.2 Results

Soil pH did not appear to differ with increasing distance from a BGD affected patch, but there were some differences down the soil profile (Table 22).

Statistically, with the pH data, none of the available models effectively modeled the correlation structure so the unstructured variance-covariance matrix was used. The Distance by Depth interaction was not significant ($P > 0.10$) so was removed and the model refitted. There was no difference ($P > 0.10$) in pH among distances but pH varied ($P <$

0.001) at different depths. The pH generally increased then decreased with depth, with a few exceptions (Table 22).

Distance / Depth (cm)	in		edge		50 cm		1 m		3 m		6 m	
	Mean	(± SE)	Mean	(± SE)	Mean	(± SE)	Mean	(± SE)	Mean	(± SE)	Mean	(± SE)
0-10	6.84	(0.067)	6.88	(0.047)	6.85	(0.047)	6.90	(0.059)	6.94	(0.120)	6.88	(0.061)
10-20	6.81	(0.040)	6.91	(0.098)	6.86	(0.043)	6.78	(0.034)	6.81	(0.112)	6.81	(0.054)
20-30	6.97	(0.106)	6.83	(0.049)	6.86	(0.031)	6.89	(0.080)	6.81	(0.075)	6.85	(0.107)
30-40	6.97	(0.082)	6.89	(0.053)	7.08	(0.145)	7.00	(0.080)	6.96	(0.087)	6.90	(0.110)
40-60	7.14	(0.121)	7.05	(0.128)	6.97	(0.056)	7.01	(0.049)	7.05	(0.073)	6.86	(0.101)
60-80	7.02	(0.175)	6.93	(0.113)	6.92	(0.069)	6.98	(0.089)	6.93	(0.246)	6.91	(0.092)
80-100	6.96	(0.110)	7.03	(0.055)	6.96	(0.127)	7.04	(0.171)	6.99	(0.113)	7.02	(0.081)

Table 22 – Mean pH of soil samples with increasing distance from the centre of a patch of buffel grass (*Cenchrus ciliaris*) affected with buffel grass dieback, and with increasing depth at each distance

Soil salinity similarly did not appear to differ with increasing distance from a BGD affected patch, but did increase down the soil profile (Table 23).

Statistically, with the salinity data, again, none of the available models effectively modeled the correlation structure so the unstructured variance-covariance matrix was used. The Distance by Depth interaction was not significant ($P > 0.10$) so was removed and the model refitted. There was no difference ($P > 0.10$) in salinity among distances but salinity varied ($P < 0.001$) for depths with salinity greater for depths below 40 cm.

Distance / Depth (cm)	in		edge		50 cm		1 m		3 m		6 m	
	Mean	(± SE)	Mean	(± SE)	Mean	(± SE)	Mean	(± SE)	Mean	(± SE)	Mean	(± SE)
0-10	18.84	(1.288)	17.94	(0.546)	18.96	(1.004)	20.58	(1.462)	23.28	(6.014)	18.16	(0.870)
10-20	16.38	(0.682)	17.48	(0.567)	18.60	(1.783)	18.70	(1.523)	16.52	(0.970)	15.96	(0.431)
20-30	16.78	(1.675)	15.58	(0.683)	15.66	(0.492)	16.12	(0.865)	15.82	(0.348)	15.98	(1.206)
30-40	16.26	(0.543)	16.76	(0.493)	16.92	(1.160)	17.62	(1.227)	16.86	(0.700)	19.44	(3.413)
40-60	25.80	(2.050)	22.00	(2.606)	22.40	(1.381)	21.40	(2.645)	22.58	(2.910)	22.54	(2.250)
60-80	29.20	(1.931)	21.68	(2.912)	23.86	(1.895)	25.06	(3.036)	23.62	(2.550)	24.56	(3.016)
80-100	26.62	(1.172)	24.28	(0.962)	25.28	(1.512)	26.40	(1.580)	24.60	(1.431)	24.64	(1.771)

Table 23 - Mean salinity (ppm) of soil samples with increasing distance from the centre of a patch of buffel grass (*Cenchrus ciliaris*) affected with buffel grass dieback, and with increasing depth at each distance

8.3.3 Discussion

There was no difference in pH or salinity with increasing distance from the BGD affected patch, although there was a significant difference in both with increasing depth. However, the difference in depth was the same for every distance from the patch, meaning that distance from the patch had no effect at any depth.

Soil pH was neutral to slightly acidic, and was within the accepted range where plant nutrients are moderately to highly available (Salisbury and Ross, 1992). Soil salinity, even at the greater depths, was at a level which, according to Graham and Humphreys (1970), should not significantly affect the growth of American buffel grass.

The lack of difference in either pH or salinity with distance from the patch resulted in two hypotheses. Firstly, that soil pH and/or salinity are not involved in the dieback condition, or secondly, that the furthermost distance of 6 m was not far enough to overtake the true boundary of the condition.

8.4 Soil Chemical Analyses

8.4.1 Materials and Methods

Methodology

As mentioned in the introduction, previous soil chemistry work had varied results. Since a nutrient imbalance may be a contributor to the BGD condition, it was decided to repeat this work, with some variations in procedure.

The focus of this study was on the plant available nutrients, namely nitrogen, phosphorus, potassium, sulfur, chloride, aluminium, boron, calcium, copper, iron, magnesium, manganese, molybdenum, sodium, and zinc. This was done as a comparative

study between BGD affected and unaffected areas using the same soil samples which were collected in the above pH and salinity experiment (part 8.3). These were the samples were collected along transects through BGD affected patches and at incremental depths. This sampling would minimise paddock variability and, if plant available nutrients were involved in the BGD condition, would aid in determining the true patch boundary.

Due to budgetary constraints, it was decided to process the samples in-house, and send the resulting extractants away for analysis. The method chosen was the Mehlich 3 soil test extractant (Mehlich, 1984). The Mehlich 3 is a multi-elemental extractant which extracts plant available phosphorus, potassium, calcium, magnesium, sodium, sulfur, boron, copper, iron, manganese, zinc, cadmium, cobalt, aluminium, nickel and molybdenum (Allen and Walton, 2003). The Mehlich 3 procedure is widely used in the United States (Allen and Walton, 2003) and is gaining popularity in Australia, with several commercial laboratories opting for this method. According to Vocasek and Friedericks (1994), Alva (1993), and Bolland *et al.* (2003), the Mehlich 3 method correlates well with other conventional soil testing methods. The main requirement for this method is that the soil must be neutral or acidic (Allen and Walton, 2003). The pH experiment (part 8.3) concluded that the BGD affected field site soil was neutral to acidic, therefore the Mehlich 3 method was considered suitable.

The Mehlich 3 method is inappropriate for the analysis of plant available nitrogen, so soil samples were sent away to an outside laboratory for processing and analysis. The chosen laboratory (CSBP Limited) offered to process and analyse the samples for ammonium-N, nitrate-N, Colwell P, Colwell K, sulfur, and chloride for a reasonable price. Therefore, the samples processed by the Mehlich 3 method were only analysed for aluminium, boron, calcium, copper, iron, magnesium, manganese, molybdenum, sodium,

and zinc. Due to further budgetary constraints, only the samples down to a depth of 60 cm were sent to CSBP Limited for processing and analysis.

Method

The soil samples utilised were a sub-sample of the soil samples collected for the pH and salinity experiment (part 8.3). A sub-sample of each of these (from the 0-10 cm to the 40-60 cm depths) was sent away to CSBP Limited, Western Australia for processing and analysis for: ammonium-N, nitrate-N, Colwell P, Colwell K, sulfur, and chloride (hitherto called the 'shallow' elements). The samples were sent in cool storage in labelled brown paper bags. CSBP Limited measure ammonium and nitrate nitrogen calorimetrically using a Lachat Flow Injection Analyser after digesting the soil in 1 M potassium chloride. They measure potassium and phosphorus using the Colwell method; sulfur is measured by ICP (Inductively coupled plasma) spectroscopy following potassium chloride extraction. Chloride is measured by ICP following water extraction.

Further sub-samples of the soil samples were processed using the Mehlich 3 method (Mehlich, 1984), and the resulting extractants were filtered to 0.2 μm by utilising the apparatus shown in Figure 60. These ensuing extractants were sent in cold storage to Symbio Alliance, Brisbane for analysis of: aluminium, boron, calcium, copper, iron, magnesium, manganese, molybdenum, sodium, and zinc (hitherto called the 'deep' elements). Symbio Alliance conduct their analyses under the APHA – Standard Methods for the Examination of Water and Waste Water, 21st Edition, 3030B. Samples are measured by ICP-AES (Inductively coupled plasma atomic emission spectroscopy).

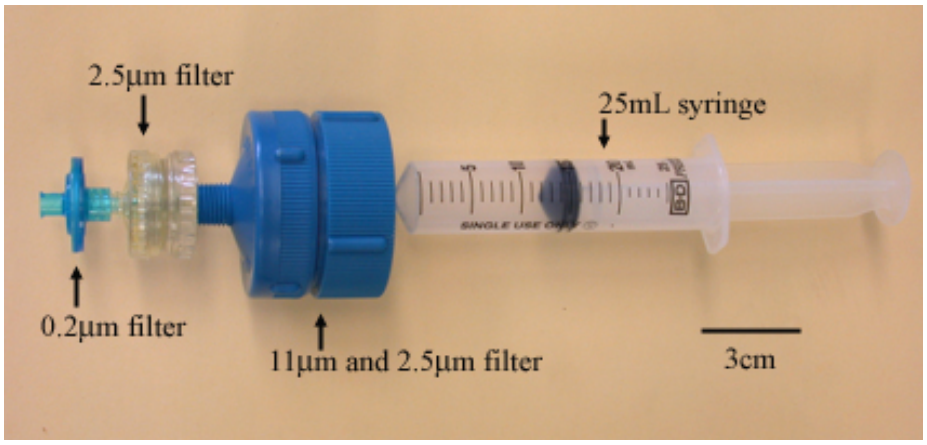


Figure 60 – Apparatus for filtering soil/extractant mixture to 0.2 µm

All data were analysed as repeated measures using REML and modelling the variance-covariance matrix to account for the correlation structure induced by sequential depths. In all cases the variance-covariance matrix was modelled satisfactorily by an ante-dependence structure of order 1. Distributional assumptions were assessed by visual inspection of residual and normal probability plots. Departures from normality and/or constant variance were observed for the 'shallow' elements (down to 60 cm) of sulfur and chloride so these data were square-root and log transformed, respectively, prior to analysis. Also, departures from normality and/or constant variance were observed for the 'deep' elements (down to 1 m) of Al, Ca, Fe, Mg, Mn and Na. A log transformation was applied to the data of Al, Ca, Fe and Mg while a square root transformation was applied to the data of Mn and Na prior to analysis. Significant differences, as well as any depth by distance interaction (DxT) are shown in the following tables.

8.4.2 Results

In Tables 24 and 25, for comparative purposes, the transformed values are shown first, with the backtransformed data (real data) shown in parentheses.

Element	Al	Ca	Cu	Fe	Mg	Mn	Na	Zn
Distance (D)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*P* =0.073
in	5.94 (377.4)	6.77 (871.2)	0.85	4.87 (129.3)	5.34 (206.9)	8.80 (77.4)	7.97 (63.4)	0.536
edge	5.92 (372.9)	6.75 (853.9)	0.86	4.83 (123.6)	5.34 (207.3)	8.60 (73.9)	7.47 (55.7)	0.383
50 cm	5.91 (365.9)	6.71 (822.9)	0.79	4.90 (133.2)	5.26 (190.7)	8.90 (79.3)	7.71 (59.5)	0.418
1 m	5.93 (373.3)	6.70 (809.8)	0.84	4.90 (133.0)	5.26 (191.5)	8.90 (79.3)	8.01 (64.1)	0.434
3 m	5.90 (362.2)	6.72 (827.8)	0.81	4.86 (127.6)	5.21 (182.8)	9.11 (83.0)	8.63 (74.5)	0.459
6 m	5.90 (363.3)	6.62 (747.4)	0.75	4.76 (115.3)	5.22 (183.6)	8.18 (66.9)	7.82 (61.2)	0.408
s.e.d.	0.04	0.07	0.05	0.11	0.06	0.53	0.49	0.053
Depth (T)	*P* <0.001	*P* <0.001	*P* <0.001	*P* <0.001	*P* <0.001	*P* <0.001	*P* <0.001	*P* <0.001
0-10 cm	5.58 b (263.5)	6.37 bc (580.1)	0.64 c	4.40 c (80.7)	4.66 bc (104.4)	12.60 a (158.8)	5.05 d (25.5)	1.231 a
10-20 cm	5.59 b (266.7)	6.32 c (554.6)	0.65 c	4.33 c (75.0)	4.40 c (80.2)	11.77 b (138.5)	5.18 d (26.8)	0.431 b
20-30 cm	5.58 b (264.3)	6.23 d (505.2)	0.59 c	4.23 c (67.7)	4.43 c (83.1)	10.98 c (120.5)	5.59 d (31.3)	0.313 c
30-40 cm	5.79 b (326.7)	6.49 b (656.9)	0.64 c	4.71 b (109.5)	5.03 b (151.3)	8.95 d (80.1)	7.14 c (50.9)	0.341 bc
40-60 cm	6.29 a (537.1)	7.16 a (1285.9)	1.00 b	5.44 a (229.4)	5.98 a (392.9)	4.99 f (24.9)	10.10 b (102.1)	0.242 c
60-80 cm	6.34 a (563.0)	7.24 a (1386.1)	1.11 a	5.41 a (222.0)	6.21 a (495.7)	5.17 f (26.7)	11.07 a (122.6)	0.291 c
80-100 cm	6.23 a (508.8)	7.19 a (1323.8)	1.08 ab	5.44 a (229.4)	6.20 a (489.8)	6.77 e (45.9)	11.41 a (130.1)	0.228 c
Ave. s.e.d.	0.04	0.06	0.04	0.13	0.08	0.51	0.45	0.062
D x T	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Table 24 – The effect of profile depth and distance from a BGD affected patch of buffel grass (*Cenchrus ciliaris*) on the concentration of plant available nutrients (mg/kg soil) in the soil (down to 1 m depth)

Note: values in parentheses are back-transformed means. Means within an effect not followed by a common letter are significantly different (*P*=<0.05). "n.s." = not significant.

Element	Ammonium	Nitrate	Colwell K	Colwell P	Sulfur	Chloride
Distance (D)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
in	2.26	1.47	212.0	17.2	1.87 (3.49)	2.10 (7.13)
edge	2.38	1.46	225.1	15.6	1.90 (3.59)	2.26 (8.55)
50 cm	2.61	1.56	236.4	14.1	1.93 (3.72)	2.19 (7.97)
1 m	2.49	1.78	226.7	15.1	1.80 (3.25)	2.28 (8.73)
3 m	2.53	1.51	231.7	15.6	1.96 (3.85)	2.23 (8.25)
6 m	2.37	1.49	248.4	20.1	2.03 (4.13)	2.19 (7.94)
s.e.d.	0.17	0.18	17.6	3.6	0.11	0.13
Depth (T)	*P*<0.001	*P*<0.001	*P*<0.001	*P*<0.001	*P*<0.001	*P*<0.001
0-10 cm	3.87 a	2.40 a	366.0 a	25.9 a	1.92 b (3.67)	2.06 c (6.85)
10-20 cm	2.40 b	1.37 b	272.9 b	17.0 b	1.72 c (2.95)	2.06 c (6.85)
20-30 cm	1.83 c	1.43 b	172.2 c	15.4 c	1.59 d (2.52)	1.98 c (6.27)
30-40 cm	1.80 c	1.37 b	140.4 d	12.3 d	1.74 c (3.03)	2.26 b (8.62)
40-60 cm	2.30 b	1.17 b	198.7 c	10.7 e	2.61 a (6.83)	2.66 a (13.34)
Ave. s.e.d.	0.18	0.17	12.0	0.8	0.08	0.08
D x T	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Table 25 - The effect of profile depth and distance from a BGD affected patch of buffel grass (Cenchrus ciliaris) on the concentration of plant available nutrients (mg/kg soil) in the soil (down to 60 cm depth)

Note: values in parentheses are back-transformed means. Means within an effect not followed by a common letter are significantly different (P=<0.05). "n.s." = not significant.

Boron and molybdenum were not reported since the data were below the detectable limits.

There was no significant difference in the concentration of any of the measured plant available nutrients in the soil with increasing distance from a BGD affected patch (Tables 24 and 25). However, all had significant differences with depth down the soil profile. The concentrations of aluminium, calcium, copper, iron, magnesium, sodium, sulfur and chloride all increased down the soil profile. The concentrations of manganese, zinc, ammonium nitrogen, nitrate nitrogen, phosphorus and potassium all decreased down the soil profile (Tables 24 and 25). There was never any depth by distance interaction (DxT), that is, the differences down the soil profile were the same both within and out of the BGD affected patches.

8.4.3 Discussion

There were no significant differences in plant available nutrients with increasing distance from the BGD affected patches, which could mean one of two things:

1. The 6 m distance from the patch was insufficient to overtake the true boundary of the condition, or
2. Soil plant-available nutrients are not the main causal agent of BGD.

Even if plant available nutrients are not the main causal agent of BGD, nutrient deficiencies may weaken the plants making them more susceptible to the causal agent(s). The reported aluminium levels are toxic to wheat and barley, though may not be toxic to pasture grasses (Slattery *et al.*, 1999). Calcium levels for pastures are critical at 240 mg/kg (Bruce, 1999) and adequate at >400 mg/kg (Brown and Grant, 2000), meaning that field levels are adequate. Copper levels in the field are above critical values for pastures according to Brennan and Best (1999). Iron levels in the field are, on average, just above

the critical iron concentration for sorghum (McFarlane, 1999), and so may be adequate for pastures. According to Aitken and Scott (1999), field magnesium levels are higher than the critical value for pastures. Similarly, according to Uren (1999), field manganese levels are adequate for grasses. Sodium field levels were at acceptable values, since Ibarra *et al.* (1995) reported buffel grass growing well with soil sodium levels up to 92 mg/kg. According to Armour and Brennan (1999), field zinc levels are low for pastures, being nearly half of what is considered adequate levels. Field nitrate levels are very low according to Strong and Mason (1999), who based their values on cereal crops such as corn and wheat, and therefore may also be low for pasture grasses. Field potassium levels are more than adequate for pastures according to Gourley (1999) and Gourley *et al.* (2007). Field phosphorus levels were low for pastures according to Moody and Bolland (1999) and Gourley *et al.* (2007), being half of what is considered adequate levels. Similarly, field sulfur levels are low for pastures accroding to Lewis (1999) and Gourley *et al.* (2007), being less than half of what is considered adequate levels. Field molybdenum and boron levels were below detectable limits and were therefore considered low by Brennan and Bruce (1999) and Bell (1999) respectively.

Overall, the field site was deficient in zinc, nitrate, potassium and sulfur. Interestingly, these nutrients were those which were identified in the nutrient deficiency study (Chapter 7) as having symptoms most closely resembling those of BGD. Plant chemical analyses will determine if BGD affected plants are deficient in these nutrients.

8.5 Plant Chemical Analyses

8.5.1 Materials and Methods

Methodology

Though the soils showed no obvious differences between BGD affected and unaffected areas, it was considered necessary to analyse the plant chemical composition. This was mainly to determine if the soil nutrient deficiencies are reflected in the plants.

Since it is plausible that the BGD affected pastures are run down or water stressed, this investigation was primarily a comparative study between BGD affected and unaffected plants, rather than a comparison to previously obtained data on buffel grass. It should be mentioned that the following results were not compared with the plant chemical tests performed on plants from the nutrient deficiency trials in Chapter 7. Since these plants were part of a nutrient omission trial, the results are not comparable, as it is unlikely that a nutrient is completely missing from field soils.

Method

BGD affected plants and unaffected plants (whole plants) were collected from the field and oven-dried at 70ºC for three days. These plants were taken from the same transects and along the same distances as the soil samples used for the pH and salinity trial and chemical analysis (parts 8.3 and 8.4). The plants were digested and analysed by CSBP Limited, Western Australia for: nitrate-N, total nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, iron, manganese, copper, boron, molybdenum, chlorine and zinc. CSBP Limited measure total nitrogen using a Leco FP-428 Nitrogen Analyser following combustion. They measure chloride and nitrate using a Lachat Flow Injection Analyser

following extraction in deionised water. The other aforementioned elements are measured by ICP-AES following digestion by nitric acid using a Milestone microwave.

All data were analysed by randomised complete block (RCB) analyses with 5 replicates of the 6 distance treatments where replicates were the different patches. Departures from normality and/or constant variance were observed for iron, manganese and molybdenum. A log transformation was applied to iron and manganese, while a square root transformation was applied to molybdenum.

8.5.2 Results

There was no significant difference in many of the nutrient concentrations with distance from the patch. However, there were significant differences in some nutrients, namely molybdenum, sodium, sulfur, total nitrogen and zinc (Table 28). The concentration of molybdenum, compared with that of the plants in the BGD affected patches, was higher in the plants from the edge of the patches to a distance of 1 m from the patches. Subsequently, plants from 3-6 m from the patch had a lower concentration of molybdenum. The plants from within the BGD affected patches and those from up to 50 cm from the patches had a higher sodium concentration compared with plants further from the patches. Plants within the BGD affected patches also had higher concentrations of sulfur compared with plants further away from the patches. Plants which were the furthest from the patches had the lowest concentrations of sulfur. Similarly, plants within the affected patches had higher concentrations of total nitrogen compared with plants at all other distances. Plants from within BGD affected patches and those up to a distance of 1 m from the patches, as well as plants 6 m from the patches, had higher concentrations of zinc compared with the plants 3 m distance from the patches (Table 26).

Element	B	Ca	Cl	Cu	Fe	K	Mg	Mn	Mo
Distance	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*P*=0.037
in	4.49	0.2578	0.321	6.22	7.107 (1251)	0.997	0.1084	5.309 (229)	24.45 a (660)
edge	3.83	0.2354	0.274	5.96	7.170 (1329)	0.716	0.0998	5.068 (162)	30.30 b (963)
50cm	3.83	0.2496	0.250	5.05	6.295 (1045)	0.811	0.1086	5.002 (150)	29.76 b (915)
1m	3.26	0.2594	0.239	5.45	7.015 (1294)	0.715	0.1038	5.078 (167)	28.42 b (825)
3m	3.72	0.2606	0.250	5.94	7.064 (1481)	0.734	0.1002	5.088 (187)	26.87 ab (759)
6m	4.19	0.2558	0.214	5.46	7.186 (1342)	0.609	0.1040	5.150 (176)	27.98 ab (805)
s.e.d.	0.512	0.025	0.037	0.578	0.219	0.134	0.008	0.193	1.739

Table 26 - The effect of distance from a BGD affected patch on the concentration of nutrients in buffel grass (*Cenchrus ciliaris*) plants

Note: values in parentheses are back-transformed means. Means within an effect not followed by a common letter are significantly different (P=<0.05). "n.s." = not significant.

Element	Na	NO_3	P	S	Total N	Zn
Distance	*P*=0.008	n.s.	n.s.	*P*<0.001	*P*=0.007	*P*=0.035
in	0.01580 a	39.80	0.1544	0.0924 a	0.788 a	33.85 a
edge	0.01580 a	40.20	0.1492	0.0756 b	0.600 b	34.18 a
50cm	0.01460 ab	40.60	0.1702	0.0656 bc	0.487 b	30.40 a
1m	0.01400 b	42.60	0.1546	0.0622 c	0.540 b	29.48 ab
3m	0.01340 b	41.60	0.1538	0.0568 c	0.463 b	23.40 b
6m	0.01400 b	39.80	0.1306	0.0608 c	0.604 b	32.48 a
s.e.d.	0.0007	1.564	0.021	0.07	0.078	3.257

(Table 26 cont.) B, Cu, Fe, Mn, NO_3, and Zn are expressed as $mg.kg^{-1}$. Mo is expressed as $\mu g.kg^{-1}$. Ca, Cl, K, Mg, Na, P, S and total N are expressed as % composition.

8.5.3 Discussion

The results do not compare well with those of Graham and Conway (2000), whose work was done on the same site. In accordance with Graham and Conway (2000), nitrogen concentrations were higher in BGD affected buffel grass. However, their work also showed lower concentrations of phosphorus and potassium in BGD affected grass, and inconclusive results on sodium and chloride levels, none of which are in agreement with these results. Perhaps the difference was due to the fact that Graham and Conway (2000) sampled unaffected buffel grass from a separate paddock to the BGD affected buffel grass, rather than nearby plants.

Molybdenum, sodium, sulfur, total nitrogen and zinc were the only nutrients which showed significant differences in concentration along the transects. The predominant trend was a higher concentration of the nutrient inside the BGD affected patch, with the exception of molybdenum. Since there were no differences in the soil concentrations of these nutrients (refer part 8.4), the differences must be attributed to differences in uptake and/or accumulation.

There is a paucity of information regarding nutrient concentration standards in buffel grass, and in pasture grasses in general. Known recommended concentrations exist for some nutrients only. Therefore, in the following discussion, recommended concentrations will only be mentioned for certain nutrients.

Molybdenum concentrations were lower in plants within a BGD affected patch, were significantly higher from the patch edge, and decreased slightly at 3 m. Though molybdenum is a component of many plant enzymes, the reason behind these findings is unknown.

Sodium concentrations were higher in plants within the patch, and decreased from 50 cm away from the edge. Sodium levels are usually higher when plants are grown in saline soils (Graham and Humphreys, 1970). However, soil salinity was not significantly different along the transects (part 8.3), nor were soil sodium levels (part 8.4). Also, sodium concentrations were quite low compared with those reported by Blanco *et al.* (2007) from buffel plants which were not salt stressed. Therefore, it is highly unlikely that high sodium concentrations are the cause or a contributor to BGD.

Sulfur concentrations were higher in plants within the patch, decreased at the edge, and further decreased at 1 m. These concentrations were similar to those measured by McIvor (2007) for buffel grass; however, even the highest concentration measured in the field was lower than the 'adequate' concentration stipulated by Pinkerton *et al.* (1997) for buffel grass.

Total nitrogen concentrations were higher in plants within the patch, and decreased from the edge. However, these measurements were generally lower than those of McIvor (2007) for buffel grass.

Zinc concentrations were higher in plants within the patch up to 1 m away from the edge, decreased at 3 m from the edge, and increased again at 6 m. These results are unusual, and elude explanation. Possible future work into BGD should encompass zinc studies. The measured zinc concentrations fall within the 'adequate' range reported for several unrelated pasture grasses (Pinkerton *et al.*, 1997), so these may also be adequate levels for buffel grass.

So far, it appears that the field plants are deficient in several nutrients. Some conclusions can also be made of the nutrients which were not significantly different. Phosphorus concentrations were similar to the lower concentrations reported by McIvor

(2007), but were lower than the 'adequate' concentration stipulated by Pinkerton *et al.* (1997) for buffel grass. Similarly, potassium concentrations of field plants were three times lower than the 'critical' concentration stipulated by Pinkerton *et al.* (1997) for buffel grass. The magnesium concentrations of field plants are also lower than the 'adequate' concentration stipulated by Pinkerton *et al.* (1997) for *Panicum*, a comparable species in the same tribe as buffel grass. Conversely, the field plants do not appear to be deficient in calcium as, according to White and Broadley (2003), plants growing with adequate calcium have concentrations between 0.1 and 5%; the field measurements obtained were all within that range (Table 28). It is obvious that the field plants, BGD affected or otherwise, have nutritional problems. However, there does appear to be a relationship between BGD and molybdenum, sodium, sulfur, nitrogen, and possibly zinc.

Comparing these results with the soil chemical analyses (Section 8.4), the low plant levels of phosphorus and sulfur can be explained by the corresponding low soil levels of these nutrients. Similarly, the low plant nitrogen levels can be attributed to the low soil nitrate levels. Whether the plant zinc levels were low, and therefore corresponding with low soil levels, is unknown due to the lack of relevant literature. The low plant levels of potassium and magnesium did not correspond to soil levels, as the latter were in the adequate range. Further work is required regarding these two nutrients.

As previously mentioned, deficiencies in zinc, nitrogen, phosphorus and sulfur were all identified as having symptoms which most closely resemble that of BGD. Both the soil and plant chemical test results show that field plants are deficient in these nutrients, with the possible exception of zinc. Therefore, it is plausible that some of the symptoms of BGD are caused by these deficiencies. However, BGD affected plants only occur in patches in the field, and there are marked differences in foliar symptoms and plant

morphology between BGD affected and unaffected plants in the same field (Chapter 4), making this possibility unlikely. If these nutrients were the sole cause of the symptoms, all plants in the field would be exhibiting symptoms, not just patches of plants.

A potential explanation for these results is the involvement of a possible biological causal agent(s) of BGD. Many plant diseases cause a change in the nutritional status of the host plants, mainly by changing the membrane permeability of root cells and increasing root lysis (Shepherd, 1994). It is possible that the causal agent(s) of BGD interferes with the uptake mechanisms for those nutrients which showed significant differences. Plant nutrition requires further work, once the causal agent(s) of BGD has been identified.

With reference to finding the true patch boundary, there is no distinct edge for these nutrient differences, as the differences in concentration occur at different distances from the patch. However, if these nutrients are involved in the BGD condition, or are simply altered as an effect of root damage caused by the causal agent(s), the true patch boundary would be located among the changes in nutrient concentrations. It is possible that one or more biological causal agents would infect a plant approximately 1 m from the patch edge, and as the infection progresses and more root damage ensues, the uptake of more nutrients is affected.

A small digression; Ford and Wilson (1981) reported that buffel grass accumulates potassium and chloride when water stressed. As the potassium and chloride levels of BGD affected and unaffected field plants were not significantly different, it can be suggested that water stress is not the major causal agent of BGD.

8.6 Concluding Statements

There were no significant differences in soil salinity, soil pH or soil nutrients between BGD affected and unaffected areas, but there were overall soil deficiencies in zinc, nitrate, phosphorus and sulfur. There were significant differences in plant molybdenum, sodium, sulfur, total nitrogen and zinc between BGD affected and unaffected areas, and overall plant nutrient deficiencies in magnesium, nitrogen, potassium, phosphorus and sulfur. Some of these can be attributed to the soil deficiencies.

The nutrients which were deficient in both plants and soil were also those identified as producing deficiency symptoms similar to symptoms of BGD (Chapter 7). However, if these were the cause of BGD, the entire paddock would be symptomatic, not just patches. The differences may be attributed to a biological causal agent(s) damaging the roots and affecting nutrient uptake.

The true patch boundary is still elusive, though there is evidence that it is further than the patch edge.

Chapter 9

Investigations Into Biological Causes of BGD

9.1 Introduction

Biological agents cause many plant disorders and diseases. These agents include vertebrate animals, insects, other plants and microorganisms. In the case of BGD, it is unlikely that the cause is vertebrate animals or insects, since these would have been detected in the field observations outlined in Chapter 2. However, this does not disregard the possibility of vertebrate animals and/or insects being carriers or vectors of the condition.

Other plant species can also cause plant disorders. Many plant species produce root exudates or other compounds which are toxic or otherwise interfere with other plants. This chemical interference is called allelopathy (Putnam and Duke, 1978). While the paddocks in which BGD occurs are predominantly colonised by buffel grass, there are other plant species present, some of which may have allelopathic effects. Therefore, an investigation into possible allelopathic interactions was considered necessary.

Certain groups of microorganisms have beneficial interactions with plants but other groups cause many diseases. These organisms collectively have the ability to infect all parts of the plants and have various morphological and physiological effects on their host. Several chapters of this work have suggested that microorganisms are a likely cause of BGD. Therefore, microbial investigations formed an important part of this study.

9.2 Allelopathic Studies

9.2.1 Materials and Methods

Methodology

While there has been much debate as to whether or not allelopathy includes beneficial interactions, this work will only include the negative aspects (Inderjit *et al.*, 1995; Narwal, 1999), that is, interactions which negatively affect the growth of other plants.

The chemicals produced by allelopathic plants generally diffuse through the soil and affect the surrounding plants in the in a number of ways. These include:

- Triggering dormancy in seeds,
- Acting as a growth inhibitor,
- Having a toxic effect on plant cells (Inderjit *et al.*, 1995).

As with many chemical based responses, allelopathic interactions are also concentration specific (Singh *et al.*, 2001).

Based on this information it is plausible that the roughly circular patches of BGD affected buffel grass could stem from allelopathic interactions from plants in the centre of the patch. The patch would spread as the chemicals diffused and as the allelopathic agent reproduced and colonised other areas. New patches would appear where the allelopathic agent germinated.

Because of this possibility, a detailed survey of other plant species in the paddock was done, especially of those plants that were present in the bare central areas of BGD affected buffel grass patches. For comparison, a survey was also done on the plant population of an unaffected paddock. As this was a pilot study, the incidence of the plants

was not recorded, only their presence or absence. Any plants that were present only in BGD affected patches and/or BGD affected areas, and which have known allelopathic effects, would bear further investigation.

Method

Surveys were made of all plant species present in three locations:

- Exclusively in the patches of BGD affected buffel grass,
- In the paddock containing these patches (24º 20.10' S; 149º 51.75' E),
- In a paddock completely unaffected by BGD (24º 19.65' S; 149º 51.25' E).

The surveys were carried out by traversing the paddocks in a series of transects and collecting specimens of the plant species present in the near vicinity of pre-determined intervals. The first transect of each paddock was along a boundary fence line. Each subsequent transect was 5 m away from the previous one, with all transects having intervals of 5 m. This resulted in each paddock being surveyed as a 5 x 5 m grid. All patches in the BGD affected paddock were surveyed. There were more than 28 of these. Specimens of all species found were collected and pressed in a plant press, and their location noted. These were later identified using Anderson (2003) and Walters (1996). Trees were not included in this study, as they were generally present only along fence lines, while the central areas of the paddocks were essentially cleared of trees.

9.2.2 Results

The tables below list the plants collected from the three areas. Not all plants reported as being found in BGD affected patches were found in every patch.

Tables 27 and 28 list the plant species surveyed and their locations. There were two plants which were exclusively found in BGD affected patches: *Indigofera linifolia* and

Sclerolaena birchii. Several plant species were present in both BGD affected patches and paddocks, but not in unaffected areas.

Herbaceous species		Presence		
Species	Common name	Unaffected paddock	Affected paddock	Affected patch
Amaranthus interruptus	Native amaranth	✓	✓	✓
Atriplex muelleri	Annual saltbush		✓	
Crinum flaccidum	Murray lilly	✓	✓	✓
Crotalaria dissitiflora	Grey rattlepod		✓	✓
Crotalaria goreensis	Gambia pea	✓		✓
Evolvulus alsinoides	Tropical speedwell	✓	✓	✓
Glycine tabacina	Glycine pea		✓	✓
Gomphrena celosioides	Gomphrena weed	✓	✓	✓
Grewia latifolia	Dysentry plant	✓		
Indigofera linifolia	Narrow-leaved indigo			✓
Indigofera linnaei	Birdsville indigo	✓	✓	
Maireana microphylla	Small leaved cotton bush	✓		✓
Neptunia gracilis	Native sensitive plant		✓	
Opuntia stricta	Prickly pear		✓	
Opuntia tomentosa	Velvety tree pear	✓		
Portulaca oleracea	Pigweed	✓	✓	✓
Salsola kali	Soft roly-poly	✓	✓	✓
Sclerolaena birchii	Galvanised burr			✓
Sclerolaena muricata	Five-spined saltbush		✓	✓
Senecio lautus	Fireweed	✓	✓	
Sida cordifolia	Flannel weed	✓		✓
Sida subspicacta	Spiked sida		✓	✓
Tribulus terrestris	Caltrop	✓	✓	✓
Verbena tenuisecta	Mayne's pest	✓	✓	✓

Table 27 – Herbaceous plant species found in BGD affected patches and paddocks, and unaffected paddocks of buffel grass (*Cenchrus ciliaris*)

Grasses		Presence		
Species	Common name	Unaffected paddock	Affected paddock	Affected patch
Aristida calycina	Dark wiregrass	✓		
Aristida caput-medusa	Many headed wire grass	✓	✓	✓
Bothriochloa decipiens	Pitted bluegrass		✓	✓
Bothriochloa ewartiana	Desert bluegrass	✓	✓	✓
Cenchrus ciliaris (Biloela)	Biloela buffel	✓	✓	✓
Chloris divaricata	Slender chloris		✓	✓
Chloris inflata	Purpletop chloris	✓	✓	
Cymbopogon refractus	Barbwire grass	✓		
Dactyloctenium radulans	Button grass		✓	✓
Eragrostis leptostachya	Paddock love grass	✓	✓	
Fimbristylis dichotoma	Common finger rush		✓	
Malinis repens	Red natal grass		✓	
Paspalum dilatatum	Paspalum	✓	✓	✓
Plectrachne schinzii	Feathertop spinifex	✓	✓	
Sorghum nitidum	Brown sorghum		✓	✓
Tragus australianus	Small burrgrass	✓	✓	✓
Urochloa mosambicensis	Sabi grass	✓	✓	✓

Table 28 – Grass species found in BGD affected patches and paddocks, and unaffected paddocks of buffel grass (*Cenchrus ciliaris*)

9.2.3 Discussion

Many of the plants were present in all areas. Of those that were only present in affected patches and/or affected paddocks, none were reported as having allelopathic effects. Many have related species that are allelopathic, so it is possible that they are allelopathic, though not yet reported. *Portulaca oleracea* has known allelopathic effects (Han *et al.*, 1992), but it was present in all areas, and is, therefore, not likely to be the causal agent of BGD.

A point worth mentioning is that buffel grass itself has been argued as being allelopathic (Smith, 1999). To some degree this accounts for its extensive usage as a pasture grass, since it not only has a high conversion ratio in livestock but also colonises vast areas rapidly. Not only does it outcompete other species, it has actually been found to affect the growth of other pasture grasses as well as weeds and legumes (Smith, 1999). In relation to BGD, the fact that so many other plant species recolonise BGD affected patches shows that the allelopathic range of buffel grass is either no more than a few centimetres, that the buffel grass on the periphery of the patch is in a weakened state, that buffel grass is only weakly allelopathic, or that these chemicals are short-lived, and only have an effect when produced by a healthy plant. Therefore, if the cause of BGD is an allelopathic plant, it must be a plant that is more aggressive than buffel grass, and must also have allelopathic effects that are specific to buffel grass, since many other plant species are present in the vicinity but remain unaffected. It is unlikely that an allelopathic plant is host specific to such an extent. The other plants present in a BGD affected patch are most probably opportunistic colonisers.

9.3 Microbial Isolations

9.3.1 Materials and Methods

Methodology

Many species of microorganisms cause plant disease. However, many more species do not cause plant disease. The challenge for the plant pathologist is to determine which, if any, microorganisms associated with a diseased plant are responsible for symptoms. This usually includes the isolation and culture of the microorganisms present on various selective and non-selective media and the satisfaction of Koch's Postulates.

When attempting to isolate plant pathogens it is often necessary to first remove or kill opportunistic colonisers and saprophytes by means of surface sterilisation. There are several methods of surface sterilisation available, although not all were suitable for this study. Some solutions, such as mercuric chloride, are very hazardous. Others, such as sodium hypochlorite, break down rapidly. Silver nitrate was chosen as it is non-hazardous in the low concentrations in which it is used, and is stable in storage. It is also advantageous in that it is neutralised when sodium chloride is added, allowing precise exposure periods. The silver chloride precipitate produced by the neutralisation is easily rinsed off with distilled water. Silver nitrate is often used to surface sterilise plant explants for tissue culture (Sigma-Aldrich, 2004), but is also used in surface sterilisation prior to isolations (Davies, 1935; Speakman and Krueger, 1983).

Since a causal pathogen for BGD has not yet been identified, non-selective media were used for microbial isolations. These included half-strength potato dextrose agar (HPDA), mainly for fungal organisms (Atlas and Parks, 1993), and nutrient agar, mainly for bacteria. As *Fusarium oxysporum* was previously thought to be the cause of BGD (Payne, 2000), a selective medium for *Fusarium*, Malachite green agar, which is a modified Nash and Snyder medium (Booth, 1971), was also included (Castella *et al.*, 1997). Some fungi require certain plant chemical compounds to grow (Richie, 2002). Therefore, V8 vegetable juice agar (Richie, 2002) was included. Plain water agar was also included to isolate microorganisms which thrive on low nutrient concentrations. In case the potential causal agent of BGD requires compounds from buffel grass, a buffel grass agar was also used. This was a modified carnation leaf agar (Fisher *et al.*, 1982) substituting carnation leaves with small pieces of buffel grass. These pieces were from both leaves and roots, and

were sterilised before being embedded. Sterilisation was by 2 min in 25% bleach, followed by two rinses with sterile distilled water.

All recovered colonies were subcultured and maintained on the same type of media from which they were isolated, in case these contained essential nutrients for their growth. All isolated microorganisms would then be identified and tested for pathogenicity and causality of BGD (in Section 9.4).

Method

For the entire duration of this study, on every field trip (32 total) BGD affected leaves and roots (entire), leaf lesions and soil from both BGD affected patches and the root zone of BGD affected plants were brought back from the field. On each field trip, at least 20 plants were sampled, with 3-4 samples being taken from each plant. Seeds from BGD affected plants were also collected when available. Similar samples were also taken from *Urochloa mosambicensis*, as this species may also be affected by the causal agent(s) of BGD (Chapter 2). Symptomatic *U. mosambicensis* plants were not always present. Therefore, these were only sampled on four occasions: twice at the beginning of this study and twice at the end, when more symptomatic plants appeared.

The plant parts targeted for isolations were:

- Leaves – the red/green symptom boundary, completely red areas, lesions (from Chapter 2) and other damaged areas,
- Roots – young roots, sections of roots (of any age) which had lesions and/or cortex damage/necrosis,
- Seeds from BGD affected plants.

Selected plant parts were cut into 5 x 5 mm sections and were surface sterilised by immersion in 1% $AgNO_3$ for two minutes, followed by a 30 sec. rinse with 5% NaCl to deactivate the silver nitrate, and two rinses with sterile distilled water (Speakman and Krueger, 1983). These plant segments were then plated onto various media, four per plate, ensuring that only four derived from same plant part were on each plate.

Several different media were used, with at least five of each medium being used for each plant part. The media used were: half-strength potato dextrose agar (HPDA), nutrient agar (NA), Malachite green agar made according to Castella (1997), V8 vegetable juice agar made according to Richie (2002), water agar, and a plain water agar with embedded sterilised leaf and root sections of unaffected buffel grass. Prior to inoculation, all media were incubated for 2 days at 28ºC in a sealed container, to ensure that they were sterile.

The inoculated plates were placed in an incubator at 28ºC. The resulting isolates were subcultured onto the medium from which they were isolated and were kept in an incubator at 28ºC. These were subcultured weekly.

Isolations from the field soil samples were attempted using standard soil dilution plating as per Larkin *et al.* (1995), with the resulting solutions being plated out onto 5 each of the above media. In addition, isolations were also attempted using the soil immersion technique as per MacLeod and Sweetingham (1997), burying plates within the root zone of BGD affected plants in the field. A total of 25 plates of the media prescribed by MacLeod and Sweetingham (1997) were used, with 5 plates being used in each of 5 sites.

9.3.2 Results

Tables 29 to 31 list the microorganisms isolated from BGD affected buffel grass, symptomatic *U. mosambicensis* plants and soil, respectively. In total, 65 isolates were

obtained from BGD affected buffel grass, 14 from *U. mosambicensis*, and 21 from soil. Most were identified to genus level using Ellis (1976) and Carmichael *et al.* (1980). Others were non-sporing isolates which could not be identified.

Genus	Location isolated	Incidence
Sclerotium	root	1
a filamentous bacterium	root	1
Thielavia	root	3
Fusarium	root	3
Alternaria	root	1
Phialophora	root	1
Papulospora	root	1
Sporothrix	root	1
Thielaviopsis	root	1
Phytophthora	root	1
Pythium	root	1
non-sporing isolates	root	8
Phoma	leaf	1
Aureobasidium	leaf	4
Curvularia	leaf	9
Acremonium	leaf	2
Alternaria	leaf	2
Colletotrichum	leaf	1
Mycosphaerella	leaf	1
Scytalidium	leaf	1
Pleospora	leaf	1
Phialophora	leaf	2
non-sporing isolates	leaf	3
Eupenicillium	lesion	1
Cladosporium	lesion	4
Penicillium	lesion	2
Fusarium	seed	3
Penicillium	seed	2
Curvularia	seed	2
Alternaria	seed	1

Table 29 – Microorganisms isolated from various plant parts of BGD affected buffel grass (*Cenchrus ciliaris*)

Genus	Location isolated	Incidence
Eurotium	root	1
Acremonium sp.1	root	1
Acremonium sp.2	root	1
non-sporing isolate	root	1
Ascochyta	leaf	1
Helminthosporium	leaf	1
Acremonium sp.3	leaf	2
Drechslera	leaf	2
Phomopsis	leaf	1
Aschochyta	leaf	1
non-sporing isolate	leaf	2

Table 30 – Microorganisms isolated from various plant parts of symptomatic *Urochloa mosambicensis*

Genus	Method	Incidence
Fusarium	soil dilution	3
	soil immersion	2
Alternaria	soil dilution	2
Curvularia	soil dilution	2
	soil immersion	1
Penicillium	soil dilution	3
	soil immersion	2
Mortierella	soil immersion	1
Absidia	soil immersion	1
Trichoderma	soil immersion	1
Cepahlosporium	soil immersion	1
Aspergillus	soil immersion	1
Gliocladium	soil immersion	1

Table 31 – Microorganisms isolated from soil from the root zone of BGD affected buffel grass (*Cenchrus ciliaris*)

9.3.3 Discussion

All but one isolate, an unidentified filamentous bacterium, were fungi. This may be due to the fungi outcompeting the bacteria either in the field or in the Petri dish. Some species would not have been isolated due to competition, dormancy, being fastidious or

simply not being present where and when samples were taken. Of those that were isolated and identified, most belong to genera that contain known plant pathogens, although all can be considered saprophytes.

Some *Sclerotium* species are responsible for white rot of onion and garlic (Dennis, 2001; Duff *et al.*, 2001), stem rot of rice (Ni *et al.*, 2001), root rot of sugarbeet (Das and Raj, 2004), stem rot of soybean (Bag and Sinha, 1997) and several others. It mostly affects the roots or stem and is transmitted through soil. Ortego (1997) reported that at least one *Sclerotium* species, *S. bataticola*, produces plant cell wall degrading enzymes.

Several *Thielavia* species are pathogenic, for example, causing stolon and root rot diseases of Japanese mint. It is also transmitted through soil (Sattar and Husain, 1982).

A number of *Fusarium* species cause many plant diseases, usually wilts and root rots. For example, they are important pathogens of maize, causing seed rot, seedling blight, root rot, stalk rot and ear rot (Asran and Buchenauer, 2003). They are usually soilborne.

Several *Alternaria* species are the cause of many root rots (Shazia and Iftikhar, 2005), but are also the cause of black spot diseases of crucifers (Guillemette *et al.*, 2004), leaf and stem blight of sunflower (Prathuangwong *et al.*, 1991), and others. They can be both soil and seed borne.

A few *Phytophthora* species are well known as the causal agents of potato blight, which led to the Irish potato famine of 1845 (Ristaino *et al.*, 2001), blight and root rot of tomato (Lingua *et al.*, 2001), and root rot of pepper plants (Matheron and Porchas, 2002) and soybean (Kaitany, 2000), among others. They are commonly soilborne.

A number of *Phoma* species are reported as causing root rot in alfalfa (Hollingsworth, 2002), black stem of sunflower (Al-Chaarani *et al.*, 2002), canker of parsnip (Cerkauskas and McGarvey, 1988), and mal secco disease of citrus trees. They

have the ability to infect many plant parts and species, and are mostly soilborne, though they can spread internally to other parts of the host plant (Muhle, 1958).

Some *Curvularia* species cause a rot of guava (Kapoor, 1983), leaf blotch disease of palmarosa (Janardhanan *et al.*, 1980), and are also reported to have caused leaf spot of buffel grass in Papua New Guinea (Lenne, 1990).

Some *Acremonium* species are endophytes commonly found in tall fescue (Joost, 1995), but others are also a wilt pathogen of sorghum (Issoufou, 2000) and melons (Martinez-Culebras *et al.*, 2004). It is generally soilborne.

A few *Colletotrichum* species are mostly known for causing anthracnose in many plant species, including banana (Thangavelu *et al.*, 2004) and citrus (Chung *et al.*, 2003). They can also cause diseases of sugar cane (Viswanathan *et al.*, 2003) and turfgrass (Khan and Hsiang, 2003). It is mostly spread via air and rain splash.

A number of *Phialophora* species are generally vascular pathogens causing brown stem rot of soybean (Chen *et al.*, 2000) and wilt diseases such as in carnations (Niemann, 1994).

Plant pathogenic *Cladosporium* species are mostly known as the cause of leaf mould of tomato (Oliver *et al.*, 2000), though it was also associated with leaf blight of buffel grass in Tanzania (Lenne, 1990).

Several *Pythium* species cause root diseases on a variety of plant hosts (Hendrix and Campbell, 1973) including large patch of zoysia grass (Aoyagi and Kageyama, 1999). They are predominantly soilborne.

A few *Penicillium* species cause rots of ornamental plant species (Wright *et al.*, 2003) and stem rot of cucumber (O'Neill *et al.*, 1991), among others. They are mainly airborne pathogens.

As can be seen from the above examples, many of the microbial genera isolated have at least one species which is a known plant pathogen. There may also be some plant pathogens among the unidentified isolates. Possibly one of the many isolates may be the causal agent of BGD. Therefore, each isolate needed to be tested for pathogenicity to buffel grass, and tested for the production of BGD symptoms.

9.4 Koch's Postulates

9.4.1 Materials and Methods

Methodology

The determination of the organism responsible for symptoms on a diseased plant is normally achieved by the application of Koch's Postulates. These state:

1. The suspected causal organism must be associated with symptoms of the disease.
2. This organism must be isolated and grown in pure culture.
3. The suspect organism must be inoculated from pure culture onto healthy test plants, and these must produce at least some of the characteristic symptoms of the disease.
4. The suspect organism must be reisolated from the test plants and be identical to the organism originally isolated (Strange, 2003).

In this case, there were 100 suspect organisms. Therefore, to apply Koch's Postulates, each should be inoculated onto healthy test plants. However, it is possible that more than one of these organisms is involved in the BGD condition. Consequently, as well as using pure cultures, mixed cultures of these organisms were used.

Some plant pathogens require a wound to enter the host plants. Threfore, both leaf and root wounding methods were used for inoculations, as well as non-wounding methods.

Since there is a possibility that soil chemistry, nutrients, or other factors are involved in the BGD condition, field soil from a BGD affected paddock was used to grow the healthy test plants.

Method

Isolates became available in groups, generally following field trips. During each spring/summer growing season, the following tests were done on isolates which had been isolated throughout the year. These tests were done when BGD symptoms were present in the field, so that conditions in the shade house would presumably be similar to those required for BGD to occur.

Commercial American buffel grass seeds obtained from Queensland Independent Seeds were planted into 12 cm diameter x 20 cm deep pots containing field soil from a BGD affected paddock. There were 5 seeds planted in each pot. One fifth of these pots each had two sterile rectangular hard plastic plant tags inserted into the soil (about 2 cm from the pot edge) so that the tags were almost completely buried, with only 1-2 cm showing above the soil surface. As the seeds germinated and grew, the roots would grow around the tags. The tags could then be removed at a later date resulting in two channels into the soil with no root damage.

The pots were placed on benches in a shade house and kept moist. The seedlings were watered as required and were allowed to grow to the third leaf stage. There were 15 of these healthy plant pots for every isolate to be tested, including three pots with inserted plant tags.

Several plates of the same physiological age were obtained for each isolate to be tested. The number of plates used depended on the reproductive output of the isolate, that is, the more spores an isolate produced, the fewer plates were used. When the plates were sporulating, the plates were flooded with a 1% Tween 80 solution and the colonies scraped with a spreader. The resulting spore suspensions were adjusted to 5×10^5 spores/mL and were poured into McCartney bottles. For non-sporing isolates, the same procedure was used resulting in suspensions of hyphal fragments, which were also adjusted to 5×10^5 fragments/mL. From these suspensions, mixed species suspensions were also produced, specifically all root isolates, all leaf isolates, all lesion isolates, and all soil isolates.

Each isolate (including the mixed cultures) was inoculated onto 15 healthy seedlings using five different methods, that is, three replicates per method. The methods were as follows:

1. Leaf inoculations – no wounding: a spore suspension was sprayed on the leaves of the seedling until run-off.
2. Leaf inoculations – stab wounds: each leaf of the seedling was wounded using a cork in which five sterile needles were inserted, thereby resulting in stabbing wounds. A spore suspension was then sprayed on the leaves of the seedling until run-off.
3. Leaf inoculations – crushing wounds: each leaf of the seedling was gently crushed with sterile forceps. A spore suspension was then sprayed on the leaves of the seedling until run-off.
4. Root inoculations – no wounding: this method used the pots with the inserted plant tags. The plant tags were removed, resulting in two channels into the pot. Ten mL

of a spore suspension was poured into each channel (the same spore suspension for each pot), and the channels were subsequently covered with pot soil.

5. Root inoculations – stab wounds: a sterile sharp scalpel blade was stabbed into the soil surrounding the seedling 5 times. Twenty mL of a spore suspension was poured around the seedling.

In addition, at each set of tests, 15 control plants were used which were inoculated with sterile water using the above methods. These methods were adapted from Dhingra and Sinclair, (1994).

The plants were randomly distributed on the benches in the shade house and were covered with plastic sleeves to avoid plant-to-plant contact (Figure 61), and to maintain humidity. Plants were watered directly into the soil to avoid splash, and were watered just enough to prevent wilting, that is, with the equivalent of *ca*. 15 mm of rain per week. The plants were observed for any symptoms for 8 weeks. In addition, 0.1 mL of each spore suspension was plated out onto HPDA, to ascertain spore viability.

Figure 61 - Individual experimental buffel grass (*Cenchrus ciliaris*) plant showing plastic sleeve to prevent plant-to-plant contact and maintain humidity

9.4.2 Results

None of the seedlings displayed buffel grass dieback symptoms. In fact, none of the seedlings produced any symptoms. All spore suspensions had > 70% germination.

9.4.3 Discussion

There are several possible explanations as to why none of the seedlings produced BGD symptoms. One obvious reason is that the causal agent of BGD was not isolated. This may be due to it being a fastidious organism, being outcompeted by other isolates, or that it cannot be cultured. The main limitation with isolations and Koch's Postulates is that it can only be done with organisms that can be cultured. This excludes all obligate pathogens including many bacteria and fungi, and also phytoplasmas, viruses and viroids (Strange, 2003). Another possibility is that the causal organism was isolated, but lost pathogenicity in culture (Strange, 2003), was isolated in a non-virulent form, or that the isolation methods were inappropriate for the causal agent(s) .

Some pathogens require vectors to infect host plants. If the causal agent of BGD was isolated but requires a vector, and the vector was not present, the test plants would not be infected and would not display symptoms. It may also be possible that physical conditions, such as available water, were not conducive to infection. Finally, it may be that the cause of BGD is not biological, though previous chapters indicate otherwise.

9.5 Concluding Statements

From the above results it seems likely that the main causal agent(s) of BGD is not an allelopathic interaction, although any allelopathic interactions present may be weakening the plants. None of the 85 microorganisms isolated from BGD affected plants produced

BGD symptoms on test plants. However, this could be due to a number of reasons, and does not eliminate the possibility of a biological causal agent. If the causal agent(s) are microbiological, they are likely to be difficult to isolate, fastidious, or are non-culturable such as obligate pathogens.

Chapter 10

Studies on the Transmission of BGD

10.1 Introduction

Previous observations and patch studies suggested that BGD is soilborne. However, this needed to be tested under controlled conditions. Therefore, to investigate this possibility, as well as to identify factors necessary for the spread of the condition, shade house studies were done supported by field trials. In addition, other possible modes of spread needed to be investigated, to determine if the condition is also spread by other means. Therefore, plant-to-plant transmission of the BGD condition was also investigated.

The possibility of BGD being seed borne also needed to be investigated. Several diseases of grasses and cereals, such as sorghum downy mildew, caused by *Peronosclerospora sorghi* (Yao *et al.*, 1990), safflower rust, caused by *Puccinia carthami* (Cappelli and Zazzerini, 1988), and barley stripe mosaic virus (Edwards, 1995) are seed borne. The possibility of the primary causal agent of BGD being a seed borne pathogen needed to be studied for two reasons. Firstly, the discovery of a seed borne pathogen would facilitate the creation of a model for the spread of patches and the emergence of new patches, as it would be based on the dispersal patterns of buffel grass. Secondly, if the condition is seed borne, selling and buying seed from neighbouring properties or from a commercial dealer may spread the condition to other properties and districts.

10.2 Transmission of Buffel Grass Dieback Through Seed

10.2.1 Materials and Methods

Methodology

While isolations are useful (Chapter 9), the causal agent (if it is biological) may not be isolated due to the wrong type of medium and/or growth conditions, or the causal agent may have been killed by the surface sterilisation. The causal agent may also be dormant in the seed, and may not grow until the seed germinates. Therefore it was necessary to plant out seeds from BGD affected plants, and determine if the seedlings developed symptoms. Self-sown seedlings in the field succumb to BGD before reaching the third leaf stage, so allowing the shade house seedlings to grow to twice that age should be sufficient for symptoms to develop. To minimise the effects of soil chemistry, which could affect the development of symptoms (as suggested in Chapter 9), field soil was used for shade house trials.

Method

Ten Styrofoam boxes (each 39 x 58 x 25 cm deep) were filled with field soil from BGD affected patches, which had been collected to a depth of 30 cm. One thousand seeds from BGD affected plants were randomly selected. One hundred seeds were planted into each Styrofoam box. The boxes were kept moist and observed for emergence. The seedlings were allowed to mature to the 6 leaf stage and observed for symptoms of BGD.

10.2.2 Results

A total of 42 seeds emerged. None of the seedlings exhibited BGD symptoms. Since there was no variance, statistical analyses were not necessary.

10.2.3 Discussion

As none of the seedlings exhibited symptoms, even in field soil from affected patches, it is reasonable to suggest that either the dieback condition is not transmitted through seed, or that it has a very low transmission rate. This also suggests that soil nutrients are not the primary causal agent of BGD.

Why the condition failed to appear in soil from a BGD affected patch is unknown. Possibly, the condition is not soilborne, otherwise perhaps other environmental factors are necessary, or disturbing the soil affects the causal agent(s) of the condition.

10.3 Shade House Studies on Symptom Expression in Field Soil

10.3.1 Materials and Methods

Soil Collection

Since the factors which cause BGD are unknown, field soil was used in shade house experiments in an attempt to reproduce field conditions. Field soil would theoretically have the chemical and/or biological composition which potentially causes the dieback condition, providing that the condition is soilborne. Sterilising field soil would remove the biological aspect, making it possible to determine if the primary causal agent is biological or chemical.

Soil was collected from the field in two places: from an area heavily affected by BGD and from an unaffected area. In each area, three randomly selected samples were taken from the top 30 cm of the soil profile. These were consequently mixed, resulting in a representative sample for the area. This generated 90 L of soil from a BGD affected area, and 90 L of soil from an unaffected area.

Both soil lots were split in half. One half of each lot was left untreated. The other half of each lot was sterilised in a steam steriliser. The sterilisation treatment consisted of two 1 h periods at minimum 80ºC, 600 kPa, with a 4 h cooling period in between. While this may have resulted in 'pasteurisation' rather than sterilisation, this was the only apparatus available at the time, and while the apparatus was operated at its maximum settings, a temperature of 100ºC could not always be reached. The resulting soil may not have been 100% sterile, so for the purposes of this experiment it will be referred to as 'steam treated'.

The treatment resulted in four soil types:

- Soil from an unaffected paddock
- Steam treated soil from an unaffected paddock
- Soil from a BGD affected paddock
- Steam treated soil from a BGD affected paddock.

Methodology

If BGD is soilborne, the causal agent(s) must be biological, chemical, or both. Planting seeds from unaffected plants (such as commercial buffel grass seed, which are pathogen tested) in the four abovementioned soil types may reveal the nature of the causal agent depending on which soil type yields plants displaying BGD symptoms. For example, if symptoms appear in the plants in non-sterile soil from a BGD affected paddock, but in no other treatments, it can be assumed that the cause is biological. If symptoms appear in both steam treated and non-sterile soil from a BGD affected area, it can be assumed that either the cause is chemical or a heat resistant pathogen. Furthermore, the development of BGD symptoms in any treatment would confirm that the condition is soilborne.

For the duration of the investigation examining the spread of the condition through soil, it was important to minimise other potential modes of spread, such as through water or plant-to-plant contact. Experimental plants were therefore isolated from each other using barriers which still allowed light to penetrate, in this case plastic sheeting.

Method

This experiment was conducted when BGD was present in the field, thereby presumably encompassing weather which was conducive to the development of BGD.

Thirty pots of each soil type (total 120 pots) were planted with commercial buffel grass seed; three to each pot. Thirty pots of each soil type were also planted with healthy seedlings (one per pot) at the 6 leaf stage, which had been grown from commercial buffel grass seed. As a control, 30 pots of each soil type were also planted with seeds from BGD affected plants (ten to each pot) and BGD affected seedlings, carefully collected from the field avoiding root damage. The seedlings were all around the 6 leaf stage, and in the middle stages of the condition, that is, only the oldest two or three leaves were displaying symptoms. All seedlings, BGD affected or unaffected, had their roots washed of all visible soil material. This resulted in a total of 480 pots.

The pots were placed on benches in a completely random design, each with an individual water tray. Clear plastic sleeves, consisting of two A4 sized cover sheets stapled lengthways to create a cylinder, were placed over each pot, thereby preventing plant-to-plant contact (illustrated in Figure 61, Chapter 9, p.229). Seedlings were watered weekly, and were watered individually to prevent soil splash. The plants were allowed to grow and were observed weekly for BGD symptoms over a 3 month period.

10.3.2 Results

None of the unaffected plants in any soil treatment displayed BGD symptoms. Four plants showed a slight reddening of the leaves, but the colour was sparsely mottled and dull, unlike the bright solid coloured areas on BGD affected plants. These symptoms most closely resembled nitrogen or phosphorus deficiency symptoms described in Chapter 7. Of the plants which had germinated from seed from BGD affected plants (total 11 plants), none displayed BGD symptoms. Due to the lack of variability, statistical analyses were not possible.

Most of the transplanted BGD affected plants had died within 6 weeks. Interestingly, some of the surviving plants recovered within a month and never displayed BGD symptoms again for the remainder of the experiment. Other plants continued displaying BGD symptoms for the entire duration of the experiment. Table 32 displays the relative numbers of these results by soil type.

Soil type	Plant Count		
	Affected	Recovered	Died
From affected patch, steam treated	4	2	24
From affected patch, non-sterile	6	1	23
From unaffected area, steam treated	4	4	22
From unaffected area, non-sterile	6	6	18

Table 32 - Number of BGD affected buffel grass (*Cenchrus ciliaris*) plants in each category after growing in various soil types

Due to the categorical nature of the data, a contingency table and chi-square analysis was done. The Pearson chi-square statistic was used.

The number of plants in each category was not significantly influenced ($P = 0.391$) by soil type.

10.3.3 Discussion

None of the unaffected plants displayed BGD symptoms. Several possibilities arise. Firstly, perhaps the condition is not spread through soil. Secondly, there may be some environmental parameter present in the field which is necessary for symptom development. Thirdly, disturbing the soil through digging and sterilising may have disrupted or destroyed the causal agent. The temporary disappearance of symptoms after tilling was also noticed by primary producers (refer Part 2.2 – Interviews/ Discussions with Primary Producers), which supports this theory. The possibility of soil disturbance and its role in the transmission of BGD should be investigated before the hypothesis that BGD is soilborne is refuted.

With the BGD affected plants, many plants died. Possibly the plants were too far along in symptom development to permit recovery. Care should be taken in future experiments to select plants in the early to middle stages of symptom development.

10.4 Effects of Soil Disturbance

10.4.1 Materials and Methods

Methodology

A possible explanation for the results of the above experiment was soil disturbance. This possibility needed to be investigated as it was imperative that the condition be reproducible in the shade house for further studies to be done.

In the above experiments, the field soil was manually disturbed through the digging process, and also when being transferred in and out of the steam steriliser. Therefore, obtaining undisturbed field soil would be difficult since the act of digging it out would

disturb it. A possible solution would be to use soil coring tubes to collect the soil (James *et al.*, 1997). While there would be some disturbance where the soil was in contact with the coring tube, the remainder would be essentially undisturbed. As transplanting also requires soil disturbance, it would be advantageous to collect experimental plants within the intact soil core. Commercially available corers, with a diameter large enough to accommodate a plant without damaging the roots, are not suitable for one or two people to use without specialised coring equipment. Therefore, suitable corers had to be made.

Method

Soil corers were made by cutting a length of 12 cm diameter PVC plumbing pipe into 40cm long lengths. Two 2.5 cm diameter holes were drilled opposite each other 3 cm from what was designated as the top end, and the bottom edge was slightly sharpened using an angle grinder (Figure 62).

Figure 62 - Photograph of the PVC implement used for obtaining intact soil cores

A field trip for plant and soil collection was undertaken one week after a moderate rainfall event, so that the soil cores would adhere and not crumble. In the field, 40 BGD

affected seedlings were selected. All were around the 6-8 leaf stage, and were in the early to middle stages of symptom development. A soil corer was placed over the seedling and a 1 cm thick steel plate was placed on top. The corer was driven approximately 20 cm into the ground by repeatedly striking the steel plate with a sledge hammer. The length of the corer ensured that the foliage was not damaged. Once the corer was at sufficient depth, a steel bar (2 cm diameter) was inserted through the two holes in the top of the corer. Using the bar as leverage, the corer was given two quarter twists and pulled out of the ground. The corer was washed in a 1% bleach solution and rinsed in water after each core was taken to minimise possible cross contamination.

To produce plants in undisturbed soil, the corer (containing soil core and plant) was placed directly over a pot (12 cm diameter x 20 cm deep) and gently tapped on the sides, so that the entire core slid out and dropped into the pot. To produce plants in disturbed soil, the corer was gently tapped so that the core slid out. The core was held over a pot (same dimensions as above) and the soil was manually disturbed so that the soil crumbled. The soil within 2 mm of the roots was left undisturbed so as not to damage them. The plant and disturbed soil was gently dropped into the pot, and the soil was lightly packed down.

Using this method, 20 plants in disturbed soil and 20 plants in undisturbed soil were produced. These were brought back to the shade house and were placed on a bench in a random design, and were all covered with a plastic sleeve to prevent contact. The plants were watered weekly and observed for symptom progression or recovery.

10.4.2 Results

In all cases, the BGD affected plants in disturbed soil recovered.

All affected plants in intact soil cores remained affected and continued symptom development.

Due to the lack of variability, statistical analyses were not possible.

It should be mentioned that this experiment was twice repeated (done a total of three times), each time producing the same results.

10.4.3 Discussion

It has been conclusively demonstrated that soil disturbance disrupts BGD. There is also a strong possibility that the condition is soilborne. There are several plant pathogens which are impeded by soil disturbance. For example, conventionally ploughed fields have a reduced disease incidence of *Rhizoctonia* root rot of wheat compared with direct drilled crops (Jarvis and Brennan, 1986; Cook, 2001).

Since BGD was now reproducible in the shade house, other investigations could be undertaken.

10.5 Plant-to-plant Transmission of BGD Through Foliage

10.5.1 Materials and Methods

Methodology

While it has been suggested that BGD is soilborne, other modes of spread should also be investigated, such as plant-to-plant transmission. Many rusts are spread by plant-to-plant contact, for example, yellow rust of wheat (Lemaire *et al.*, 2002). Whilst it is unlikely that the cause of BGD is a rust (refer Chapter 2, Part 2.3), other disease causing organisms also use this method of spread, such as *Xanthomonas campestris*, causal agent of bacterial

leaf spot of pepper (Bernal and Berger, 1996). An understanding of the spread of BGD is essential for further work on the possible control of the condition.

In studying the possible spread of the condition through foliar contact, the possibility of other p methods of spread, such as through soil or water, must be minimised.

Method

Twenty unaffected plants were grown from commercial seeds in field soil, which was collected to a depth of 30 cm from a BGD unaffected paddock. Twenty BGD affected plants were collected from a BGD affected paddock with intact soil cores, using the method described in Section 10.4. All plants were in individual pots and around the 8 leaf stage at the start of the trial. The plants were placed on a bench in two 4 x 5 blocks, each of which was in an alternating pattern. The plants were placed directly adjacent to each other so that there was foliar contact. Each plant had its own watering tray to minimise the chances of the condition spreading through water. As some pathogens, such as *Xanthomonas campestris*, require leaf moisture for successful transmission to occur (Bernal and Berger, 1996), one of these blocks was covered with clear plastic sheeting so as to maintain high humidity. The plants were watered weekly on an individual basis to prevent water or soil splash, and were observed for 12 weeks for signs of symptom spread.

10.5.2 Results

None of the unaffected plants developed BGD symptoms. The BGD affected plants remained affected and continued symptom development.

Statistical analyses were not possible due to the lack of variance.

10.5.3 Discussion

There is a strong likelihood that BGD is not spread by plant-to-plant contact, at least not through foliage. As the plants were in a shade house, where wind is not impeded, there is also a strong likelihood that BGD is not transmitted by wind dispersal. Therefore, BGD must be transmitted through soil, water, vectors or other unidentified means.

10.6 Soil Core Transmission

10.6.1 Materials and Methods

Methodology

The above studies have suggested that the causal agent(s) of BGD are soilborne. Testing this theory is difficult since the condition only persists in the shade house in intact soil cores. To overcome this problem, seeds were planted in the intact cores, and the resulting seedlings observed for the development of symptoms. Planting seeds causes minimal disturbance; planting seedlings may disturb the soil, removing the condition.

If the primary causal agent of BGD is a disease causing organism, it may require a host plant to be actively growing. This is always the situation with viruses, which are obligate pathogens, but it can also be true of parasitic disease causing organisms, such as the plant parasitic bacterium *Clavibacter xyli* subsp. *cynodontis*, isolated from Bermuda grass (Haapalainen *et al.*, 2000). For this reason, soil cores both with and without BGD affected plants were included in this study. Apart from determining whether or not the condition is spread through soil, it might provide important information as to the nature of the causal agent(s).

Method

Forty soil cores were collected from the field in the manner described in Section 10.4. Twenty of these contained an affected plant in the 6-8 leaf stage and in the middle stages of BGD symptom progression. The other twenty soil cores were from within BGD affected patches but without affected plants. These were placed on a bench in a shade house in a completely randomised design. Three commercial seeds were planted into each pot and the pots were watered weekly. Germinated seedlings were allowed to grow to the 6 leaf stage and observed for symptom development.

10.6.2 Results

The seedlings in the ten pots containing soil cores only did not develop BGD symptoms. The seedlings in the ten pots with a BGD affected plant all developed BGD symptoms around the third leaf stage.

This experiment was repeated at a later date, at the next time when symptoms were present in the field, producing the same results.

Statistical analyses were not possible due to the lack of variability.

10.6.3 Discussion

There is evidence to propose that BGD is soilborne, though it seems to require the presence of other affected plants to spread. It is possible that BGD is reliant on root spread. There is also a strong possibility that it is caused primarily by a soilborne pathogen. Whether this pathogen is an obligate pathogen or parasite, or that it requires the presence of a host to become pathogenic, either through a biochemical trigger, or through growing on the host to reach a critical infective dose, has not been determined.

10.7 Soil Transmission of BGD

10.7.1 Materials and Methods

Methodology

It has been proposed that BGD is transmitted through soil in the shade house. The most logical method to investigate this possibility would be to transplant BGD affected plants in intact soil cores into pots with healthy buffel growth, with some of these cores being surrounded by a soil barrier. It was suggested to use pots rather than a complete soil barrier, as a complete soil barrier may create drainage problems and cause other symptoms which may compromise the results. Pots with small drainage holes still provide a *ca.* 98% soil barrier while still allowing drainage.

Method

Twenty styrofoam boxes, 40 cm x 60 cm x 30 cm deep, were filled with soil from a BGD affected patch, making sure that there were no plant parts in the soil. All boxes were sown with commercial buffel seed (*ca.*10 per 100 cm^2), and watered weekly. The seedlings were allowed to grow to the 6 leaf stage.

Twenty affected buffel seedlings were obtained from the field with intact soil cores, as described in Section 10.4. All were around the 6-8 leaf stage and in the middle stages of symptom development. In each of the twenty styrofoam boxes, a core was taken from the centre. The affected buffel seedlings were transplanted into these boxes; half of them were transplanted with the pot, the other half without.

These boxes were placed four to a bench on five benches in a randomised block design. The plants were watered weekly and observed for symptom spread over 6 months.

10.7.2 Results

There was an initial two month period in which symptoms did not spread. Following this, red foliar symptoms appeared on the healthy plants in all of the boxes without soil barriers (pots). Two days later, in all but two boxes red foliar symptoms appeared on the healthy plants of the boxes with soil barriers.

The two boxes in which symptoms did not spread both had affected plants without pots; the transplanted BGD affected plant in both of these boxes died in the early stages of the experiment.

Another point of interest is that if the plants had more than adequate water, they temporarily recovered from symptoms.

Statistical analyses were not possible.

10.7.3 Discussion

The results show strong evidence that the causal agent(s) of BGD is soilborne. The initial delay could be from soil disturbance, as initially the soil in the boxes was disturbed. This confirms results from previous studies.

The two day delay between symptom appearance in boxes with and without soil barriers suggests again that the condition is soilborne. The small delay was possibly due to the drainage holes in the pots and the fact that the Styrofoam boxes did not have drainage holes. Any excess water from watering would have stayed in the bottom of the containers, providing water contact between the plant in the pot and those on the other side. This may have facilitated the spread of a soilborne pathogen.

The results from the two boxes where there was no spread of symptoms suggest that the primary causal agent is either an obligate pathogen or parasite, or requires the host to

either trigger pathogenicity or grow in large enough numbers to create a critical infective dose. Further work is needed to identify potential pathogens.

Similarly to field conditions, plants recovered if more than adequate water was available (refer Chapter 2). This is possibly due to the increase in growth rate, resulting in the plants growing faster than symptom progression, and/or that the causal agent(s) is disrupted by this amount of water. This also means that if BGD affected plants are required for future work, they should be kept under slight water stress.

A possible improvement to this experiment would be to use a fine mesh as a root barrier instead of pots with small drainage holes; this is outlined in Section 10.9.

10.8 Soil Transmission of BGD in the Field

10.8.1 Materials and Methods

Methodology

It has been proposed that BGD is transmitted through soil in the shade house. However, this needs to be investigated in the field. The most practical method would be to plant healthy buffel grass in or near BGD affected patches, both with and without soil barriers, and observe these plants for symptom development. The appropriate conditions may not exist in the field for the germination of seeds. Therefore, it may be more feasible to use seedlings. As in the shade house experiment (Section 10.7), to minimise the effects of soil disturbance, seedlings should be placed in the ground with their intact potting medium.

Planting the seedlings outside the periphery of the affected patches would serve two purposes. Firstly, it would determine whether the condition spreads through soil in the field, and if it does, the seedlings would develop symptoms as the patch periphery expands

past them. Secondly, it may also determine if the disturbance has an effect on the rate of spread of the patch. However, since patch spread has been found to be uneven (Chapter 3), the seedlings should be planted close to the periphery to somewhat negate this effect.

Method

In the shade house, 120 seedlings were germinated in pots from commercial seed. These were allowed to grow to the 4 leaf stage, and then taken to the field site. In the field, 6 BGD affected patches were selected (located at 24º 20.10' S; 149º 51.75' E). All were 3-5 m in diameter, roughly circular, and were noticeably isolated from other patches. For each patch, a circle concentric to the periphery of the patch, but with a radius larger by 20 cm, was marked. Ten holes were made (by removing a soil core) at regular intervals around the marked circle, each hole being the approximate dimensions of the pots (15 cm diameter x 25 cm deep).

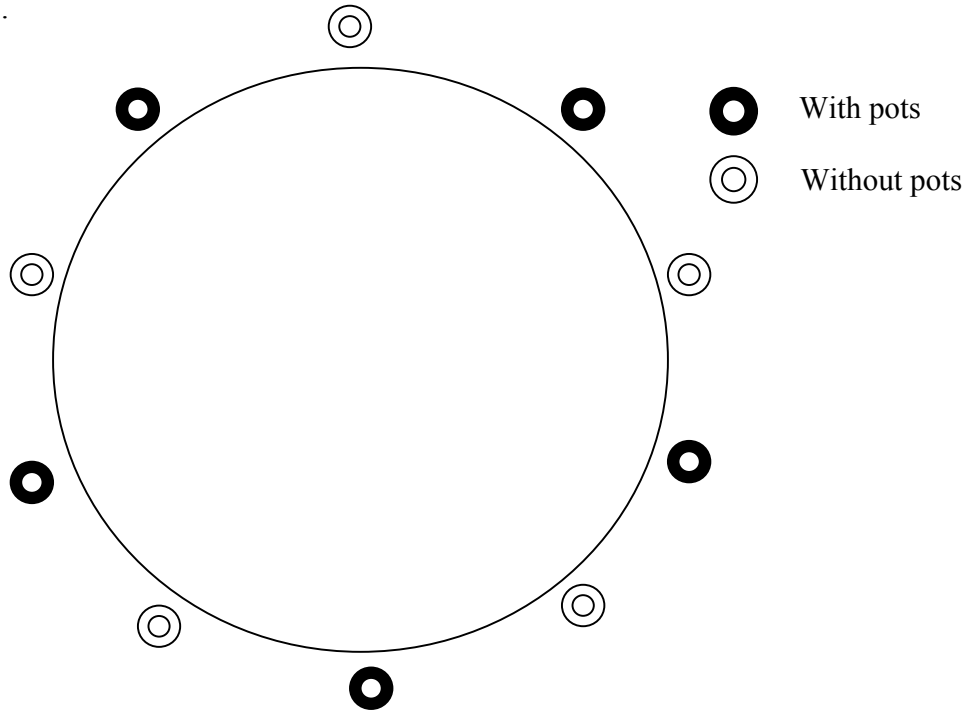


Figure 63 - Diagram of the field set up of a soil transmission experiment on dieback of buffel grass (*Cenchrus ciliaris*)

A seedling was planted into each of these holes: half (5) with pots, the other half (5) without, in an alternating pattern (Figure 63). Care was taken to ensure the soil was disturbed as little as possible.

To test the effects of transplanting into the field, 6 control 'patches' were set up in an unaffected paddock. Similarly to above, for each 'patch', 10 seedlings were planted in a circle approximately 5 m in diameter, alternating between plants with pots and those without. It should be mentioned that the pots had 5 small drainage holes at the bottom, and were therefore not total soil barriers.

The seedlings were observed for symptoms over an 8½ month period. At one stage during a prolonged drought period, the seedlings were watered weekly by hand using a mobile tanker

10.8.2 Results

At no stage were symptoms seen in the unaffected paddock. At the BGD affected site, red foliar symptoms were first seen in the plants around the affected patch 30 days after planting. They then progressed in the manner detailed in Chapter 2.

Around day 100 there was a large rainfall event. Several affected transplanted seedlings recovered along with paddock plants. BGD symptoms later re-emerged. After this rainfall event, foliar lesions also emerged on several plants.

BGD symptoms occurred mostly in the transplanted plants without a root barrier (Figure 64). Symptom emergence in transplanted seedlings with a root barrier was also considerably delayed following the rainfall event.

Symptom development in all transplanted seedlings was delayed compared with the symptom progression of the patch, that is, the visible patch boundary spread past the transplanted seedlings before these exhibited symptoms.

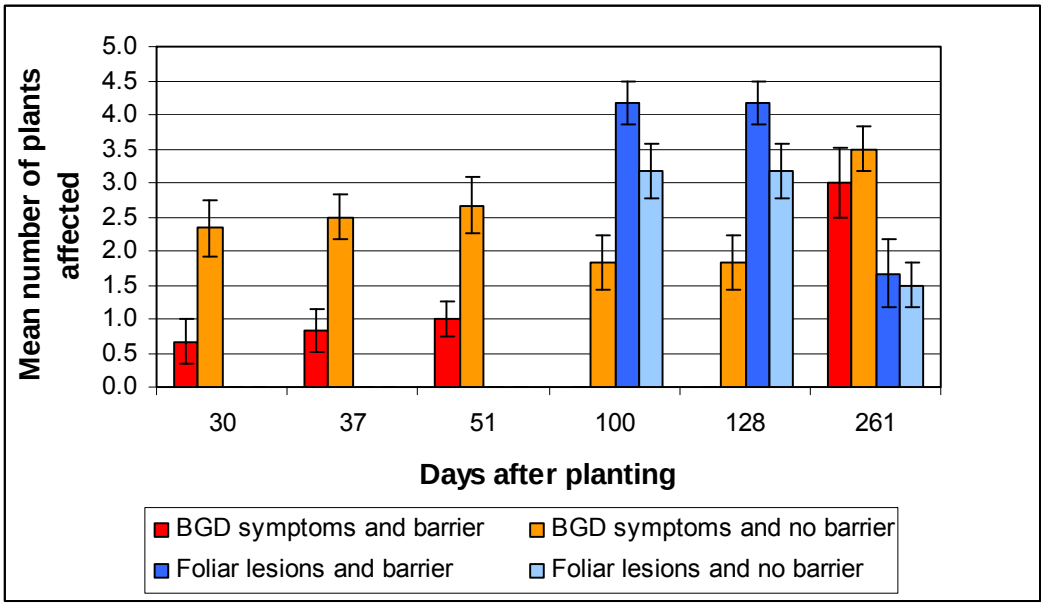


Figure 64 – Mean number of transplanted buffel grass (*Cenchrus ciliaris*) plants with BGD symptoms or foliar lesions, in both the presence and absence of a soil barrier (± SE)

Statistics:

Foliar lesions were not observed until day 100 with no further lesions by day 128. Between day 128 and 261, the number of seedlings with lesions was reduced in all patches. Therefore, the proportion of seedlings with lesions on day 100 and day 261 was analysed using a GLM assuming a binomial error and a logit link function to determine the difference between seedlings with pots and those without. There was evidence of under-dispersion for lesions on day 261 so the dispersion parameter was estimated.

The proportion of seedlings with lesions did not differ ($P = 0.070$) between those with pots and those without at day 100 with 83% and 63%, respectively. At day 261 there

was no difference ($P > 0.10$) between seedlings with pots and those without (33% and 30% respectively).

The proportions of seedlings with BGD symptoms were quite similar for days 30, 37 and 51. The number of seedlings with symptoms often decreased by day 100, stayed similar by day 128, and then increased by day 261. Thus, critical times would appear to be days 51 and 261. Therefore, the proportion of seedlings with symptoms on day 51 and day 261 were analysed using a GLM assuming a binomial error and a logit link function to determine the difference between seedlings with pots and those without. There was evidence of under-dispersion for symptoms on day 261 so the dispersion parameter was estimated.

The proportion of seedlings with symptoms differed ($P < 0.01$) between those with pots and those without at day 51 with 20% and 53%, respectively. By day 261 there was no difference ($P > 0.10$) between seedlings with pots and those without (60% and 70% respectively).

10.8.3 Discussion

The appearance of lesions was probably due to an opportunistic pathogen taking advantage of weakened host plants, as well as the increased moisture. The incidence of lesions appears to follow weather patterns more so than the incidence of BGD.

The results suggest that the pots (soil barrier) delayed symptom progression. It therefore appears that BGD is soilborne. The observations also suggest that the 'disturbance' in soil created when planting the seedlings was enough to also delay symptom progression.

There was a greater delay of symptom appearance between plants with, and without, a soil barrier in the field compared with the shade house studies (Section 10.7). The two main suggestions to explain this are that, firstly, field conditions are more variable than shade house conditions and, secondly, field plants had better drainage of water and, therefore, there was less potential for soilborne microorganisms to be spread by soil water. Future experiments should explore the rate of transmission with soil water movement.

In both this section and the previous section on shade house studies (10.7), the soil barriers had drainage holes. Therefore, it is possible that the condition can spread via root contact, rather than just through soil.

10.9 Transmission of BGD Through Root Contact

Attempts were made to replicate the experiments in Sections 10.7 and 10.8 using root barriers instead of soil barriers. 'Pots' were made of Termimesh©, a fine stainless steel mesh with a pore size of 2 x 3 mm. This mesh would act as a strengthening medium. The pots were then lined with a nylon root barrier (pore size 90 μm) (Figure 65).

The experiments were conducted in the same manner as in Sections 10.7 and 10.8, with the exception that, as a shade house was not available, the 'shade house' experiment was conducted on outside benches near the shade house. Unfortunately, heavy storm rains washed out both experiments, and there was insufficient time to replicate them. Future work should definitely investigate the possibility of root transmission.

Figure 65 – 'Pot' for root barrier studies made of nylon root mesh and Termimesh©

10.10 Concluding Statements

It is highly probable that BGD is not seed borne. This eliminates the possibility of wind borne seed dispersal, and dispersal through the commercial sale of seed.

It was conclusively proven that BGD is transmitted through the soil, though whether or not root contact is necessary is still unknown. It is most likely that the condition is caused by a pathogen, which at this stage appears to require a host to be pathogenic. The condition is disrupted and temporarily delayed by soil disturbance, which could be a possible method of control.

To keep affected plants in the shade house, they must be obtained within an intact soil core, and must be kept under slight water stress.

Chapter 11

Studies on Possible Control Methods for BGD

11.1 Introduction

Though the cause of BGD is not yet clear, possible control measures may still be tested, and a successful control will provide clues as to the identity of the cause. Previous chapters suggested that the cause was microbiological. However, plant nutrition may still be a contributing factor to the BGD condition. Therefore, controls for both biotic and abiotic causes were tested.

The control of abiotic disorders is usually achieved by the application of chemicals such as fertilisers. The control of a plant disease can take place prior to infection or after the infection process. In the former, the inoculum is controlled, whether by eradicating it, or influencing its germination and growth. In the latter, the inoculum is controlled and plant defences may be activated, whether constitutive or induced (Strange, 2003).

The application of fertiliser is straightforward, both in the field and in the shade house. On the other hand, a plant disease can be more difficult to control. Since the cause of BGD is unknown, both the control of the inoculum and activating plant defences should be tested. Although activating some plant defences can be achieved through chemical means (Strange, 2003), eradicating inoculum in field soil can be difficult due to the huge volume and the density of soil. For that reason, chemical sterilisation methods were used.

Graham and Conway (2000) undertook a similar study at a nearby site whereby strips of BGD affected buffel grass were treated with: complete fertiliser, nitrogen fertiliser,

tyne renovation, a soil application of a systemic insecticide/nematocide (*Temik*©), *Temik*© plus complete fertiliser, and a control plot. The effect of these treatments was measured as plant growth by harvesting plant tops. In Graham and Conway's study, none of these treatments had an effect on the incidence of BGD, although the application of fertiliser improved growth. However, Graham and Conway (2000) did not report on the spread of symptoms, only on the effect on already affected plants. It is possible that some of their treatments resulted in affected plants remaining affected, but hindered patch expansion. Based on these results, it was decided to repeat the work with some differences, such as attempting to control the condition both in the field and in controlled conditions in the shade house, as well as attempting some different control measures to those used by Graham and Conway (Graham and Conway, 2000).

11.2 Field Studies

11.2.1 Materials and Methods

Methodology

As with the work by Graham and Conway (2000), complete fertiliser was used as one of the treatments. The fertiliser used was Miracle-Gro®, a water soluble plant food. Although there were no differences in soil nutrients (Chapter 9), it was possible that the pastures were run down (lacking in overall nutrients), contributing to the BGD condition. Also used was a nematocide, though *Nemacur*® instead of *Temik*©. *Nemacur*® is a systemic nematocide (active ingredient 400 g/L fenamiphos). While previous work (Graham and Conway, 2000) had suggested that nematodes are not the causal agent of BGD, they may act as a vector and aid in the spread of the condition. Treatments which were different from Graham and Conway's work included: soil applications of $KMnO_4$,

H_2O_2, Agrifos® (a systemic fungicide, active ingredients 45.8% mono- and di-potassium salts of phosphorus acid), and copper oxychloride (a contact fungicide and bactericide), as well as foliar applications of Agrifos® and copper oxychloride.

Potassium permanganate ($KMnO_4$) at certain concentrations is known to increase the fungistatic activity of soil (Pandey, 1976). It has also been used to control root fungi in pine plantings (Alleksev, 1973), and has been found to have a viricidal effect (Eskarous and Habib, 1972).

Hydrogen peroxide (H_2O_2) has bactericidal, fungicidal and sporicidal properties (Baldry, 1983). It is also believed to be involved in plant defence responses (Huang *et al.*, 1998), as it has been detected in epidermal cells, extracellular spaces and hypersensitive reaction cells shortly after infection (Huang *et al.*, 1998). The accumulation of hydrogen peroxide is a characteristic early feature of the hypersensitive response (Lamb and Dixon, 1997), which it has been found to induce (Huang *et al.*, 1998). Hydrogen peroxide is a part of the post-infectional oxidative burst (Kachroo *et al.*, 2003), which is also a key component of plant defence responses. It has been shown to induce resistance against anthracnose in cucumber (Byun and Choi, 2004), and has been deliberately applied to plants to induce disease resistance, such as downy mildew in pearl millet (Geetha and Shetty, 2002).

Both *Agrifos*® and copper oxychloride are used commercially to control or prevent diseases of crops. For example, *Agrifos*® has been used for control of *Phytophthora* blight of vegetables (Babadoost and Islam, 2004), and *Phytophthora* root rot of olive (Spooner-Hart, 2005). Copper oxychloride has been used to control anthracnose in olive, caused by *Colletotrichum gloeosporioides* (Spooner-Hart, 2005) and powdery mildew in grapevines, caused by *Uncinula necator* (Gadoury *et al.*, 1994).

As the rate of patch spread will be measured, applying treatment strips on large areas of affected buffel grass is not conducive to assessing the spread of the condition as the patches have coalesced, thereby masking individual patch spread. Therefore, discrete patches were used for this experiment. To ensure that all treatments were applied on all stages of the BGD progression, the treatments were applied in sectors rather than strips (Figure 66). To prevent contamination between treatments, buffer zones were included between each treatment (Figure 66). The absence of buffer zones at the centre of the patch is irrelevant, since there are no living buffel grass plants present there. Since it is possible that the causal agent(s) of BGD exist further than the patch boundary, treatments were applied from the centre of the patch to 50 cm past the patch boundary (Figure 66).

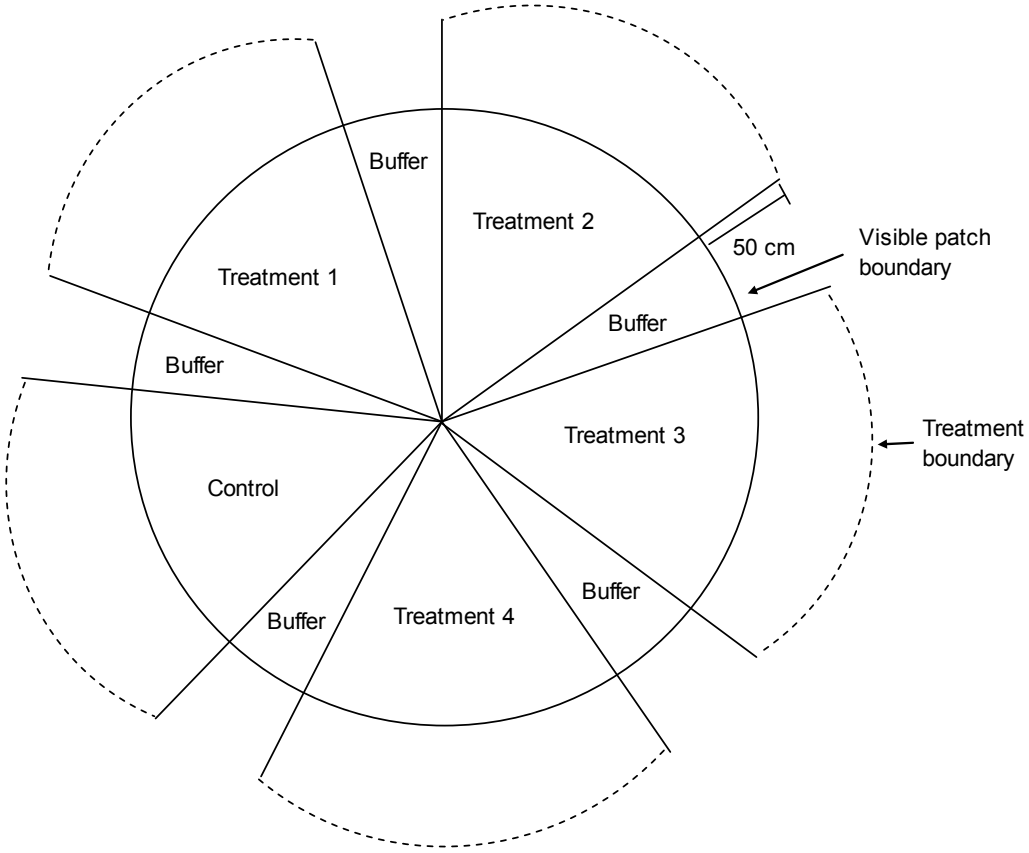


Figure 66 - Representative diagram showing treatment sectors and buffer zones in a BGD affected patch of buffel grass (*Cenchrus ciliaris*)

There were a total of 8 treatments plus a control in this experiment. Discrete patches were fairly small (*ca.*10 m diameter) in the field. It was thought that if all treatments were included in one patch, each treatment sector would be too narrow to produce accurate results. Therefore, the treatments were divided so that four treatments plus a control were applied to 5 replicate patches (Set 1), and the other four treatments plus a second control were applied to another 5 replicate patches (Set 2).

Method

The field trial was carried out from January to March 2004, at a time when soil was moist due to adequate rainfall and BGD red symptoms were prevalent.

Ten discrete BGD affected patches of buffel grass were selected from a BGD affected paddock (24º 20.10' S; 149º 51.75' E). These patches were separated from other patches by at least 10 m. A wooden stake was hammered into the centre of each patch. For each patch, five treatment sectors were measured, each of 47°, with five buffer sectors in between, each of 25°. A string from the centre stake to a stake 50 cm beyond the patch boundary identified each ray. The area of each treatment sector was calculated to 50 cm beyond the patch boundary. The volume of soil of each treatment sector was also calculated down to 50 cm depth. A sample of soil of known volume was collected and weighed, so as to obtain an approximate measure of weight per unit volume.

A line was drawn on each treatment sector describing the patch boundary at the last plants exhibiting BGD symptoms. The lines were drawn in red spray paint and were drawn directly on the soil. The plants were sufficiently dispersed so as to avoid spraying foliage.

Five of the ten patches were selected at random to have one set of treatments (Set 1); the other five patches had the other set of treatments (Set 2). Set 1 consisted of:

$KMnO_4$, H_2O_2, fertiliser, *Nemacur*®, and a control. Set 2 consisted of: soil application of *Agrifos*®, foliar application of *Agrifos*®, soil application of copper oxychloride, foliar application of copper oxychloride, and a control. The order of the treatment sectors was randomised for each patch. All treatment sectors were labelled.

The potassium permanganate as a 0.1% solution was added to the soil of its designated treatment sectors at 1:10 (vol/wt), as prescribed in Pandey (1976). A 1:100 solution of 35% hydrogen peroxide was added to the soil of its designated treatment sectors at a rate of 3.125 L/m^3, as prescribed in the patent by Nippon Peroxide Co., Ltd, Japan (1982). The fertiliser (Miracle-Gro®) was added to the soil of its designated treatment sectors as per manufacturer's recommendations, that is, 1 scoop/4 L/m^2. *Nemacur*® was also added to the soil of its designated treatment sectors as per manufacturer's recommendations, that is, at the rate of 10 L/ha. *Agrifos*® and copper oxychloride were also added to the soil of their designated treatment sectors as per instructions, at the rates of 750 mL/m^2 and 4 kg/1000 L/ha respectively. Care was taken to apply these chemicals to the soil only. Foliar application of both of these chemicals in their designated treatment sectors was done using the same concentrations as was applied to the soil, but was sprayed using spray bottles onto individual plants within the treatment sectors until run-off. This was used following the instructions on the respective containers.

The patches were monitored fortnightly for 10 weeks. Any movement of the patch boundary was measured from the original drawn boundary for each treatment sector. A qualitative evaluation was also made of symptom severity and plant growth for each treatment sector compared with the treatment sector of each patch. There were no cattle in the paddock during this period.

11.2.2 Results

In the following tables, all treatments are compared with the control, not to the results of the previous fortnight. Tables 33 to 36 display the results of the first set of treatments; Tables 37 to 39 display the results of the second set of treatments. Each table represents the results of the five replicate patches for each set of treatments. There was no difference in results between patches of each set of treatments.

Treatment	Patch boundary increase	Observations
Control	nil	increased red symptom severity/progression
$KMnO_4$	nil	less red symptoms
H_2O_2	nil	less red symptoms, slight growth increase
Fertiliser	nil	better plant growth, slightly less red symptoms
Nemacur®	nil	increased red symptom severity/progression

Table 33 – Observations on BGD affected buffel grass (*Cenchrus ciliaris*) with the first set of treatments after the first fortnight

There was no change from the above table in the 2nd fortnight.

Treatment	Patch boundary increase	Observations
Control	10 cm	increased red symptom severity/progression
$KMnO_4$	nil	red symptom severity still low in centre of sectors, but increasing on edges
H_2O_2	nil	red symptom severity still low in centre of sectors, but increasing on edges
Fertiliser	10 cm	red symptoms increased, almost same severity as control
Nemacur®	10 cm	increased red symptom severity/progression

Table 34 – Observations on BGD affected buffel grass (*Cenchrus ciliaris*) with the first set of treatments after the third fortnight

Treatment	Patch boundary increase	Observations
Control	10 cm	increased red symptom severity/progression
$KMnO_4$	nil	red symptoms increased, almost same severity as control
H_2O_2	nil	red symptoms increased, almost same severity as control
Fertiliser	10 cm	red symptoms same as control, though plants larger
Nemacur®	10 cm	increased red symptom severity/progression

Table 35 – Observations on BGD affected buffel grass (*Cenchrus ciliaris*) with the first set of treatments after the fourth fortnight

Treatment	Patch boundary increase	Observations
Control	20 cm	increased red symptom severity/progression
$KMnO_4$	10 cm	red symptoms almost same severity as control
H_2O_2	10 cm	red symptoms almost same severity as control
Fertiliser	20 cm	increased red symptom severity/progression
Nemacur®	20 cm	increased red symptom severity/progression

Table 36 – Observations on BGD affected buffel grass (*Cenchrus ciliaris*) with the first set of treatments after the fifth fortnight

Sectors with potassium permanganate and hydrogen peroxide had a decrease in BGD symptom severity during the first month, with symptom severity again increasing after 6 weeks. Fertiliser application increased the growth of the plants but otherwise had no effect on symptom severity. Sectors with Nemacur® were the same as the controls.

Treatment	Patch boundary increase	Observations
Control	nil	increased red symptom severity/progression
Agrifos® (soil)	nil	increased red symptom severity/progression
Agrifos® (foliar)	nil	slightly less red symptoms
Copper oxychloride (soil)	nil	slightly less red symptoms
Copper oxychloride (foliar)	nil	increased red symptom severity/progression

Table 37 – Observations on BGD affected buffel grass (*Cenchrus ciliaris*) with the second set of treatments after the first fortnight

There was no change from the above table in the 2nd fortnight.

Treatment	Patch boundary increase	Observations
Control	10 cm	increased red symptom severity/progression
Agrifos® (soil)	10 cm	increased red symptom severity/progression
Agrifos® (foliar)	nil	red symptoms increased, almost same severity as control
Copper oxychloride (soil)	nil	red symptoms increased, almost same severity as control
Copper oxychloride (foliar)	10 cm	increased red symptom severity/progression

Table 38 – Observations on BGD affected buffel grass (*Cenchrus ciliaris*) with the second set of treatments after the third fortnight

There was no change from the above table in the 4th fortnight.

Treatment	Patch boundary increase	Observations
Control	20 cm	increased red symptom severity/progression
Agrifos® (soil)	20 cm	increased red symptom severity/progression
Agrifos® (foliar)	10 cm	red symptoms almost same severity as control
Copper oxychloride (soil)	10 cm	red symptoms almost same severity as control
Copper oxychloride (foliar)	20 cm	increased red symptom severity/progression

Table 39 – Observations on BGD affected buffel grass (*Cenchrus ciliaris*) with the second set of treatments after the fifth fortnight

Sectors with foliar application of Agrifos® and soil application of copper oxychloride had a decrease in BGD symptom severity in the first month, with symptom severity again increasing after 6 weeks. None of the other treatments had any discernible effects on symptom severity.

11.2.3 Discussion

None of the treatments cured the BGD condition, and none completely halted its spread, either on individual plants or in patches. However, some of the treatments appeared to cause a decrease in symptom severity and a delay in patch spread. While it may be argued that patch growth is highly variable in different directions, as evidenced in Chapter 3, the randomisation of treatment sectors somewhat reduces this effect.

Similarly to Graham and Conway's (2000) work, the application of a nematocide had no apparent effect on BGD symptoms or patch spread. This implies that nematodes are not the causal agent of BGD, and have no noticeable role in its spread. Yet it is possible that they still have a role as wounding agents, allowing the causal agent(s) to affect the host plant, or as alternative vectors of the causal agent(s), allowing it to spread more rapidly when nematodes are present. Until nematodes are definitively collected, counted and studied in relation to BGD, they may remain as a possible contributor to the condition.

Also similarly to Graham and Conway's (2000) work, the application of fertiliser improved growth. However, unlike Graham and Conway's work, there was also a temporary decrease in red symptom severity. This may be a similar effect to that found in Chapter 2, where BGD affected plants seemed to outgrow the BGD condition after a rainfall event. Since the BGD symptom severity in the added fertiliser sectors subsequently increased to levels similar to the control plots, it is unlikely that nutrient deficiencies are the cause of BGD. However, pasture run-down may still be a contributor by making plants more susceptible to the causal agent(s).

Potassium permanganate appeared to have an effect on both symptom severity and patch spread. Symptom severity was lower than the control for six weeks, especially in the centre of the sector, where it is assumed that potassium permanganate concentrations would remain higher for longer. This treatment also seemed to delay patch spread. This suggests that the causal agent(s) of BGD is affected by potassium permanganate, and is most likely a disease-causing microorganism.

Hydrogen peroxide also appeared to have an effect on both symptom severity and patch spread, in a manner similar to potassium permanganate. This suggests that the causal agent(s) of BGD is affected by hydrogen peroxide, whether directly or through induced plant defences, again implying a disease-causing microorganism.

Soil application of *Agrifos*® seemed to have no effect on the BGD condition. However, foliar application appeared to have an effect on both symptom severity and patch spread. Being a systemic fungicide more would have entered the plant through foliar contact than via root contact. The results for foliar application supports the suggestion that the causal agent(s) of BGD is a disease-causing microorganism.

Foliar application of copper oxychloride had no apparent effect on the BGD condition. However, soil application appeared to have an effect on both symptom severity and patch spread. Since copper oxychloride is a contact fungicide/bactericide, this reinforces the hypothesis that the cause of BGD is soilborne. It also implies once again that the causal agent(s) of BGD is a disease-causing microorganism.

There are several possible reasons as to why none of the treatments eliminated the BGD condition or halted its spread. Firstly, the treatments used may not have had a substantial effect on the causal agent(s) of BGD. Perhaps other treatments would provide better results. Secondly, the treatments may not have been in a strong enough concentration to affect the causal agent(s) of BGD. Further studies are needed in this area.

Due to the paucity of distinct BGD affected patches in the field, this experiment could not have been replicated without using the same patches, which may have influenced the results due to residual chemicals in the soil.

11.3 Shadehouse Studies

11.3.1 Materials and Methods

Methodology

Field studies suggested that some of the treatments used, namely potassium permanganate, hydrogen peroxide, foliar application of *Agrifos*® and soil application of copper oxychloride, had an effect on BGD symptom severity and patch spread. None of these treatments eliminated the condition or halted its spread, but this may be due to the wrong types of chemicals used or in the wrong concentrations. It is also possible that the various treatments were diluted in the field due to the large volume of soil, thereby diminishing the full effect of said treatments. Therefore, a similar study was undertaken in

controlled conditions with known volumes of soil. The application of the same treatments used in the field study, and in the same concentrations, to BGD affected plants grown in pots may reveal whether the field results were due to a dilution effect, or were accurate representations of treatment effect. Additional treatments using stronger concentrations could also be used, in case field results were not due to a dilution effect.

As discovered in Chapter 10, BGD affected plants can only be maintained in the shade house if collected with intact soil cores. Therefore, field plants collected in the manner prescribed in Chapter 10 (Section 10.4) must be used for this trial.

Method

A field trip for plant collection was undertaken one week after a moderate rainfall event, so that the soil cores would adhere and not crumble. This field trip was in March 2004, when new growth and BGD symptoms were prevalent. BGD affected plants were collected in a BGD affected paddock (24º 20.10' S; 149º 51.75' E). There were 90 plants in total, each of approximately the same age (5^{th} leaf stage). The plants were collected with intact soil cores using the PVC soil corers described in Chapter 10, in the same manner prescribed in Chapter 10 (Section 10.4).

The plants were brought back to a shade house and were randomly divided into six groups of fifteen plants. The treatments were the same as those which appeared to decrease symptom severity in the field trial, that is, potassium permanganate, hydrogen peroxide, soil application of copper oxychloride, and foliar applications of *Agrifos*®. An additional treatment was also used; Amistar (active ingredient 200 g/L azoxystrobin), which was not available at the time of the field trial. Amistar is a systemic, broad-spectrum fungicide with activity against the four main groups of plant pathogenic fungi including the Ascomycetes,

Basidiomycetes, Deuteromycetes and Oomycetes (considered as fungi here). A control was also added, in which water was added to the plant. This resulted in a total of six treatments with fifteen plants per treatment. For each treatment, the plants were divided into three groups of five. In one group, the same treatment concentration as in the field trial (part 11.2) was used, that is, the recommended concentrations. The second and third groups used 20% and 40% stronger concentrations, respectively. Therefore, the amounts of each chemical used were as follows:

Treatment	Percentage of recommended dosage		
	100%	120%	140%
Control	10 mL	12 mL	14 mL
$KMnO_4$ (1% solution)	20 mL	24 mL	28 mL
H_2O_2 (35% solution)	6 mL	7.2 mL	8.4 mL
Agrifos® (foliar)	0.009 mL	0.011 mL	0.013 mL
Copper oxychloride (soil)	0.005 g	0.006 g	0.007 g
Amistar	0.009 mL	0.011 mL	0.013 mL

Table 40 – Amounts of each chemical, at three different treatment concentrations, applied to dieback affected buffel grass (*Cenchrus ciliaris*) plants

The plants were randomly distributed on a bench in a shade house and were covered with plastic sleeves to avoid plant-to-plant contact. Plants were watered directly into the soil to avoid splash, and were watered just enough to prevent wilting. The plants were observed for 12 weeks.

11.3.2 Results

The control plants continued red symptom progression. The other treatments are reported compared with these control plants.

The plants treated with potassium permanganate, at all concentrations, showed an initial decrease in symptom severity but symptom progression resumed after 6 weeks. The

plants treated with hydrogen peroxide had similar results. The plants treated with *Agrifos*®, at recommended concentrations and at 20% stronger, also had decreased in symptom severity but symptom progression resumed after 8 weeks. The plants treated with *Agrifos*® at 40% stronger concentrations all died within 1 week of the experiment. Similarly, the plants treated with copper oxychloride at recommended concentrations and 20% stronger had decreased in symptom severity, which resumed after 8 weeks. With both treatments, the plants treated with 40% stronger concentrations looked unhealthy, with a marked tip necrosis.

In all of these cases where there was a decrease in symptom severity, symptoms resembled those of plants in which BGD symptoms were emerging (Table 5, p.37), as compared with the control plants which resembled moderately affected BGD plants. At the end of the 12 week period, most of the plants (which had not died) had BGD symptom severity close to that of the control plants.

The plants treated with the recommended concentration of Amistar, and those treated with 20% stronger concentrations, showed a decrease in symptom severity similar to those of plants treated with *Agrifos*® and copper oxychloride. Symptom progression resumed after 8 weeks. Plants treated with 40% stronger concentrations showed a greater decrease in symptom severity to the other treatments, with only one older leaf per plant displaying BGD red foliar symptoms. Symptom progression resumed after 11 weeks, but appeared to progress more slowly compared with the control plants.

This experiment was repeated and produced the same results.

11.3.3 Discussion

The results were similar to those of the field trial in that potassium permanganate, foliar application of *Agrifos*® and soil application of copper oxychloride resulted in decreased severity of red BGD symptoms. Similarly, potassium permanganate and hydrogen peroxide both resulted in decreased severity of red BGD symptoms. This implies that the field results (Section 11.2) were reporting treatment effects. Increasing the concentration of these chemicals had no effect on symptom severity, and in some cases possibly killed the plants.

Amistar produced the most promising results at the strongest concentrations, with red foliar symptoms almost disappearing. This suggests that the causal agent(s) of BGD is one of the microorganisms which is susceptible to this chemical. More trials, both in the shade house and in the field, should be done with Amistar with further increases in concentration. Unfortunately, there was insufficient time to do these in this study.

Even in controlled pot trials, none of the treatments succeeded in completely eradicating the BGD condition. There are two possible reasons for this. Firstly, as previously mentioned, the treatments used may not have had a substantial effect on the causal agent(s) of BGD. Secondly, the treatments may not be suitable for the causal agent(s) of BGD.

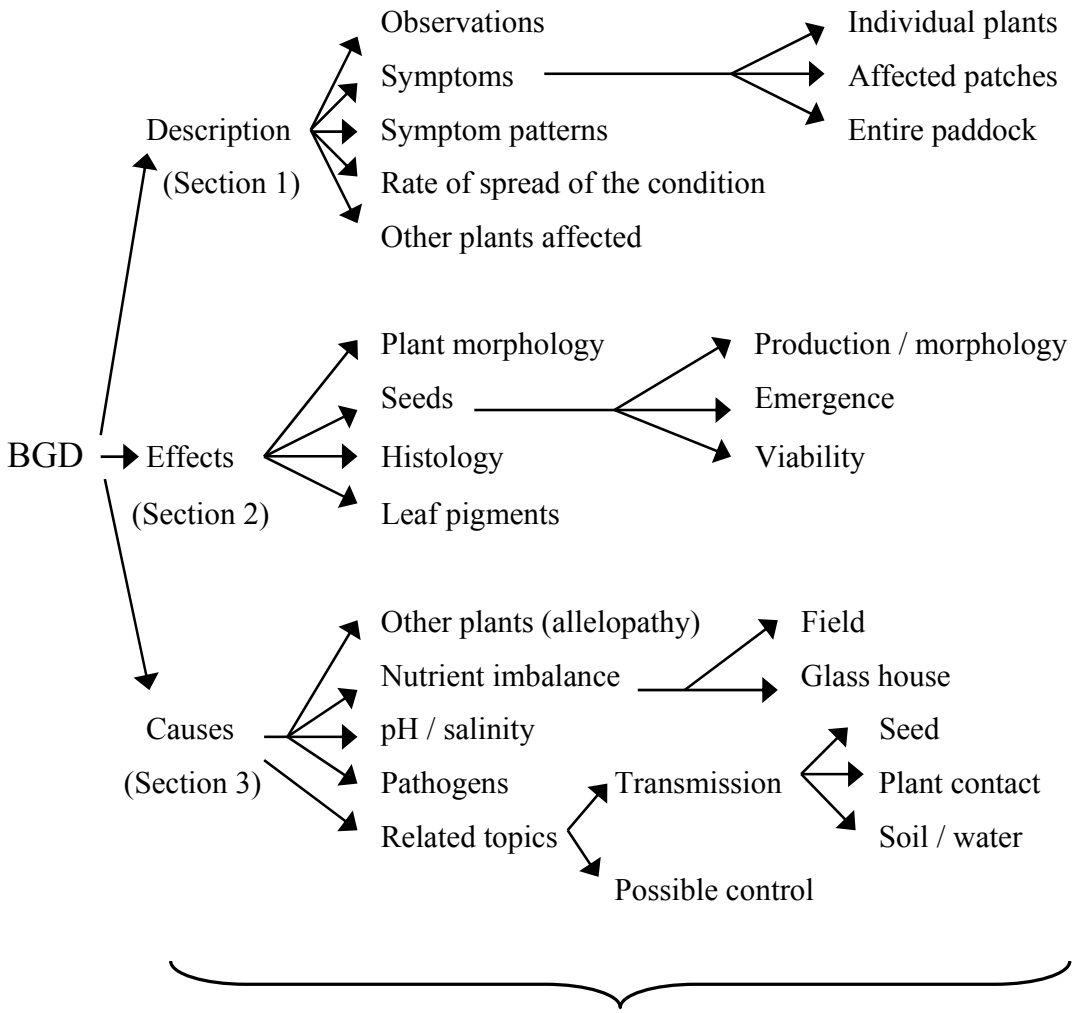
A third possible explanation for these results and those of the field trial is that BGD is caused by several organisms and other factors. It is possible that the treatments used affected some of these organisms, resulting in a decrease in symptom severity but not in eradication. As the treatment chemicals became more dilute, populations of these organisms were re-established, resulting in an increase in symptom severity.

11.4 Concluding Statements

Though a successful control measure was not found, there is evidence that chemical control of the condition is possible, potentially using Amistar. The results imply that the causal agent(s) of BGD is a disease-causing microorganism, or possibly several.

Section 4

Comparative Discussion of All Topics



Chapter 12

General Discussion

The introduction (Chapter 1) outlined three aims for this work:

1. to describe the plant and field symptoms of BGD, including spread,
2. to determine the effects of BGD on buffel grass,
3. to identify the causal agent(s) of BGD, in addition to factors responsible for symptoms and plant death.

These will be discussed separately along with possible control measures for the BGD condition and areas of future work.

12.1 Plant and Field Symptoms of BGD

This first aim was successfully completed, with the following being a brief summary of BGD symptoms.

Symptoms of BGD presented as a reddening of the leaves starting from the tip and progressively moving towards the ligule. The red symptoms range from bright red, to dark red, to bronze (RHSPCC red group 45: A, B; 46: A, B; greyed-orange group 166: A; 177; A) (The Royal Horticultural Society, 2001). Symptoms first appeared on the tips of the older leaves and progressively moved down the leaf. The next oldest leaf then showed symptoms, and so on, with the youngest leaf showing symptoms last. Any tillers followed the same pattern, regardless of whether symptoms on the primary shoot had progressed past the point at which the tiller was produced. The amount of time from new growth to the appearance of the red symptoms seemed to be directly proportional to the amount of

rainfall, that is, the more rain, the longer it took for symptoms to develop. The amount of subsequent rainfall seemed to influence the time it took for plants to succumb to the condition, that is, when there was adequate water and lush growth plants grew faster than the spread of the condition. When plants became water stressed, the condition overtook growth and the plants succumbed.

Symptomatic leaves did not always have a clear red-green boundary. Occasionally, BGD symptoms progressed faster down one half of the leaf. Red symptoms were invariably more vivid on the adaxial surface of the leaves than on the abaxial surface. Roots of affected plants appeared stunted compared with roots of unaffected plants. Roots of affected plants often displayed soft, darker, ovoid sunken regions, which were possibly lesions.

The BGD condition appeared to become dormant as buffel grass became dormant, that is, if the dieback condition killed the plant before the onset of dormancy, no new shoots were produced subsequent to a rainfall event. However, if dormancy occurred before the plant succumbed to the condition, new shoots were produced after rain, and the cycle repeated with symptoms first appearing in the oldest leaf.

Of the varieties of buffel grass observed, American buffel seemed to be the most susceptible to BGD. Symptoms were not apparent on the taller Biloela variety.

Patches were roughly circular and ranged from 2 m diameter to over 60 m diameter. Adjacent patches often coalesced and further enlarged. Symptoms first appeared on the periphery of an existing patch, where during the last cycle the plants had become dormant before succumbing to the condition. Symptoms progressively moved outwards from the periphery of the patch, at a rate of approximately 5 cm per week. Patch spread was

irregular and did not correspond with soil compaction or land slope, though the condition may spread more rapidly downhill due to runoff.

12.2 The Effects of BGD on Buffel Grass

The BGD condition was found to have several effects on buffel grass plants. However, whether these were directly caused by the causal agent(s) of BGD or whether they were secondary symptoms is uncertain.

BGD affected plants weighed approximately two thirds the weight of unaffected plants. They were noticeably shorter and had shorter leaves and internodes, with the difference in height attributed to internodes rather than leaf length. BGD affected plants also had fewer tillers than unaffected plants of the same age. Although the numbers of leaves per tiller were the same as unaffected plants, the overall result was a decreased amount of foliage available for grazing, thereby decreasing productivity of livestock. In fact, the loss of productivity was twofold, since cattle had been observed to selectively graze unaffected plants.

BGD affected plants had fewer seed heads, shorter seed fascicles, and a higher proportion of non-viable embryos compared with unaffected plants. Percent germination was very low compared with that of commercial seed. Therefore, not only did BGD affected plants succumb and die, but there were fewer seedlings to replace them. This could have detrimental consequences for the sustainability of an improved pasture.

At the cellular level, there was no discernable difference in cell size between BGD affected plants and unaffected plants in either roots or leaves. However, the roots of BGD affected plants were more damaged at the cellular level, with the cortex mostly sloughed off and the mesophyll cells disrupted.

The bulliform and mesophyll cells of BGD affected leaves were more irregular in shape. The bundle sheath cells of BGD affected leaves appeared disrupted, with chloroplasts not in their usual alignment. There also seemed to be a breakdown of chloroplasts.

The leaf pigment data concurred with the premise of a breakdown of chloroplasts. Red symptomatic leaves had lower concentrations of chlorophylls *a* and *b* compared with green leaves on the same plant. Red symptomatic leaves also had higher concentrations of anthocyanins and carotenoids. It appears that, in red symptomatic leaves, chlorophylls were being destroyed and anthocyanins were being excessively produced.

There was no discernible difference in the phloem vessels of BGD affected and unaffected plants, either in the roots or the leaves. However, the xylem of both roots and leaves was partially occluded by structures tentatively identified as tyloses. These structures could also have been local accumulations of phenols or polyphenols, or in some cases the remnants of partially decomposed cells. These occlusions seemed more severe in the roots than in the leaves. Possible inclusion bodies were also found in the mesophyll cells of BGD affected leaves. Tyloses and inclusion bodies are usually a sign of pathogen infection. However, there were no pathogens detected in the histology work.

Whether these symptoms and effects are a direct result of the causal agent(s) or whether they are secondary symptoms is difficult to determine in some cases. For example, are the stunted plants a result of the stunted roots? This is feasible since stunted, rotted roots would interfere with the water and nutrient supply. The stunting may also be a result of xylem occlusions or tyloses, since these would also interfere with water and nutrient supply.

The BGD affected plants were stunted, but according to the histological work, there was no discernible size difference between cells. Consequently, it can be concluded that the BGD condition influences cell production, not cell size, although it is possible that it affects cell elongation since longitudinal sections were not done. This suggests the involvement of plant growth regulators.

Overall, BGD has marked effects on buffel grass plants, both in morphology and histology. While there are no known causes which produce an identical range of symptoms, there are several causal agents that can cause one or more of these symptoms in other plant species.

12.3 Causal Agent(s) of BGD

Though the causal agent(s) of BGD was not found, there are several clues as to their identity. The nutrient studies (Chapter 7) concluded that deficiency symptoms of nitrogen, phosphorus, sulfur, zinc and calcium most closely resembled the morphological and foliar colour symptoms of BGD. Soil and plant chemical tests confirmed that field plants and soil were deficient in nitrogen, phosphorus, sulfur and zinc. However, these results also applied to plants and soil which were in the immediate surroundings of BGD affected patches. As the results were not limited to BGD affected plants and soil from affected patches, it was concluded that nutritional problems are not the main cause of BGD. Nevertheless, it is possible that this underlying nutrient deficiency weakens the plants, making them more susceptible to the causal agent(s) of BGD. Whether the red foliar symptoms are caused directly by the causal agent or secondarily by these nutrient deficiencies is unknown. Several plant disease causing organisms can cause a change in the nutritional status of the host plants, mainly by changing the membrane permeability of root cells and increasing

root lysis (Shepherd, 1994). It is possible that a microbiological causal agent disrupts the nutrient uptake of BGD affected plants, exacerbating the already existing nutrient deficiencies and thereby creating red foliar symptoms.

Soil nutritional status was not significantly different within and outside of BGD affected patches. However, there were significant differences in molybdenum, sodium, sulfur, total nitrogen and zinc concentrations between BGD affected plants and plants adjacent to an affected patch. Since there were no soil differences, these were regarded as symptoms, not causes. The most likely explanation is that of a plant pathogen interfering with nutrient uptake and/or translocation in the plant.

Pathogens can have numerous effects on host plants. Many plant pathogens interfere in one or more ways with the translocation of water and inorganic nutrients through plants. Some pathogens affect the integrity or function of the roots, thereby affecting the absorption of water and nutrients. Others, by growing in the xylem, interfere with water and nutrient transport through the stem (Agrios, 2005). Pathogens can also cause decreased photosynthesis through degeneration of chloroplasts. The overall chlorophyll content of leaves in many fungal and bacterial diseases is reduced. In some, photosynthesis is reduced because of toxins which inhibit enzymes involved in photosynthesis. In plants infected with vascular pathogens, the stomata remain partially closed, chlorophyll is reduced, and photosynthesis stops before the plant wilts (Strange, 2003). Many pathogens cause extensive damage to the roots before symptoms appear on the aboveground parts of the plant (Agrios, 2005), and they can also interfere with plant growth regulators causing abnormal growth (Parry, 1990). Many of these effects are similar to the symptoms of BGD, supporting the hypothesis that the cause is a disease-causing microorganism. The question is, which disease-causing microorganism could be

the causal agent(s) of BGD? The answer, or clues to the answer, may lie in the method of spread of the condition.

It has been established that the BGD condition is not transmitted through seeds, plant contact, or by wind. It is also unlikely that it is spread by sap sucking insects. Therefore, it is unlikely that the causal agent is a phytoplasma, which are transmitted by grafting or insect vectors (Lucas, 1998). This supports previous work on the BGD condition (Graham and Conway, 2000), where no phytoplasmas were found in BGD affected plants. The BGD condition was found to be transmitted through soil, with soil water possibly aiding dispersal. Whether root contact is necessary for successful transmission was not established.

Unlike nutrient disorders, soilborne pathogens generally form circular patches or foci (Lucas, 1998; Gilligan, 1995). While soil may not offer the same opportunities for rapid long distance dispersal as do other means of dispersal, it does compensate by providing a better medium for growth. Soilborne pathogens, because of the physical restrictions imposed by soil, are usually unable to produce sudden epidemics, but local slow spreading diseases of considerable severity (Agrios, 2005). Soilborne plant pathogens can spread through soil by their own movement, by growing through the soil, by growing along plant roots, and by the action of vectors. They may also be passively spread by soil erosion, flash flooding, and by the actions of humans and animals, such as soil in hooves and shoes, and agricultural practices such as ploughing (Lucas, 1998).

In the case of BGD, the condition was disrupted when the soil was disturbed; temporarily disrupted in the field, and permanently disrupted in the shade house. Soil disturbance, such as tillage, disrupts the soil structure, disturbs the soil ecology and also decreases soil moisture through evaporation (Bockus and Shroyer, 1998). A plant pathogen

that occupies a specific ecological niche, has certain environmental requirements, and/or requires a certain amount of soil water, will therefore be impeded by soil disturbance. For example, the web of mycelium that *Rhizoctonia* produces in undisturbed soil is important for successful infection (Bockus and Shroyer, 1998). As an added example, *Pythium*, which produces motile spores which 'swim' through soil water, has reduced activity in disturbed soils due to the decrease in soil water (Bockus and Shroyer, 1998). In addition, soil disturbance disperses inoculum. Many plant diseases have a threshold concentration of inoculum required for successful infection. Below this threshold, no symptoms are apparent, though the disease-causing organism amplifies within the host (Truscott and Gilligan, 2001). Therefore, it appears that the causal agent(s) of BGD may be one of the pathogens which are 'disrupted' by soil disturbance. In the shade house, the time of spread from an affected plant to an unaffected plant ranged from 1 week to 2 months, depending on the proximity of the plants and the amount of soil disturbance (those with soil disturbance took 2 months). This was also witnessed in field trials. Therefore, it appears that soil disturbance can delay the onset of symptoms, potentially for a long time.

BGD could only be replicated in the shade house when using BGD affected plants brought back from the field with intact soil cores. This led to the hypothesis that the causal agent(s) of BGD was a hemi-biotroph, in that it requires a host to spread. However, BGD was not localised to one grazing property. It had been reported in many other areas, as seen in Figure 1, p5. This somewhat discredits the above hypothesis since it is highly unlikely that BGD affected plants with intact soil cores are being moved from one property to another. However, the reported incidences of the BGD condition on several non-adjacent properties indicate that the condition is somehow spread, and is spread through the movement of soil since it is soilborne. Nevertheless, the above hypothesis may have been

correct in that the causal agent(s) of BGD require a host and/or undisturbed soil in which to spread. There was no experimentation done on the size of the intact soil (including a plant) required to spread the condition. It is possible that BGD is spread via the movement of relatively undisturbed clods of soil containing sections of roots from BGD affected plants; clods of soil which are easily carried by hooves or tyres. This would explain the incidence of BGD on various properties. It may also explain how new BGD affected patches in a paddock are formed. But, how does the causal agent(s) of BGD spread though the soil?

It has been demonstrated that the spread of BGD through soil is irregular. It has also been observed that the spread of the condition is delayed at cattle and vehicle trails. These findings may be explained by the above hypothesis that BGD requires a host in order to spread. It is possible that the causal agent(s) of BGD grows along plant roots, or is carried by a vector which moves along plant roots. In this manner, the required nutrients are supplied by the host rather than the soil. An example of this is the pathogen *Armillaria*, which grows along the roots of trees (Lucas, 1998). Buffel grass plants in pastures are not evenly dispersed, and there are no plants present on cattle and vehicle trails. Therefore, the spread of BGD may be irregular or delayed due to the absence of a host (Burdon and Chilvers, 1982). Supporting the hypothesis of the dependence of the causal agent(s) of BGD on its host is the 'ring' effect seen in the field, in which BGD symptoms always appear first on previously affected plants after a rainfall event following a dry period. If the causal agent(s) of BGD could spread through the soil without the presence of a growing host, it would be expected that, after a rainfall event following a dry period when all plants are producing new growth, symptoms would appear in other areas of the paddock, and not exclusively on previously affected buffel grass. So how does the causal agent(s) of BGD survive in dry periods when plants are dead or dormant?

When pathogens are outside a host, they face survival problems. There are four main mechanisms to aid survival: two active and two inactive. The active mechanisms are to act as parasites or saprophytes. The inactive mechanisms are to create dormant propagules or quiescent vegetative forms (Lucas, 1998). Some pathogens have alternate or alternative hosts (Lucas, 1998; Agrios, 2005). Other pathogens become inactive in the dormant host, such as powdery mildews in dormant buds in winter (Lucas, 1998). Which mechanism is used by the causal agent(s) of BGD?

BGD appears to affect *Urochloa mosambicensis* in a similar manner to buffel grass, though this needs to be confirmed in future trials. No other plants in the paddock were observed to be affected. Therefore, it is possible that *U. mosambicensis* is an alternative host for BGD. It is also possible that the causal agent(s) of BGD survives as a saprophyte in the soil. However, this is discredited by the observed 'ring' effect, in which BGD symptoms always appear first on previously affected plants after a rainfall event following a dry period. If the causal agent(s) of BGD could survive as a saprophyte, it would be expected to cause symptoms in other plants in the paddock. It is most likely that the causal agent(s) of BGD creates dormant or otherwise resistant propagules when buffel grass plants become dormant.

To this point, the causal agent(s) of BGD appears to be a pathogen which is soilborne, is disrupted by soil disturbance, probably requires a host plant on which to survive and in order to spread, and creates dormant propagules when hosts are unavailable. So what is the causal agent(s) of BGD? It is unlikely to be allelopathic interactions or nematodes. The remaining possibilities are protozoa, bacteria, fungi and viruses, as well as others such as viroids and some diseases of unknown aetiology.

Protozoans tend to infect the phloem (Agrios, 2005). Many ailments of tropical perennial crops are associated with the presence of flagellate protozoa, often in the phloem tissue (Lucas, 1998). They can also serve as vectors of viruses (Strange, 2003). Since these organisms are difficult to culture, they remain a possible cause of BGD, and are recommended for further work.

Bacteria generally grow best in warm, alkaline conditions with high nitrogen levels. Because they are unicellular their spread within a host is enhanced by a circulatory system (Strange, 2003). Compared with fungi, relatively few bacteria have been recorded as plant pathogens and many are limited to the xylem (Lucas, 1998). Bacteria generally require a wound for entry into the host and are mostly spread by water, insects, animals and humans (Agrios, 2005). They may cause obstructions in the plant vasculature, cause various changes in the levels of plant growth regulators, and can aggregate to form cankers, crown gall, and soft rots (Lucas, 1998).

While it is possible that the causal agent(s) of BGD is a bacterium, it is unlikely for several reasons. Firstly, bacteria do not usually cause patch diseases. There are a few species that do cause patch disease, such as some turfgrass diseases, but the patches are highly irregular in shape (Couch, 2000), unlike the ovoid to circular shape of BGD affected patches. Secondly, without the aid of soil water or a vector, bacteria do not travel quickly through the soil (Strange, 2003); certainly not at the rate of patch spread observed with BGD. Lastly, bacteria generally cause wilting and/or abnormal growths such as cankers, which are not part of the symptoms of BGD. Nevertheless, while it seems unlikely that bacteria are the primary causal agent of BGD, they may be a secondary causal agent or a contributor to the condition.

Most fungi have a filamentous mycelium which grows through the substrate. Almost all plant pathogenic fungi spend part of their life cycle in their host plants, and part of their life cycle in the soil or in plant debris in the soil. Some fungi are biotrophic, that is, they spend their active lives on the living host, and only their spores land on soil, where they die or are inactive until carried to a host. Other fungi are hemi-biotrophs, that is, they must live some of their life on a host and the other part of their life on the dead tissue of the same host as saprophytes (Mendgen and Hahn, 2002). A third group of fungi are facultative saprophytes, that is, they grow on the host, but can also move into the soil or other decaying plant material (Strange, 2003; Agrios, 2005).

Fungi and fungal like organisms reproduce and spread through the use of asexual and sexual spores. Some, such as *Phytophthora* , produce zoospores which exhibit chemotaxis towards the roots of the host plants (Hardham, 2001). However, the great majority of plant pathogenic soil fungi depend on hyphal growth to spread from host to host (Agrios, 2005). The hyphae act as transport systems from the older parts of the mycelium to the hyphal tips. This is essential when fungi try to grow from one host to another, unless the fungus is able to grow as a saprophyte and compete with other saprophytes (Lucas, 1998).

Some fungi create their own entry wound through penetrating hyphae, whereas others enter through natural openings. In general, they cause local or general necrosis of tissue, and often cause stunting (Parry, 1990; Agrios, 2005). Fungi are responsible for many patch diseases (Couch, 2000), as well as root rots, wilts, cankers, rusts, and many others (Strange, 2003; Agrios, 2005). A few fungi produce excessive growth of infected plant parts. Fungi can also produce toxins which spread throughout the host plant causing permeability changes, disruption of normal metabolic processes, the blocking of

vasculature, whether physically or plant induced, the interference of plant growth regulators, and with some toxins the inhibition of root development (Tarr, 1972; Strange, 2003).

In relation to BGD, it seems that fungi are a likely candidate as the causal agent(s). Their frequent association with patch diseases, their rapid spread through soil, and the range of symptoms they may cause all concur with BGD symptoms and characteristics.

Viruses are obligate pathogens or parasites, and as such cannot reproduce without a host. There are few viruses which exist in the soil. Some of these are dispersed through the movement of soil or soil water. Others are spread through the soil by nematodes, insects and parasitic fungi vectors (Fulton *et al.*, 1987; Brown *et al.*, 1995; Campbell, 1996). For example, the raspberry ringspot virus (RRV) is spread by a nematode, and the tobacco stunt virus (TSV) is spread by *Olpidium*, a root infecting fungus (Lucas, 1998). Most plant virus vectors are themselves plant pathogens or parasites (Lucas, 1998). This is a necessary adaptation since, to be successfully transmitted, viruses must come into contact with wounded living cells (Lucas *et al.*, 1985).

Almost all viral diseases cause some stunting (Parry, 1990). Obvious symptoms also appear on leaves as colour deviations. The most common of these are mosaics and ring spots (Bos, 1978; Agrios, 2005). They may also cause abnormal growths such as tumours, local lesions, and other systemic symptoms (Bos, 1978; Agrios, 2005).

Certain fungi produce similar symptoms to viruses. For example, leaf rolling, chlorosis and anthocyanin formation in potato may be caused by either potato leaf roll virus or *Rhizoctonia solani* (Bos, 1978). Sometimes virus diseases are confused with disorders from other microbes, especially when the syndrome results from the combined effect of virus and fungus or bacteria (Bos, 1978).

It is possible that the causal agent(s) of BGD is a virus. Due to the nature of the spread of BGD, this virus would have to be spread by a vector. The experiments on possible control, as well as previous work on the BGD condition (Graham and Conway, 2000), suggested that BGD is not affected by nematodes. However, possible vectors could include other soil insects and fungi.

Viroids have been shown to cause potato spindle tuber disease and several other plant diseases. There are also many severe diseases of plants, particularly of trees, for which no causal organism has been found (Agrios, 2005). An example of this is Mundulla Yellows of eucalypts, wattles, and other native Australian shrubs (Hanold *et al.*, 2006). Mundulla yellows is characterised by progressive yellowing and dieback of foliage, and is generally fatal. Studies have indicated that an interaction of soil conditions contributes to Mundulla yellows, but it is unlikely to be the sole cause (Hanold *et al.*, 2006). It is possible that one of the above may be the causal agent(s) of BGD, but this possibility must be reserved for future work.

Frequently, plant diseases, especially those induced by soilborne pathogens, involve more than one pathogen (Lucas *et al.*, 1985). This is generally referred to as a disease complex. Disease complexes can be caused by a combination of several pathogens, or by a combination of a pest and a pathogen (Tarr, 1972; Agrios, 2005). Pests can have a relationship with a pathogen, or may simply create a wound, providing entry (Parry, 1990). As an example of disease complexes, the *Rhizoctonia* disease complex of wheat and barley is associated with many fungi, bacteria, viruses and nematodes (Moen and Harris, 1983). Similarly, wilt complex of french bean is caused by *Sclerotium rolfsii*, *Rhizoctonia solani*, and *Fusarium oxysporum* f. sp. *phaseoli* (Mukherjee and Tripathi, 2000). Frequently, one pathogen causes more damage to the host plant than others (Tarr, 1972). Disease

complexes have also been reported in Australian pastures, examples being root rot of subterranean clover, caused by a nematode and several fungi (Flett and Clarke, 1996).

It is possible that BGD is caused by a disease complex. This would explain the difficulty in reproducing the condition in the shade house. It also provides another explanation as to why the condition is disrupted when soil is disturbed. All involved pathogens would have to be present for symptoms to occur; soil disturbance would disperse the causal agents.

From the above discussion, the most likely cause of BGD is a disease complex. This complex would consist of several pathogens, each causing a certain set of symptoms, collectively causing BGD symptoms. The complex may include the soil nutrient deficiencies identified earlier.

12.4 Possible Control Measures for BGD

Disease complexes, if that is the cause of BGD, are notoriously difficult to control when the individual causal agents are unknown. Even single causal agents can be difficult to control when unknown. However, certain suggestions can be made based on the results of this study.

The control trials (Chapter 11) suggested that chemical control of the BGD condition is possible, though perhaps not with the treatments tested, or with stronger concentrations of them, namely with Amistar. Nonetheless, further work is needed in this area to find a more effective chemical control. Controlling one of the causal agents in this potential disease complex may be enough to prevent the BGD condition (Tarr, 1972). However, care should be taken as chemical control may adversely affect beneficial

microbes in the soil (Agrios, 2005), which may be partially controlling or hindering the causal agents of BGD.

Soil disturbance disrupts the BGD condition. However, it is believed that agricultural practices such as ploughing should not be used as a sole means of control. While this may temporarily stop the progress of the condition, it may ultimately result in further spread. Soil disturbance may be used in conjunction with chemical control methods, once a suitable chemical control is found. The application of fertiliser to alleviate the identified nutrient deficiencies may also aid in the control of this condition.

Another possible control measure would be the production of suppressive soils (Agrios, 2005), where certain microorganisms which act as biological control agents are encouraged to increase in number (Agrios, 2005), thereby outcompeting the pathogens. A third option is to plant resistant buffel grass varieties such as the Biloela cultivar. However, this is somewhat impractical, since sustainable buffel grass pastures may take years to establish (Cavaye, 1991).

12.5 Future Work

It is likely that BGD is caused by a disease complex, involving both microorganisms and soil chemistry. Therefore, future work needs to concentrate on identifying the individual causal agents. Since the causal agents have proved difficult to culture, molecular methods such as PCR could be used (McCartney *et al.*, 2003; Ma and Michailides, 2007; Sharma *et al*., 2007).

Another possible area of future work is in testing for viruses. This can be achieved by virus transmission tests to host plants by sap inoculation or grafting, sometimes by a specific vector if known. Viruses can also be diagnosed by serodiagnostic tests, electron

microscopy, microscopic examination of infected cells for crystalline or amorphous inclusions, electrophoretic tests and the hybridisation of commercially available radioactive DNA which is complementary to certain virus DNA or RNA (Hill, 1985; Agrios, 2005; Boonham *et al*., 2007). The use of electron microscopes, both scanning and transmission, may also reveal the presence of pathogens not found in the histology work.

In addition, further chemical work can be done to identify any pathogen products such as toxins (Lucas, 1998). Chemical analyses of xylem and phloem content could be done, firstly to identify any toxins, and secondly to more thoroughly investigate the differences in plant chemical composition found in Chapter 8. Trials could also be done involving the application of plant growth regulators, to determine if these are involved in the BGD condition.

The microorganisms isolated from the field site could be tested to determine if any of these could act as a biological control agent. This could result in the production of a suppressive soil.

Further work needs to be done regarding the transmission of BGD, the volume of undisturbed soil necessary for spread, and whether or not root contact is required. Finally, further work is needed in identifying a more effective means of chemical or biological control.

Bibliography

Agrios, G.N. (2005) *Plant Pathology.* 5th ed. Burlington: Elsevier Academic Press.

Aitken, R.L. & Scott, B.J. (1999) Magnesium. In: *Soil Analysis: An Interpretation Manual*. K.I. Peverill, L.A. Sparrow & D.J. Reuter (eds). Collingwood: CSIRO Publishing.

Al-Chaarani, G.R., Roustaee, A., Gentzbittel, L., Mokrani, L., Barrault, G., Dechamp-Guillaume, G. & Sarrafi, A. (2002) A QTL analysis of sunflower partial resistance to downy mildew (*Plasmopara halstedii*) and black stem (*Phoma macdonaldii*) by the use of recombinant inbred lines. *Theoretical and Applied Genetics*. **104**(2-3): 490-496.

Alleksev, I.A. (1973) Use of chemical substances and mineral fertilisers to control root fungus in pine plantings. *Visnik Sil's'kogospodars'koi Nauki*. **5**: 66-70.

Allen, D. & Walton, K. (2003) Mehlich 3 - a new comprehensive soil test. In *Agribusiness Crop Updates*: Department of Agriculture, Western Australia.

Alva, A.K. (1993) Comparison of Mehlich 3, Mehlich 1, ammonium bicarbonate-DTPA, 1.0M ammonium acetate, and 0.2M ammonium chloride for extraction of calcium, magnesium, phosphorus, and potassium for a wide range of soils. *Communications in Soil Science and Plant Analysis*. **24**(7-8): 603-612.

Alves, V.G., Andrade, M.J.B., Correa, J.B.D., Moraes, A.R. & Silva, M.V. (2003) Common bean (*Phaseolus vulgaris* L.) plant macronutrient concentration affected by compaction and soil classes. *Ciencia e Agrotecnologia*. **27**(1): 44-53.

Aoyagi, A. & Kageyama, K. (1999) Isolation of *Pythium* species from zoysia grass and their effect on large patch disease. *Plant Disease*. **83**(2): 171-175.

Anderson, E.R. (2003) *Plants of Central Queensland: Their Identification and Uses.* Brisbane: Queensland Department of Primary Industries.

Armour, J.D. & Brennan, R.F. (1999) Zinc. In: *Soil Analysis: An Interpretation Manual*. K.I. Peverill, L.A. Sparrow & D.J. Reuter (eds). Collingwood: CSIRO Publishing.

Asran, M.R. & Buchenauer, H. (2003) Pathogenicity of *Fusarium graminearum* isolates on maize (*Zea mays* L.) cultivars and relation with deoxynivalenol and ergosterol content. *Zeitschrift fuer Pflanzenkrankheiten und Pflanzenschutz*. **110**(3): 209-219.

Atlas, R.M. & Parks, L.C. (1993) *Handbook of Microbiological Media.* Boca Raton: CRC Press.

Ayres, P.G. (1978) Water relations of diseased plants. In *Water Deficits and Plant Growth*. Kozlowski, T.T. (ed.), New York: Academic Press.

Babadoost, M. & Islam, S.Z. (2004) Fungicides for control of *Phytophthora* blight (*Phytophthora capsici*) of vegetables. *Phytopathology*. **94**(6).

Bag, T.K. & Sinha, A.K. (1997) Control of stem rot of soybean incited by *Sclerotium rolfsii* through metal salts. *Indian Phytopathology*. **50**(3): 350-357.

Baldry, M.G.C. (1983) The bactericidal, fungicidal and sporicidal properties of hydrogen peroxide and peracetic acid. *Journal of Applied Bacteriology*. **54**: 417-423.

Bashaw, E.C. (1962) Apomixis and sexuality in buffel grass. *Crop Science*. **85**: 652.

Beckman, C.H. (2000) Phenolic-storing cells: keys to programmed cell death and periderm formation in wilt disease resistance and in general defence response in plants? *Physiological and Molecular Plant Pathology*. **57**(3): 101-110.

Bell, R.W. (1999) Boron. In: *Soil Analysis: An Interpretation Manual*. K.I. Peverill, L.A. Sparrow & D.J. Reuter (eds). Collingwood: CSIRO Publishing.

Bernal, R.F. & Berger, R.D. (1996) The spread of epiphytic populations of *Xanthomonas campestris* pv. *vesicatoria* on pepper in the field. *Journal of Phytopathology*. **144**(9-10): 479-484.

Blanco, F., Machado, H. & Segui, E. (2001) Characteristics and variations on sodium content in buffel grass hybrids at two locations. *Pastos y Forrajes*. **15**(1): 47-54.

Blazier, S.R. & Conway, K.E. (2004) Characterization of *Rhizoctonia solani* isolates associated with patch diseases on turfgrass. *Proceedings of the Academy of Science*. **Vol 84**: 41-51.

Bloem, E., Haneklaus, S. & Schnug, E. (2005) Significance of sulfur compounds in the protection of plants against pests and diseases. *Journal of Plant Nutrition*. **28**(5): 763-784.

Bockus, W.W. & Shroyer, J.P. (1998) The impact of reduced tillage on soilborne plant pathogens. *Annual Review of Phytopathology*. **36**: 485-500.

Boehm, M.J. & Hoitink, H.A.J. (1992) Sustenance of microbial activity in potting mixes and its impact on severity of *Pythium* root rot of poinsettia. *Phytopathology*. **82**(3): 259-264.

Bolland, M.D.A., Allen, D. & Walton, K. (2003) Soil testing for phosphorus: comparing the Mehlich 3 and Colwell procedures for soils of south-western Australia. *Australian Journal of Soil Research*. **41**(6): 1185-1200.

Boonham, N., Tomlinson, J. & Mumford, R. (2007) Micro arrays for the rapid identification of plant viruses. *Annual Review of Phytopathology*. **45**: 307-328.

Booth, C. (1971) *The Genus Fusarium.* Kew: Commonwealth Mycological Institute.

Bos, L. (1978) *Symptoms of Virus Diseases in Plants.* 3rd edition. Wageningen: Research Institute for Plant Protection.

Boyer, J.S. (1995) Biochemical and biophysical aspects of water deficits and the predisposition to disease. *Annual Review of Phytopathology*. **33**: 251-274.

Brennan, R.F. & Best, E. (1999) Copper. In: *Soil Analysis: An Interpretation Manual*. K.I. Peverill, L.A. Sparrow & D.J. Reuter (eds). Collingwood: CSIRO Publishing.

Brennan, R.F. & Bruce, R.C. (1999) Molybdenum. In: *Soil Analysis: An Interpretation Manual*. K.I. Peverill, L.A. Sparrow & D.J. Reuter (eds). Collingwood: CSIRO Publishing.

Brown, D.J.F., Robertson, W.M. & Trudgill, D.L. (1995) Transmission of viruses by plant nematodes. *Annual Review of Phytopathology*. **33**: 223-249.

Brown, G.M.C & Grant, C.D. (2000) Nutrient status of pasture ecosystems established on rehabilitated overburden and topsoil sites in the Hunter Valley, New South Wales. *Australian Journal of Soil Research*. **38**: 479-491.

Bruce, R.C. (1999) Calcium. In: *Soil Analysis: An Interpretation Manual*. K.I. Peverill, L.A. Sparrow & D.J. Reuter (eds). Collingwood: CSIRO Publishing.

Buchanan-Wollaston, V. (1997) The molecular biology of leaf senescence. *Journal of Experimental Botany*. **48**(307): 181-199.

Burdon, J.J. & Chilvers, G.A. (1982) Host density as a factor in plant disease ecology. *Annual Review of Phytopathology*. **20**: 143-166.

Byun, H. & Choi, S. (2004) Hydrogen peroxide induces resistance against anthracnose in cucumber (*Cucumis sativus*). *Journal of the Korean Society for Horticultural Science*. **45**(1): 21-26.

Campbell, R.N. (1996) Fungal transmission of plant viruses. *Annual Review of Phytopathology*. **34**: 87-108.

Cappelli, C. & Zazzerini, A. (1988) Safflower rust (*Puccinia carthami* Cda.) in Italy: seed-plant transmission, and seed dressing for disease control. *Phytopathologia Mediterranea*. **27**(3): 145-147.

Carmichael, J.W., Bryce Kendrick, W., Conners, I.L. & Sigler, L. (1980) *Genera of Hyphomycetes.* Edmonton: The University of Alberta Press.

Castella, G., Bragulat, M.R., Rubiales, M.V. & Cabanes, F.J. (1997) Malachite green agar, a new selective medium for *Fusarium* spp. *Mycopathologia*. **137**: 173-178.

Cavaye, J.M. (1988) Buffel grass basics. *Queensland Agricultural Journal*. **114**: 69-72.

Cavaye, J.M. (1991) *The buffel book: a guide to buffel grass pasture development in Queensland.* Brisbane: Queensland Department of Primary Industries.

Cerkauskas, R.F. & McGarvey, B.D. (1988) Fungicidal control of *Phoma* canker of parsnip. *Canadian Journal of Plant Pathology*. **10**(3): 252-258.

Chen, W., Grau, C.R., Adee, E.A. & Meng, X. (2000) A molecular marker identifying subspecific populations of the soybean brown stem rot pathogen, *Phialophora gregata*. *Phytopathology*. **90**(8): 875-883.

Christie, E.K. (1975) A study of phosphorus nutrition and water supply on the early growth and survival of buffel grass grown on a sandy red earth from south-west Queensland. *Australian Journal of Experimental Agriculture and Animal Husbandry*. **15**: 239-249.

Chung, K., Shilts, T., Erturk, U., Timmer, L.W. & Ueng, P.P. (2003) Indole derivatives produced by the fungus *Colletotrichum acutatum* causing lime anthracnose and postbloom fruit drop of citrus. *FEMS Microbiology Letters*. **226**(1): 23-30.

Clerivet, A., Alami, I., Breton, Garcia, D. & Sanier, C. (1996) Phenolic compounds and plant resistance to pathogenic microorganisms. *Acta Botanica Gallica*. **143**(6): 531-538.

Close, D.C. & Beadle, C.L. (2003) The ecophysiology of foliar anthocyanin. *The Botanical Review*. **69**(2): 149-161.

Cook, R.J. (2001) Management of wheat and barley root diseases in modern farming systems. *Australasian Plant Pathology*. **30**: 119-126.

Cordoba, A., Seffino, L.G., Moreno, H., Arias, C., Grunberg, K., Zenoff, A. & Taleisnik, E. (2001) Characterisation of the effect of high salinity on roots of *Chloris gayana* Kunth: carbohydrate and lipid accumulation and growth. *Grass and Forage Science*. **56**: 162-168.

Costacurta, A. & Vanderleyden, J. (1995) Synthesis of phytohormones by plant-associated bacteria. *Critical Reviews in Microbiology*. **21**(1): 1-18.

Couch, H.B. (2000) *The Turfgrass Disease Handbook.* Malabar: Krieger Publishing Company.

Craft, C.M. & Nelson, E.B. (1996) Microbial properties of compost that suppress damping-off and root rot of creeping bentgrass caused by *Pythium graminicola*. *Applied Environmental Microbiology*. **62**(5): 1550-1557.

Craig, J. & Hignight, K.W. (1991) Control of ergot in buffel grass with triadimefon. *Plant Disease*. **75**: 627-629.

Cromey, M.G., Parkes, R.A. & Fraser, P.M. (2006) Factors associated with stem base and root diseases of New Zealand wheat and barley crops. *Australasian Plant Pathology*. **35**: 391-400.

Czapik, R. (2000) Apomixis in monocotyledons. In: *Grasses: Systematics and Evolution*. W.L. Jacobs & J. Everett (eds). Collingwood: CSIRO Publishing.

Dantzman, C.L., Richter, M.F. & Martin, F.G. (1983) Chemical elements in soils under cattle pens. *Journal of Environmental Quality*. **12**(2): 164-168.

Das, S. & Raj, S.K. (2004) Management of *Sclerotium* root rot of sugarbeet through seed soaking with non-conventional chemicals. *Indian Phytopathology*. **57**(1): 112-113.

Davies, F.R. (1935) Superiority of silver nitrate over mercuric chloride for surface sterilization in the isolation of *Ophiobolus graminis* Sacc. *Canadian Journal of Research*. **13**: 168-173.

Dell, B. & Huang, L. (1997) Physiological responses of plants to low boron. *Plant and Soil*. **193**: 103-120.

De Neve, S. & Hofman, G. (2000) Influence of soil compaction on carbon and nitrogen mineralization of soil organic matter and crop residues. *Biology and Fertility of Soils*. **30**(5-6): 544-549.

Del Rio, J.A., Gonzalez, A., Fuster, M.D., Botia, J.M., Gomez, P., Frias, V. & Ortuno, A. (2001) Tyloses formation and changes in phenolic compounds of grape roots infected with *Phaeomoniella chlamydospora* and *Phaeoacremonium* species. *Phytopathologia Mediterranea*. **40**: 394-399.

Dennis, J.J. (2001) Progress towards an integrated control strategy for onion white rot disease, including the use of artificial germination stimulants. In *Acta Horticulturae: Proceedings of the Second International Symposium on Edible Alliaceae, 1997*, pp. 117-121.

Dhingra, O.D. & Sinclair, J.B. (1994) *Basic Plant Pathology Methods*. 2nd edition. Boca Raton: CRC Press.

Duff, A.A., Jackson, K.J. & O'Donnell, W.E. (2001) Tebuconazole (Folicur) shows potential in the control of white rot (*Sclerotium cepivorum*) in garlic in subtropical Queensland, Australia. In *Acta Horticulturae: Proceedings of the Second International Symposium on Edible Alliaceae, 1997*, pp. 247-250.

Durbin, R.D. (1978) Abiotic diseases induced by unfavorable water relations. In *Water Deficits and Plant Growth*. Kozlowski, T.T. (ed.), New York: Academic Press.

Eagles, D.A., Hacker, J.B. & Pengelly, B.C. (1992) Morphological and agronomic attributes of a collection of buffel grass (*Cenchrus ciliaris*) and related species. *Genetic Resources Communication*(14): 1-15.

Edwards, M.C. (1995) Mapping of seed transmission determinants of barley stripe mozaic virus. *Molecular Plant-Microbe Interactions*. **8**(6): 906-915.

Ellis, M.B. (1976) *More Dematiaceous Hyphomycetes.* Kew: Commonwealth Mycological Institute.

El-Shintinawy, F. (1999) Structural and functional damage caused by boron deficiency in sunflower leaves. *Photosynthetica*. **36**(4): 565-573.

Esdale, C.R. (2001) Assessing the impact of introduced tropical pasture plants in northern Australia (buffel grass). In *Buffel Grass Symposium*. Cook, B. (ed.), Brisbane: DPI Publications, pp. 31-33.

Eskarous, J.K. & Habib, H.M. (1972) Effect of potassium permanganate and toluquinone on mozaic symptoms and necrosis caused by tomato streak virus on tobacco. *Advancing Frontiers of Plant Sciences*. **29**: 125-169.

Fensham, R. & McCosker, J. (2001) Environmental concerns of buffel grasslands. In *Buffel Grass Symposium*. Cook, B. (ed.), Brisbane: DPI Publications, pp. 34-35.

Field, T.S., Lee, D.W. & Holbrook, N.M. (2001) Why leaves turn red in autumn. The role of anthocyanins in senescing leaves of red-osier dogwood. *Plant Physiology*. **127**(2): 566-574.

Fisher, N.L., Burgess, L.W., Toussoun, T.A. & Nelson, P.E. (1982) Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. *Phytopathology*. **87**: 480-484.

Flett, S.P. & Clarke, R.G. (1996) Disease complexes in Australian Pastures. In: *Pasture and Forage Crop Pathology*. S. Chakraborty, K.T. Leath, R.A. Skipp, G.A. Pederson, R.A. Bray, G.C.M. Latch & F.W. Nutter (eds). Madison: American Society of Agronomy.

Food and Agriculture Organisation of the United Nations (Accessed 2001), *Cenchrus ciliaris* L. - species description, Food and Agriculture Organisation of the United Nations, http://www.fao.org/ag/AGP/AGPC/doc/Gbase/DATA/Pf000196.htm

Ford, C.W. & Wilson, J.R. (1981) Changes in levels of solutes during osmotic adjustment to water stress in leaves of four tropical pasture species. *Australian Journal of Plant Physiology*. **8**: 77-91.

Forde, B.J. (1966) Translocation in grasses. *New Zealand Journal of Botany*. **4**: 479-495.

Franks, A., Butler, D. & Fairfax, R. (2000) A weed by any other name. In *Wildlife Australia*, pp. 24.

Fulton, J.P., Gergerich, R.C. & Scott, H.A. (1987) Beetle transmission of plant viruses. *Annual Review of Phytopathology*. **25**: 111-123.

Gadoury, D.M., Pearson, R.C., Riegel, D.G., Seem, R.C., Becker, C.M. & Pscheidt, J.W. (1994) Reduction of powdery mildew and other diseases by over-the-trellis applications of lime sulfur to dormant grapevines. *Plant Disease*. **78**(1): 83-87.

Gardner, P.A., Angus, J.F., Pitson, G.D. & Wong, P.T.W. (1998) A comparison of six methods to control take-all in wheat. *Australian Journal of Agricultural Research*. **49**: 1225-1240.

Gaudin, V., Vrain, T. & Jouanin, L. (1994) Bacterial genes modifying hormonal balances in plants. *Plant Physiology and Biochemistry*. **32**(1): 11-29.

Geetha, H.M. & Shetty, H.S. (2002) Induction of resistance in pearl millet against downy mildew disease cuased by *Sclerospora graminicola* using benzothiadiazole, calcium chloride and hydrogen peroxide - a comparative evaluation. *Crop Protection*. **21**(8): 601-610.

Gilligan, C.A. (1995) Modelling soil-borne plant pathogens: reaction-diffusion model. *Canadian Journal of Plant Pathology*. **17**: 96-108.

Gould, K.S., McKelvie, J. & Markham, K.R. (2002) Do anthocyanins function as antioxidants in leaves? Imaging of H_2O_2 in red and green leaves after mechanical injury. *Plant, Cell and Environment*. **25**(10): 1261-1269.

Gourley, C.J.P. (1999) Potassium. In: *Soil Analysis: An Interpretation Manual*. K.I. Peverill, L.A. Sparrow & D.J. Reuter (eds). Collingwood: CSIRO Publishing.

Gourley, C.J.P., Melland, A.R., Waller, R.A., Awty, I.M., Smith, A.P., Peverill, K.I. & Hannah, M.C. (2007) *Making Better Fertiliser Decisions for Grazed Pastures in Australia*. Melbourne: Department of Primary Industries.

Graham, T.W.G. & Conway, M. (1998) Some sick buffel. *TGS News and Views*. **14**(4): 6.

Graham, T.W.G. & Conway, M. (2000) Sustainable production from sown pastures. In *Producer Demonstration Site Annual Report 1999-2000*. Middleton, C. (ed.): Queensland Department of Primary Industries/ Meat & Livestock Australia, pp. 58-69.

Graham, T.W.G. & Humphreys, L.R. (1970) Salinity response of cultivars of buffel grass (*Cenchrus ciliaris*). *Australian Journal of Experimental Agriculture and Animal Husbandry*. **10**: 725-728.

Greber, R.S. (1979) *Digitaria* striate virus – a rhabdovirus of grasses transmitted by *Sogatella kolophon*. *Australian Journal of Agricultural Research*. **30**: 43-51.

Grundon, N.J., Robson, A.D., Lambert, M.J. & Snowball, K.A. (1997) Nutrient deficiency and toxicity symptoms. In: *Plant Analysis: An Interpretation Manual*. D.J. Reuter & J.B. Robinson (eds). Collingwood: CSIRO Publishing.

Guillemette, T., Iacomi-Vasilescu, B. & Simoneau, P. (2004) Conventional and real time PCR-based assay for detecting pathogenic *Alternaria brassicae* in cruciferous seed. *Plant Disease*. **88**(5): 490-496.

Guo, X., Chen, D., Fang, Z. & Gao, Y. (2004) Study on the properties of anthocyanin from *Impatiens hawkeri*. *Hebei Nongye Daxue Xuebao*. **27**(3): 33-35.

Guy, P.L. (2006) New Zealand revisited: identification of *Cocksfoot mild mosaic virus*. *Australasian Plant Pathology*. **35**: 461-464.

Haapalainen, M., Mattinen, J. & Meltzler, M.C. (2000) The growth of plant-parasitic bacterium, *Clavibacter xyli* subsp. *cynodontis*, is enhanced by xylem fluid components. *Physiological and Molecular Plant Pathology*. **56**(4): 147-155.

Hacker, J.B. & Ratcliff, D. (1989) Seed dormancy and factors controlling dormancy breakdown in buffel grass accessions from contrasting provenances. *Journal of Applied Ecology*. **26**: 201-212.

Hall, T.J. (2001) History and development of buffel grass pasture lands in Queensland. In *Buffel grass symposium*. Cook, B. (ed.), Brisbane: DPI Publications, pp. 2-12.

Han, S.G., Oh, D.H., Ryu, J., Hwang, N.Y., Na, J.S., Park, K.H. & Choi, B.J. (1992) Allelopathic effects on upland weeds on plant growth and properties in soils. *Han'guk T'oyang Piryo Hakhoechi*. **25**(3): 263-269.

Hanold, D., Gowanlock, D., Stukely, M.J.C., Habili, N. & Randles, J.W. (2006) Mundulla Yellows disease of eucalypts: descriptors and preliminary studies on distribution and etiology. *Australasian Plant Pathology*. **35**(2): 199-215.

Hardham, A.R. (2001) The cell biology behind *Phytophthora* pathogenicity. *Australasian Plant Pathology*. **30**: 91-98.

Hawkes, J.R. & Jones, R.A.C. (1995) Incidence and distribution of Barley yellow dwarf virus and cereal yellow dwarf virus in over-summering grasses in a Mediterranean-type environment. *Australian Journal of Agricultural Research*. **56**: 257-270.

Hawkesford, M.J. (2000) Plant responses to sulphur deficiency and the genetic manipulation of sulphate transporters to improve S-utilisation efficiency. *Journal of Experimental Botany*. **51**(342): 131-138.

Hendrix, F.F. & Campbell, W.A. (1973) Pythiums as plant pathogens. *Annual Review of Phytopathology*. **11**: 77-98.

Hewitt, E.J. & Smith, T.A. (1975) *Plant Mineral Nutrition.* London: The English Universities Press Ltd.

Hill, S.A. (1985) *Methods in Plant Virology*. Oxford: Blackwell Science Inc.

Hollingsworth, C.R. (2002) Assessing heritability of brown root rot (*Phoma scerotioides*) resistance and forage yield in nine alfalfa (*Medicago sativa* ssp. *sativa*) populations. *Dissertation Abstracts International*. **63**(5): 2132.

Hopkins, W.G. (1995) *Introduction to Plant Physiology.* Toronto: John Wiley & Sons, Inc.

Huang, J., Zhou, F., Thordal-Christensen, H. & Collinge, D.B. (1998) H_2O_2 involved in defence responses of barley to powdery mildew infection. *Huazhong Nongye Daxue Xuebao*. **17**(5): 435-441.

Humphreys, L.R. (1967) Buffel grass (*Cenchrus ciliaris*) in Australia. *Tropical Grasslands*. **1**(2): 123-134.

Hynes, H.J. (1933) "Purple patch" of wheat and oats. *Agricultural Gazette of NSW*. Dec. 1, 1933: 879-883.

Ibarra, F.A., Cox, J.R., Martin, M.H., Crowl, T.A. & Call, C.A. (1995) Predicting buffel grass survival across a geographical and environmental gradient. *Journal of Range Management*. **48**(January): 53-59.

Ikeda, K., Toyota, K. & Kimura, M. (1997) Effects of soil compaction on the microbial populations of melon and maize rhizoplane. *Plant and Soil*. **189**(1): 91-96.

Inderjit, Dakshini, K.M.M. & Einhellig, F.A. (eds.) (1995) *Allelopathy - Organisms, Processes, and Applications*. Washington DC: American Chemical Society.

International Seed Testing Association (1999) International Rules for Seed Testing. In *International Seed Testing Congress*: International Seed Testing Association

Issoufou, K.A. (2000) *Acremonium* wilt complex of sorghum in Niger, etiology and control. *Dissertation Abstracts International*. **61**(7): 3343.

James, R.A., Kirkegaard, J.A. & Munns, R. (1997) Contribution of *Rhizoctonia* to reduce seedling growth of direct-drilled wheat: studies with intact cores. *Australian Journal of Agricultural Research*. **48**: 1231-1239.

Janardhanan, K.K., Gupta, M.L. & Husain, A. (1980) Effect of *Curvularia* leaf blotch disease on the essential oil contents of palmarosa. *Indian Journal of Experimental Biology*. **18**(4): 439-440.

Jarvis, R.J. & Brennan, R.F. (1986) Timing and intensity of surface cultivation and depth if cultivation affect *Rhizoctonia* patch and wheat yield. *Australian Journal of Experimental Agriculture*. **26**: 703-708.

Johansen, C. (1978) Responses of some tropical grasses to molybdenum application. *Australian Journal of Experimental Agriculture and Animal Husbandry*. **18**: 732-736.

Joost, R.E. (1995) *Acremonium* in fescue and ryegrass: Boon or bane? A review. *Journal of Animal Science*. **73**(3): 881-888.

Jones, J.B. (1998) *Plant Nutrition Manual*. Boston: CRC Press.

Jones, R.A.C. (1996) Virus diseases of Australian pastures. In: *Pasture and Forage Crop Pathology*. S. Chakraborty, K.T. Leath, R.A. Skipp, G.A. Pederson, R.A. Bray, G.C.M. Latch & F.W. Nutter (eds). Madison: American Society of Agronomy.

Kachroo, A., He, Z., Patkar, R., Zhu, Q., Zhong, J., Li, D., Ronald, P., Lamb, C. & Chattoo, B.B. (2003) Induction of H_2O_2 in transgenic rice leads to cell death and enhanced resistance to both bacterial and fungal pathogens. *Transgenic Research*. **12**(5): 577-586.

Kaitany, R.C. (2000) Races of the pathogen *Phytophthora sojae* found in Michigan and factors affecting root rot of soybean. *Dissertation Abstracts International*. **61**(5): 2297.

Kapoor, I.J. (1983) Pathological and biochemical studies on *Curvularia* rot of guava. *Zeitschrift fuer Pflanzenkrankheiten und Pflanzenschutz*. **90**(6): 591-598.

Keates, S.E., Kostman, T.A., Anderson, J.D. & Bailey, B.A. (2003) Altered gene expression in three plant species in response to treatment with Nep1, a fungal protein that causes necrosis. *Plant Physiology*. **132**: 1610-1622.

Khan, A. & Hsiang, T. (2003) The infection process of *Colletotrichum graminicola* and relative aggressiveness on four turfgrass species. *Canadian Journal of Microbiology*. **49**(7): 433-442.

Khan, M.W. & Pathak, K.N. (1993) Nematodes as vectors of bacterial and fungal plant pathogens. In *Nematode Interactions*. Khan, M.W. (ed.), London: Chapman & Hall.

Kirkegaard, J.A., Munns, R., James, R.A. & Neate, S.M. (1999) Does water and phosphorus uptake limit leaf growth of *Rhizoctonia*-infected seedlings? *Plant and Soil*. **209**: 157-166.

Kozlowski, T.T. (1999) Soil compaction and growth of woody plants. *Scandinavian Journal of Forest Research*. **14**(6): 596-619.

Lamb, C. & Dixon, R.A. (1997) The oxidative burst in plant disease resistance. *Annual Review of Plant Physiology and Plant Molecular Biology*. **48**: 251-275.

Larkin, R.P., Ristaino, J.B. & Campbell, C.L. (1995) Detection and quantification of *Phytophthora capsici* in soil. *Phytopathology*. **85**(10): 1057-1063.

Lazarides, M. (1970) *The Grasses of Central Australia.* Canberra: Australian National University Press.

Lee, D.W., O'Keefe, J., Holbrook, N.M. & Feild, T.S. (2003) Pigment dynamics and autumn leaf senescence in a New England deciduous forest, eastern USA. *Ecological Research*. **18**: 677-694.

Lemaire, D., Huret, S., Calay, F. & Maraite, H. (2002) Understanding *Puccinia striiformis* West. epidemics on winter wheat. *Mededelingen*. **67**(2): 251-256.

Lenne, J.M. (1990) A world list of fungal diseases of tropical pasture species. *Phytopathological Papers*, 31.

Lewis, D.C. (1999) Sulfur. In: *Soil Analysis: An Interpretation Manual*. K.I. Peverill, L.A. Sparrow & D.J. Reuter (eds). Collingwood: CSIRO Publishing.

Li, C.H., Ma, B.L. & Zhang, T.Q. (2002) Soil bulk density effects on soil microbial populations and enzyme activities during the growth of maize (*Zea mays* L.) planted in large pots under field exposure. *Canadian Journal of Soil Science*. **82**(2): 147-154.

Liberato, J.R., Barreto, R.W. & Shivas, R.G. (2005) Leaf-clearing and staining techniques for the observation of conidiophores in the *Phyllactinioideae* (*Erysiphaceae*). *Australasian Plant Pathology*. **34**: 401-404.

Lingua, G., D'Agostino, G., Fusconi, A. & Berta, G. (2001) Nuclear changes in pathogen-infected tomato roots. *European Journal of Histochemistry*. **45**(1): 21-30.

Loch, D. (1998) Buffel blight in southern USA. *TGS News and Views*. **14**(4): 7.

Loch, D.S. (1999) *Cenchrus ciliaris* in Australia. *Forage Seed Production*. **2**: 351-361.

Low, T. (1997) Tropical pasture plants as weeds. *Tropical Grasslands*. **31**: 337-343.

Lucas, G.B., Campbell, C.L. & Lucas, L.T. (1985) *Introduction to Plant Diseases - Identification and Management.* 2nd edition. New York: Van Nostrand Reinhold.

Lucas, J.A. (1998) *Plant Pathology and Plant Pathogens.* 3rd edition. Oxford: Blackwell Science, Ltd.

MacLeod, W.J. & Sweetingham, M.W. (1997) A root disease of *Lupinus angustifolius* caused by a new species of binucleate *Rhizoctonia*. *Australian Journal of Agricultural Research*. **48**: 21-30.

MacNish, G.C. & O'Brien, P.A. (2003) The thin, binucleate *Rhizoctonia* causing Eradu patch is widespread in lupin and cereal crops in Western Australia. *Australasian Plant Pathology*. **32**: 299-304.

Ma, Z. & Michailides, T.J. (2007) Approaches for eliminating PCR inhibitors and designing PCR primers for the detection of phytopathogenic fungi. *Crop Protection*. **26**: 145-161.

Maloy, O.C. (1993) *Plant Disease Control – Principles and Practice*. New York: John Wiley & Sons, Inc..

Manners, J.G. & Myers, A. (1981) Effects on host growth and physiology. In *Biology and Control of Take-all*. Asher, M.J.C., and Shipton, P.J. (eds.), London: Academic Press.

Marten, G.C. (1978) The animal-plant complex in forage palatability phenomena. *Journal of Animal Science*. **46**(5): 1470-1477.

Martinez, H.E.P. & Barbosa, J.G. (1999) Substrates for hydroponics. *Informe Agropecuario*. **20**: 200-201.

Martinez-Culebras, P.V., Abad-Campos, P. & Garcia-Jimenez, J. (2004) Molecular characterization and PCR detection of the melon pathogen *Acremonium cucurbitacearum*. *European Journal of Plant Pathology*. **110**(8): 801-809.

Massicotte, H.B., Melville, L.H. & Peterson, R.L. (2005) Structural characteristics of root-fungal interactions for five ericaceous species in eastern Canada. *Canadian Journal of Botany*. **83**: 1057-1064.

Matern, U. & Kneusel, R.E. (1988) Phenolic compounds in plant disease resistance. *Phytoparasitica*. **16**(2): 153-170.

Matheron, M.E. & Matejka, J.C. (1993) Seasonal differences in susceptibility of three citrus rootstocks to root lesions caused by *Phytophthora citrophthora* and *P. parasitica*. *Plant Disease*. **77**(7): 729-732.

Matheron, M.E. & Porchas, M. (2002) Suppression of *Phytophthora* root and crown rot on pepper plants treated with acibenzolar-S-methyl. *Plant Disease*. **86**(3): 292-297.

Matile, P., Hortensteiner, S. & Thomas, H. (1999) Chlorophyll degradation. *Annual Review of Plant Physiology and Plant Molecular Biology*. **50**: 67-95.

Mauseth, J.D. (1988) *Plant Anatomy.* Menlo Park: Benjamin/Cummings Publishing Company Inc.

McCartner, H.A. & Fitt, B.D.L. (1998) Dispersal of foliar fungal pathogens: mechanisms, gradients and special patterns. In: *The Epidemiology of Plant Diseases*. D.G. Jones (ed.). Dordrecht: Kluwer Academic Publishers.

McCartney, H.A. (1991) Airborne dissemination of plant fungal pathogens. In: *Pathogens in the Environment*. B. Austin (ed.). Oxford: Blackwell Scientific Publications.

McCosker, T. (2000) Cell grazing – the first 10 years in Australia. *Tropical Grasslands*. **34**: 207-218.

McElhany, P., Real, L.A. & Power, A.G. (1995) Vector preference and disease dynamics: a study of barley yellow dwarf virus. *Ecology*. **76**(2): 444-457.

McFarlane, J.D. (1999) Iron. In: *Soil Analysis: An Interpretation Manual*. K.I. Peverill, L.A. Sparrow & D.J. Reuter (eds). Collingwood: CSIRO Publishing.

McIvor, J.G. (2007) Pasture management in semiarid tropical woodlands: improving the herbage quality of stylos and grasses. *Australian Journal of Experimental Agriculture*. **47**: 1359-1367.

Meenakshi, G., Solomon, J.J. & Sulochana, C.B. (1972) Toluidine blue meta chromasia. Means for early detection of plant virus infection. *Stain Technology*. **47**(5): 267-268.

Mehlich, A. (1984) Mehlich 3 soil test extractant: a modification of Mehlich 2 extractant. *Communications in Soil Science and Plant Analysis*. **15**(12): 1409-1416.

Mendgen, K. & Hahn, M. (2002) Plant infection and the establishment of fungal biotrophy. *Trends in Plant Science*. pp.1-5.

Michniewicz, M. (1982) Role of growth regulators in the higher plant-pathogen system. *Postepy Nauk Rolniczych*. **29**(5): 27-45.

Moen, R. & Harris, J.R. (1983) The *Rhizoctonia* disease complex of wheat. In *Proceedings of Section 5 of the Fourth International Congress of Plant Pathology*. Parker, C.A., Rovira, A.D., Moore, K.J., and Wong, P.T.W. (eds.), University of Melbourne, 17-24th August 1983: The American Phytopathological Society

Moody, P.W. & Bolland, M.D.A. (1999) Phosphorus. In: *Soil Analysis: An Interpretation Manual*. K.I. Peverill, L.A. Sparrow & D.J. Reuter (eds). Collingwood: CSIRO Publishing.

Muhle, E. (1958) *Phoma* wilting of valerian. *Planta Medica*. **6**: 89-91.

Muller, P.G. (2001) Soil properties of the buffel grass pasture lands. In *Buffel Grass Symposium*. Cook, B. (ed.), Brisbane: DPI Publications, pp. 13-17.

Mulholland, B. & Fullen, M.A. (1991) Cattle trampling and soil compaction on loamy sands. *Soil Use and Management*. **7**(4): 189-193.

Murray, G.M. & Davis, R.D. (1996) Fungal, bacterial and nematode diseases of Australian pastures. In: *Pasture and Forage Crop Pathology*. S. Chakraborty, K.T. Leath, R.A. Skipp,

G.A. Pederson, R.A. Bray, G.C.M. Latch & F.W. Nutter (eds). Madison: American Society of Agronomy.

Musetti, R., Favali, M.A. & Pressacco, L. (2000) Histopathology and polyphenol content in plants infected by phytoplasmas. *Cytobios*. **102**: 133-147.

Nable, R.O., Banuelos, G.S. & Paull, J.G. (1997) Boron toxicity. *Plant and Soil*. **193**: 181-198.

Naidu, R. & Rengasamy, P. (1993) Ion interactions and constraints to plant nutrition in Australian sodic soils. *Australian Journal of Soil Research*. **31**(6): 801-819.

Narayanasamy, P. (2001) *Plant Pathogen Detection and Disease Diagnoses.* New York: Marcel Dekker Inc.

Narwal, S.S. (ed.) (1999) *Allelopathy Update*. New Hampshire: Science Publishers Inc.

Nelson, E.B. (1990) Exudate molecules initiating fungal responses to seeds and roots. *Plant and Soil*. **129**: 61-73.

Ng, T.T., Wilson, J.R. & Ludlow, M.M. (1975) Influence of water stress on water relations and growth of a tropical (C_4) grass, *Panicum maximum* var. *trichoglume*. *Australian Journal of Plant Physiology*. **2**: 581-595.

Ni, J., Colowit, P.M., Oster, J.J., Xu, K. & Mackill, D.J. (2001) Molecular markers linked to stem rot resistance in rice. *Theoretical and Applied Genetics*. **102**(4): 511-516.

Niemann, G.J. (1994) A crucial role of phenolic metabolism in resistance of carnation of wilt disease. *Acta Horticulturae*. **381**(2): 565-571.

Nippon Peroxide Co Ltd (1982) Soil Fungus Control With Hydrogen Peroxide. *Japan Kokai Tokyo Koho*: 2.

O'Brien, T.P. & McCully, M.E. (1981) *The Study of Plant Structure - Principles and Selected Methods.* Melbourne: Termarcarphi Pty Ltd.

O'Brien, P.A. & Zamani, M. (2003) Production of pectic enzymes by barepatch isolates of *Rhizoctonia solani* AG 8. *Australasian Plant Pathology*. **32**: 65-72.

Ogle, H. & Brown, J. (1997) Vector-borne inoculum. In: *Plant Pathogens and Plant Diseases*. J.F. Brown & H.J. Ogle (eds). Armidale: University of New England Printery.

Oliver, R.P., Henricot, B. & Segers, G. (2000) *Cladosporium fulvum*, cause of leaf mold of tomato. *Fungal Pathology*: 65-91.

O'Neill, T.M., Bagabe, M. & Ann, D.M. (1991) Aspects of biology and control of a stem rot of cucumber caused by *Penicillium oxalicum*. *Plant Pathology*. **40**(1): 78-84.

Ortego, J. (1997) Plant cell wall degrading enzymes produced by the phytopathogenic fungus *Scerotium bataticola*. *Texas Journal of Science*. **49**(3): 235-242.

Otten, W. & Gilligan, C.A. (2006) Soil structure and soil-borne diseases: using epidemiological concepts to scale from fungal spread to plant epidemics. *European Journal of Soil Science*. **57**: 26-37.

Pagliai, M. (1987) Micromorphometric and micromorphological investigations on the effect of compaction by pressures and deformations resulting from tillage and wheeled traffic. In *Soil Compaction and Regeneration*. Monnier, G., and Goss, M.J. (eds.), Rotterdam: A.A.Balkema.

Pais, I. & Benton Jones Jr., J. (1997) *The Handbook of Trace Elements.* Boca Raton: St. Lucie Press.

Pandey, K.K. (1976) Effect of certain chemicals on soil fungistasis. *Plant and Soil*. **44**(2): 487-489.

Parry, D.W. (1990) *Plant Pathology in Agriculture.* New York: Cambridge University Press.

Paull, C.J. & Lee, G.R. (1978) Buffel grass in Queensland. *Queensland Agricultural Journal*. **104**: 57-75.

Payne, W. (2000) *Fusarium oxysporum Schlect. and Buffel Grass (Cenchrus ciliaris) Dieback.* Unpublished project report, Central Queensland University, Rockhampton.

Perrott, R. (2001) Diseases of buffel grass. In *Buffel Grass Symposium*. Cook, B. (ed.), Brisbane: DPI Publications, pp. 19-20.

Perrott, R. & Chakraborty, S. (1999) *Pyricularia grisea* causes blight of buffel grass (*Cenchrus ciliaris*) in Queensland, Australia. *Tropical Grasslands*. **33**: 201-206.

Petrie, J.M. (1924) Plant pigments II: The red pigments produced by insect injury in *Eucalyptus stricta*. *Proceedings of the Linnean Society of New South Wales*. **49**: 386-394.

Pinkerton, A., Smith, F.W. & Lewis, D.C. (1997) Pasture Species. In: *Plant Analysis: An Interpretation Manual*. D.J. Reuter & J.B. Robinson (eds). Collingwood: CSIRO Publishing.

Prathuangwong, S., Kao, S.W., Sommartya, T. & Sinchaisri, P. (1991) Role of four *Alternaria* spp. causing leaf and stem blight of sunflower in Thailand and their chemical controls. *Kasetsart Journal*. **25**(1): 112-124.

ProSciTech (accessed 2003), L.R. White - user notes, ProSciTech, http://www.proscitech.com

Putnam, A.R. & Duke, W.B. (1978) Allelopathy in agroecosystems. *Annual Review of Phytopathology*. **16**: 431-451.

Ragazzi, A., Moricca, S., Dellavalle, I. & Mancini, F. (1995) Infection of cotton by *Fusarium oxysporum* f.sp. *vasinfectum* as affected by water stress. *Phytoparasitica*. **23**(4): 315-321.

Rahman, A., Burney, B. & Manson, B.E. (1978) Effect of soil compaction on phytotoxicity and persistence of soil-applied herbicides. *Weed Research*. **18**(2): 93-97.

Rayment, G.E. & Higginson, F.R. (1992) *Australian Laboratory Handbook of Soil and Water Chemical Methods.* Melbourne: Reed International Books Australia Pty Limited.

Reid, R.J., Hayes, J.E., Post, A., Strangoulis, J.C.R. & Graham, R.D. (2004) A critical analysis of boron toxicity in plants. *Plant, Cell and Environment*. **25**: 1405-1414.

Reis, E.M., Cook, R.J. & NcNeal, B.L. (1982) Effect of mineral nutrition on take-all of wheat. *Phytopathology*. **72**(2): 224-229.

Rhoades, C.C., Brosi, S.L., Dattilo, A.J. & Vincelli, P. (2003) Effect of soil compaction and moisture on incidence of *Phytophthora* root rot on American chestnut (*Castanea dentata*) seedlings. *Forest Ecology and Management*. **184**(1/3): 47-54.

Richie, B.J. (2002) Mycological Media and Methods. In *Plant Pathologist's Pocketbook*. Waller, J.M., Lenne, J.M., and Waller, S.J. (eds.), New York: CABI Publishing.

Ristaino, J.B., Groves, C.T. & Parra, G.R. (2001) PCR amplification of the Irish potato famine pathogen from historic specimens. *Nature*. **411**(6838): 695-697.

Roberts, J. & Whitehouse, D.J. (1976) *Practical Plant Physiology.* London: Longman Group Limited.

Rodriguez, O., Gonzalez-Dominguez, J., Krausz, J.P., Odvody, G.N., Wilson, J.P., Hanna, W.W. & Levy, M. (1999) First report and epidemics of buffel grass blight caused by *Pyricularia grisea* in South Texas. *Plant Disease*. **83**(4): 398.

Russel, J.S. (1976) Comparative salt tolerance of some tropical and temperate legumes and tropical grasses. *Australian Journal of Experimental Agriculture and Animal Husbandry*. **16**: 103-109.

Ryley, M.J., Lloyd, D.L., Johnson, B., Teasdale, K.C. & Mackie, J.M. (2004) *Rhizoctonia* crown and root rot of the pasture legume, sulla (*Hedysarum coronarium*). *Australasian Plant Pathology*. **33**: 183-188.

Sakai, W.S. (1973) Method for differential staining of paraffin embedded plant material using toluidine blue. *Stain Technology*. **48**(5): 247-249.

Salisbury, F.B. & Ross, C.W. (1992) *Plant Physiology*. 4th ed. Belmont: Wadsworth Publishing Company.

Sattar, A. & Husain, A. (1982) Chemical control of *Thielavia basicola* causing stolon and root-rot disease of Japanese mint. *Indian Phytopathology*. **35**(1): 106-110.

Schoeneweiss, D.F. (1978) Water stress as a predisposing factor in plant disease. In *Water Deficits and Plant Growth*. Kozlowski, T.T. (ed.), New York: Academic Press.

Schwartz, P.A. & Safaya, N.M. (1978) Some nutrient deficiency and toxicity symptoms in slender wheatgrass. *Proceedings of the North Dakota Academy of Science*. **31**(2): 50-57.

Seifert, K. (1996), Fusarium interactive key, http://www.ctu.edu.vn/colleges/agri/gtrinh/fuskey.pdf

Settle, D.M., Fry, J.D. & Tisserat, N.A. (2001) Development of brown patch and *Pythium* blight in tall fescue as affected by irrigation frequency, clipping removal, and fungicide application. *Plant Disease*. **85**(5): 543-546.

Sharma, S., Radl, V., Hai, B., Kloos, K., Fuca, M.M., Engel, M., Schauss, K. & Schloter, M. (2007) Quantification of functional genes from procaryotes in soil by PCR. *Journal of Microbiological Methods*. **68**: 445-452.

Shaul, O. (2002) Magnesium transport and function in plants: the tip of the iceberg. *BioMetals*. **15**: 309-323.

Shazia, I. & Iftikhar, A. (2005) Analysis of variation in *Alternaria alternata* by pathogenicity and RAPD study. *Polish Journal of Microbiology*. **54**(1): 13-19.

Shepherd, T. (1994) The microchemistry of plant/microorganism interactions. In: *Ecology of Plant Pathogens*. J.P. Blakeman & B. Williamson (eds). Wallingford: CAB International.

Sigma-Aldrich (2004), Surface Sterilisation of Plant Explants and Orchid Seeds, Sigma-Aldrich, www.sigmaaldrich.com/Area_of_Interest/Life_Science/Plant_Biotechnology

Sims, D.A. & Gamon, J.A. (2002) Relationship between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. *Remote Sensing of Environment*. **81**: 337-354.

Singh, H.P., Batish, D.R. & Kohli, R.K. (2001) Allelopathy in Agroecosystems: an Overview. In: *Allelopathy in Agroecosystems*. R.K. Kohli, H.P. Singh & D.R. Batish (eds). Binghampton: Food Products Press.

Slattery, W.J., Conyers, M.K. & Aitken, R.L. (1999) Soil pH, aluminium, manganese and lime requirements. In: *Soil Analysis: An Interpretation Manual*. K.I. Peverill, L.A. Sparrow & D.J. Reuter (eds). Collingwood: CSIRO Publishing.

Smith, A.E. (1999) Allelopathy in the grassland ecosystems. In *Allelopathy Update*. Narwal, S.S. (ed.), New Hampshire: Science Publishers Inc.

Smith, F.W. (1972) Foliar symptoms of nutrient disorders in *Panicum maximum* var. *trichoglume* cv. Petrie. In *Division of Tropical Pastures Technical Paper No 9* Melbourne: Commonwealth Scientific and Industrial Research Organisation.

Smith, F.W. (1974) Foliar Symptoms of Nutrient Disorders in *Cenchrus ciliaris*. In: CSIRO Australia, Division of Tropical Agronomy Technical Paper No. 16.

Smith, J.D., Gossen, B.D. & Evans, I. (2001) *Ascochyta phleina* causes a patch disease on turfgrass. *Canadian Journal of Plant Pathology*. **23**: 181-183.

Speakman, J.B. & Krueger, W. (1983) A comparison of methods to surface sterilise wheat seeds. *Transactions of the British Mycological Society*. **80**(2): 374-376.

Spooner-Hart, R. (2005) Sustainable Pest and Disease Management in Australian Olive Production. *RIRDC Publication*, 05/080.

Standards Australia International Limited (1997) *Methods of testing soils for engineering purposes (AS 1289.6.3.2 - 1997).* Sydney: Standards Australia International Limited.

Stebbins, G.L. (1967) Variation and evolution in plants. In *Columbia Biological Series*. Dunn, L. (ed.), New York: Columbia University Press.

Strange, R.N. (2003) *Introduction to Plant Pathology.* Chichester: John Wiley & Sons, Ltd.

Strong, W.M. & Mason, M.G. (1999) Nitrogen. In: *Soil Analysis: An Interpretation Manual*. K.I. Peverill, L.A. Sparrow & D.J. Reuter (eds). Collingwood: CSIRO Publishing.

Suslow, T.V. & Schroth, M.N. (1982) Role of deleterious rhizobacteria as minor pathogens in reducing crop growth. *Phytopathology*. **72**(1): 111-115.

Tamura, S., Kimura, Y., Inoue, T., Hirota, A., Katagiri, K. & Suzuki, A. (1974) Novel plant growth regulators produced by phytopathogenic fungi. In *Plant Growth Substances, Proceedings of the 8th International Conference*, Tokyo: Hirokawa Publishing Company

Tarr, S.A.J. (1972) *Principles of Plant Pathology.* London: The Macmillan Press.

Teakle, D.S. & Grylls, N.E. (1973) Four strains of sugar-cane mosaic virus infecting cereals and other grasses in Australia. *Australian Journal of Agricultural Research*. **24**: 465-477.

Terry, N. (1977) Photosynthesis, growth, and the role of chloride. *Plant Physiology*. **60**: 69-75.

Thangavelu, R., Sundararaju, P. & Sathiamoorthy, S. (2004) Management of anthracnose disease of banana caused by *Colletotrichum musae* using plant extracts. *Journal of Horticultural Science and Biotechnology*. **79**(4): 664-668.

The Royal Horticultural Society (2001) *Royal Horticultural Society Plant Colour Chart.* London: The Royal Horticultural Society.

Thompson, L.N., Adkins, S.W. & Bellairs, S.M. (2001) Implications of native seed viability. In *Fourth Australian Workshop on Native Seed Biology for Revegetation*. Adkins, S.W., Bellairs, S.M., and Bell, L.C. (eds.), Mildura: Australian Centre for Mining Environmental Research, pp. 37-44.

'tMannetje, L.M. & Jones, R.M. (1990) Pasture and animal productivity of buffel grass with Siratro, lucerne or nitrogen fertiliser. *Tropical Grasslands*. **24**: 269-281.

Tothill, J.C. & Hacker, J.B. (1983) *The Grasses of Southern Queensland.* Brisbane: University of Queensland Press.

Truscott, J.E. & Gilligan, C.A. (2001) The effect of cultivation on the size, shape, and persistence of disease patches in fields. *Proceedings of the National Academy of Sciences of the United States of America*. **98**(13): 7128-7133.

Uren, N.C. (1999) Manganese. In: *Soil Analysis: An Interpretation Manual*. K.I. Peverill, L.A. Sparrow & D.J. Reuter (eds). Collingwood: CSIRO Publishing.

Val, J., Monge, E., Heras, L. & Abadia, J. (1987) Changes in photosynthetic pigment composition in higher plants as affected by iron nutrient status. *Journal of Plant Nutrition*. **10**: 9-16.

Van Andel, O.M. & Fuchs, A. (1972) Interference with plant growth regulators by microbial metabolites. Proceedings of the NATO Advanced Study Institute - *Phytotoxins Plant Disease*. pp. 227-249.

Vanderplank, J.E. (1982) *Host-Pathogen Interactions in Plant Disease.* London: Academic Press Inc.

Visser, N.C., Spies, J.J. & Venter, H.J.T. (1998a) Meiotic chromosome behaviour in *Cenchrus ciliaris* (Poaceae: Panicoideae). *Bothalia*. **28**(1): 83-90.

Visser, N.C., Spies, J.J. & Venter, H.J.T. (1998b) Uneven segregation of chromosomes: a possible source of aneuploidy in *Cenchrus ciliaris* (Poaceae: Paniceae). *South African Journal of Botany*. **64**(2): 130-136.

Visser, N.C., Spies, J.J. & Venter, H.J.T. (1998c) Aneuploidy in *Cenchrus ciliaris* (Poaceae, Panicoideae, Paniceae): truth or fiction? *South African Journal of Botany*. **64**(6): 337-345.

Viswanathan, R., Nandakumar, R. & Samiyappan, R. (2003) Role of pathogenesis-related proteins in rhizobacteria-mediated induced systemic resistance against *Colletotrichum falcatum* in sugarcane. *Zeitschrift fuer Pflanzenkrankheiten und Pflanzenschutz*. **110**(6): 524-534.

Vocasek, F.F. & Friedericks, J.B. (1994) Soil micronutrient extraction by Mehlich 3 compared to $CaCl_2$-DTPA. *Communications in Soil Science and Plant Analysis*. **25**(9-10): 1583-1593.

Wallace, T. (1947) Visual diagnosis of mineral deficiencies in plants. *Annals of Applied Biology*. **34**: 146-147.

Wallace, T. (1961) *The Diagnosis of Mineral Deficiencies in Plants by Visual Symptoms.* 3rd ed. London: H.M. Stationery Office.

Walters, D.R. (1996) *Vascular Plant Taxonomy.* Dubuque: Kendall/Hunt Publishing.

Webb, A.A., Dowling, A.J. & Nugent, P.J. (1982) Studies on solodic soils under *Acacia harpophylla-Eucalyptus cambageana* forests in central Queensland. *Queensland Journal of Agricultural and Animal Sciences*. **39**(2): 109-123.

Welch, R.M. (1986) Effects of nutrient deficiencies on seed production and quality. *Advances in Plant Nutrition*. **2**: 205-247.

Werner, T., Motyka, V., Strnad, M. & Schmulling, T. (2001) Regulation of plant growth by cytokinin. *Proceedings of the National Academy of Sciences of the United States of America*. **98**(18): 10487-10492.

White, P.J. & Broadley, M.R. (2003) Calcium in plants. *Annals of Botany*. **92**: 487-511.

Wilson, J.R., Ludlow, M.M., Fisher, M.J. & Schulze, E.D. (1980) Adaptation to water stress of the leaf water relations of four tropical forage species. *Australian Journal of Plant Physiology*. **7**: 207-220.

Winter, E. (1958) The formation of a red pigment in injured plant tissue. *Planta*. **52**: 187-194.

Wong, P.T.W., Tan, M.K. & Beehag, G.W. (2000) Confirmation of take-all patch disease in Tifdwarf hybrid couch grass (bermudagrass) by morphological and DNA methods. *Australasian Plant Pathology*. **29**(1): 19-23.

Wright, E.R., Palmucci, H.E., Vero, S., Pianzzola, M.J., Moscatelli, M.R. & Cappucchio, S. (2003) Rot of bulbs of ornamental species caused by *Penicillium minioluteum* in Argentina. *Phyton*: 241-250.

Wrolstad, R.E. (2004) Anthocyanin pigments. Bioactivity and coloring properties. *Journal of Food Science*. **69**(5): 419-421.

Yao, C.L., Frederiksen, R.A. & Magill, C.W. (1990) Seed transmission of sorghum downy mildew: detection by DNA hybridisation. *Seed Science and Technology*. **18**(2): 201-207.

York, K. (1996) Take-all patch in cereals and amenity turf. *International Turfgrass Bulletin*. **193**: 29-30.

Zimmermann, M.H. (1983) *Xylem Structure and the Ascent of Sap.* New York: Springer-Verlag.