Germination and Storage Studies of Selected Australian Tropical Native Grasses in Relation to their Ecology and Use in Land Rehabilitation

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To

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DECLARATION

I hereby declare that the main text in this thesis is an original work and no part of this thesis has been previously submitted for the award of any other degree.

I also declare that to my best knowledge any assistance I received in the experimentation presented in this thesis and all sources of information used in this thesis have been acknowledged.

Sam Fesuk

Abstract

A study was financed by the Victoria River District Conservation Association (VRDCA) and Greening Australia Northern Territory (GANT) to examine the germination and storage requirements of a range of grasses indigenous to the northern part of the Northern Territory. The aim was to increase understanding of the germination requirements and dormancy cycles of species that could be utilised for restoration and rehabilitation purposes, and be commercially grown and harvested for seed supply to pastoralists (initially through a co-operative), mining companies and contractors involved in the construction of railway lines and raised banks, roads and bridges.

In 2000 and 2001, seeds of twenty-five grass species were collected from across the Northern Territory and were placed into long-term storage trials. Results showed that some tropical seeds retained their viability when frozen (*Astrebla squarrosa*), while others achieved their germination potential after storage in a garden shed (*Brachyachne convergens*).

A trial was undertaken to test for the suitability of three indigenous grass species in batter stabilisation, using an exotic pasture grass as a control. The species were monitored for their ability to germinate rapidly, produce stolons and to mature and seed quickly. The study found that while two of the species (*Brachyachne convergens* and *Chloris pectinata*) showed great potential, they were infringed upon by the invasive *Chloris gayana* (Rhodes grass) during the second season.

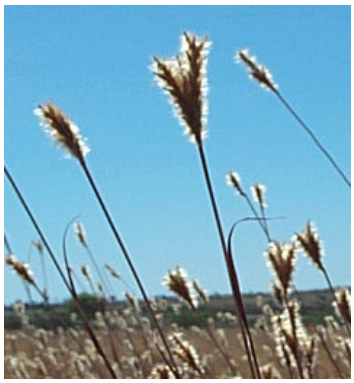
Studies were also conducted using Scanning Electron Microscopy to examine the surface of the seeds in their storage treatments. This study provided a database of the morphological features of all of the seed species in storage, the information thaof which could be utilised by other researchers.

One dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was used to extract and analyse the molecular mass of proteins in the seed of *Triodia bitextura* at various stages of imbibition prior to germination. The results demonstrated that storage condition affects the seed viability of this species possibly due to the structural break-down of stored macro-proteins prior to imbibition.

Field observations were also conducted on ten sites across the top of the North Territory. GANT has been monitoring sites at which they had planted selected species of grasses to test their suitability for rehabilitation work across a range of environmental conditions. These observations, in combination with separate GANT observations led to the selection of four indigenous species, three of which (*Chrysopogon elongatus, Alloteropsis* semialata and *Dichanthium fecundum*) are now under commercial cultivation. The fourth species, *Brachyachne convergens*, is undergoing further experimentation.

The end result of this study is an increased understanding of the dormancy cycles of some species of seed, their optimum storage requirements and testing their suitability for use in rehabilitation and restoration of disturbed land.

Chapter 1
Introduction



1.1 Introduction to the topic

The topic “Germination and Storage Studies of Selected Australian Tropical Native Grasses in Relation to their Ecology and Use in Land Rehabilitation” encompasses a wide range of experiments in the fields of grass seed biology and physiology. Hence, the literature reviewed is primarily concerned with seed germination treatments, effects of storage, dormancy mechanisms, enzyme pathways, seed structure in relation to edaphic conditions, and the use of native grasses in environmental rehabilitation and restoration.

1.2 Uses of Australian Native Grasses

1.2.1 Pastures

Australian native grasses are adapted to the poor soils, and climatic extremes of the Australian outback. Grasses provide feed for cattle with some species of grasses achieving digestibility levels of 70% (*Digitaria bicornis* (Lam.) Roemer & Schultes*)* and crude protein levels in excess of 15% *(Dactyloctenium radulans* (R.Br) P. Beauvois). These characteristics

combine to make some native grass species a very valuable resource (Vallance et al. 1993; Shaw and Fesuk 2003).

1.2.2 Rehabilitation/Revegetation

Australia has a wide diversity of native grass species many of which are restricted to specific soil types or geographic regions. To date, trials have been conducted using exotic species such as Rhodes grass (*Chloris gayana* Kunth) which has been extensively used in the tropics in revegetation of road sides, railway batters (Gyasi-Agyei et al. 2001) and mine sites (Ashwath et al. 1994; Huxtable and Waters 2001) and Vetiver grass (*Vetiveria zizanioides* (L) Nash*)* which has been used in the stabilisation of batters in Queensland (Truong and Loch 2004). Yet little has been trialled on the use of native grasses such as *Brachyachne convergens* (F.Muell.) Stapf*, Chloris pectinata* (Benth) or *Iseilema vaginiflorum* (Domin) the species which have been investigated and reported on in Chapter 4.

1.3 Ecology of Australian Native Grasses

1.3.1 Description

The fruit of members of the family Poaceae is referred to as a caryopsis. A caryopsis is an indehiscent dry fruit in which the layers of the integument and the pericarp are fused together to form the seed coat. In grasses, the embryo, which is very small relative to the seed size, is usually laterally positioned to one side of the seed, beside a large starchy endosperm (Baskin and Baskin 1998a).

The majority of Australian native grasses are classed as ‘chaffy’ grasses, having awns, glumes, bristles and hairs associated with, or part of the seed

coat. These features in some species play an important role in the dispersion of the seed, or in the imbibition of water during germination (Paterson et al. 2001).

1.3.2 Dispersion

Grasses, as a whole, utilise a wide range of dispersal methods. Some use the inflorescence itself as the tool of dispersal for the seeds, while in others the inflorescence structure remains attached to the parent plant. Where the spikelets contain more than one seed, the rachillas may disarticulate between each floret, dropping a naked caryopsis (e.g. *Dactyloctenium radulans*) or still be enclosed within the palea and lemma (e.g. *Triodia basedowii* (E. Pritz)). Some inflorescences have special structures to aid wind dispersal, while other species employ sharp calluses (e.g. *Aristida* spp.), or scabrous lemmas (e.g. *Cenchrus* spp.) to embed in, or to stick to, passing animals. Others have hygroscopically active awns (e.g. *Sorghum intrans* (F. Muell) ex. Benth) to drill into the sub-soil moisture zone. Some species will have large awns (e.g. *Aristida* spp.), whereas other species have fine seed, easily blown on by the wind (e.g. *Sporobolus* spp.)(Peart 1984).

1.3.3 Environmental (Edaphic) Conditions

The Northern Territory is the region that exhibits great plant diversity that has been established upon ancient, weathered and leached soils and exist under a strong wet-dry seasonality (Woinarski 1999).

The arid lands in the south of the Northern Territory have a wide variation in the amount of rainfall received from year to year, yet they still contain a wide

range of species hidden within the dominant vegetation types of hummock grasslands and open woodlands and desert scrubs (Woinarski 1999).

Unlike southern Australia, the northern regions have been exempted from heavy land clearing for cropping. Instead their vast plains have been utilised for grazing. It was through over-utilisation and the subsequent erosion effects that prompted the realisation of how fragile these skeletal soils really are, and how unprepared graziers were for the sudden loss of productivity and ensuing dominance of unpalatable pasture species (Scattini et al. 1988).

Studies conducted over the past decades have highlighted the inter-dependency of the vegetation types with the underlying soils and rock strata, as well as the vegetation dependence upon the varying climatic factors of the Northern Territory (Thackway and Cresswell 1995).

Perry (1960) has categorised eight basic groups of soils all of which are typified by both low phosphate and nitrogen concentrations.

i. *Red sands and clayey sands* comprise all the sand plains and dune fields which occupy over half of the area of the centre of Australia and are covered with hummock grasslands primarily composed of *Triodia* spp.

ii. *Red earths* are the second most extensive type of soils and extend into the high rainfall areas. In drier regions they carry short grass/forb pastures and in higher rainfall areas support *Themeda* spp and *Sorghum* spp.

iii. *Grey and brown heavy texture soils* that mainly occur as a crescent shaped belt that stretches across the Northern

Territory from the Barkly Tableland to the headwaters of the Victoria River. Usually treeless where rainfall is less than 450 mm per annum, supporting plains of *Astrebla* spp. and other associated grass species, such as *Iseilema* spp., *Eulalia aurea* (Bory) Kunth and *Spathia neurosa* (Ewart and Archer).

iv. *Lateritic podzols* are extensive in the northern part of the Northern Territory, carrying species such as *Sorghum intrans* and *Brachyachne convergens* (Shaw and Fesuk 2003).

v. *Stony soils* restricted to the low rainfall areas south of 23^0S. Mainly chenopodiaceous shrubland, with the occasional short grass species such as *Eriachne* spp., and forbs dominate these soils (Milson 2000).

vi. *Calcareous desert soils* occur in relatively small isolated pockets in regions of 350 mm annual rainfall and support grasses such as *Enneapogon* spp. (Vallance et al. 1993).

vii. *Yellow podzols* (podosols) also occur in small patches carrying mainly *Themeda triandra* (Forssk.) and *Sorghum* spp.

viii. *Meadow podzols* (podosols) are present in small areas in the far north of the Northern Territory and support grasses such as *Themeda* spp. and *Eriachne* spp.

Australian native pasture species are adapted to low nutrient soils and are able to reach maturity in about three months while containing low concentrations of nutrients in their plant tissues (Scattini et al. 1988).

In 1996, Australia's Commonwealth Scientific and Industrial Research Organisation (CSIRO) published a different (to the traditional) method of soil classification, using the concept of pedalogic organisation, where soils are distinguished by the changes in the soil materials as a result of the effects of physical, chemical and biological actions occurring in the soil. This includes the formation of horizons, peds (or lumps), colour, texture and the effects of man such as the spreading of overburden (Isbell 1996).

Isbell (1996) categorised 13 main orders of soils, 11 of which occur in the Northern Territory and whose distribution is illustrated in Figure1. These soils are (Isbell 1996):-

Rudosols – soils that have little pedologic organisation but will vary tremendously in terms of texture, depth, stratification and salinity. Essentially, these are young soils and whose occurrences are found scattered throughout the Northern Territory.

Tenosols – soils that have a structured (albeit weakly) A horizon and little or no pedologic structure in the B horizons. Often these soils will overlie a hard layer of calcrete soil, or unweathered rock. This group along with Rudosols and Kandosols are one of the dominant soil types in the Northern Territory, being most predominant across the Tanami Shield in the west.

Kandosols are soils which lack a strong texture contrast, have weakly structured B horizons and are not calcareous. They too are distributed across the Northern Territory, with a slightly lower occurrence across the Tanami Shield.

Vertosols are cracking clay soils with shrink-swell properties. The dominant forms of this order are the black soil plains on the northern part of the Barkly Tableland. The cracking nature of the soils precludes the establishment of trees with deep root systems, resulting in vast “seas” of *Iseilema* and *Astrebla* spp. of grasses.

Chromosols are soils that have a strong texture contrast between the A and B horizons and are neither strongly acidic nor sodic. These little-utilised agricultural soils occur in a northwest-southeast strip situated between 15.5^0S and 16.5^0S and 131.4^0E and 132.3^0E; the eastern extremity is the Victoria River District Conservation Association pastoral district.

Dermosols, after Rudosols, are the next dominant soil group of the Eastern Kimberley region. These soils have little contrast between their A and B horizons, yet have a strongly structured B2 horizon. These soils will generally have a red-brown or a calcrete hard pan underlying the B horizon.

Hydrosols are common in the coastal regions around the Northern Territory and along 13^0S between 130.4^0E and 132.0^0E. These soils are either seasonally (for at least 2-3 months of the year) or permanently wet. This may be caused by either poor table drainage or the influence of tidal flows.

Sodosols are soils which have a strong texture contrast between the A horizon and a sodic B horizon that is not strongly acidic. These soils occur mainly in the southern central region of the Northern Territory and in the eastern portion of the Territory just to the north of the Barkly Tableland.

Calcarosols are soils that are calcareous throughout the soil without a clear B horizon, and are most common in the southern regions of the Northern

Territory, with some limited occurrence on the eastern edge of the Eastern Kimberley's.

The compaction of the upper surface of the soil often determines the survival of seeds in the soil seed bank, and in turn the floristic composition of the environment. Examples of edaphically influenced speciation are illustrated in the following relationships

The density of stands of *S. intrans* growing on gravelly earth 10 km east of Darwin is greatly affected by the seed's ability to penetrate the soil layer after seed drop. Where the soil surface has been compacted, ants either collect or devour seeds on top of the ground, or seeds lose their viability at a rate faster than that of seeds which have been able to be partially buried. Where population densities of the *S. intrans* is low, other species such as *T. bitextura* (Lazarides) and *Aristida browniana* (Henrard) will grow into and colonise the vacant space (Andrew 1986).

Loose sandy alluvial soils in forest and spinifex country suit the seed characteristics of the loosely tufted annual *Aristida hygrometrica* (R. Br.)(Wheeler 1992). The narrow leaf blades are rolled inward conserving moisture in the arid conditions. It has a large seed structure with the seed being 1.5 cm long, the twisted column up to 8 cm long and the awns are each 8-15 cm long (Milson 2000). The large awns facilitate wind dispersal of the seed, and the pointed callus and scabrous awns facilitate adherence to the covering of passing animals. The awns are twisted into a column, and when wet, unwind at the same time twisting the pointed seed into the ground, thus positioning it for germination (Whalley 1987). .

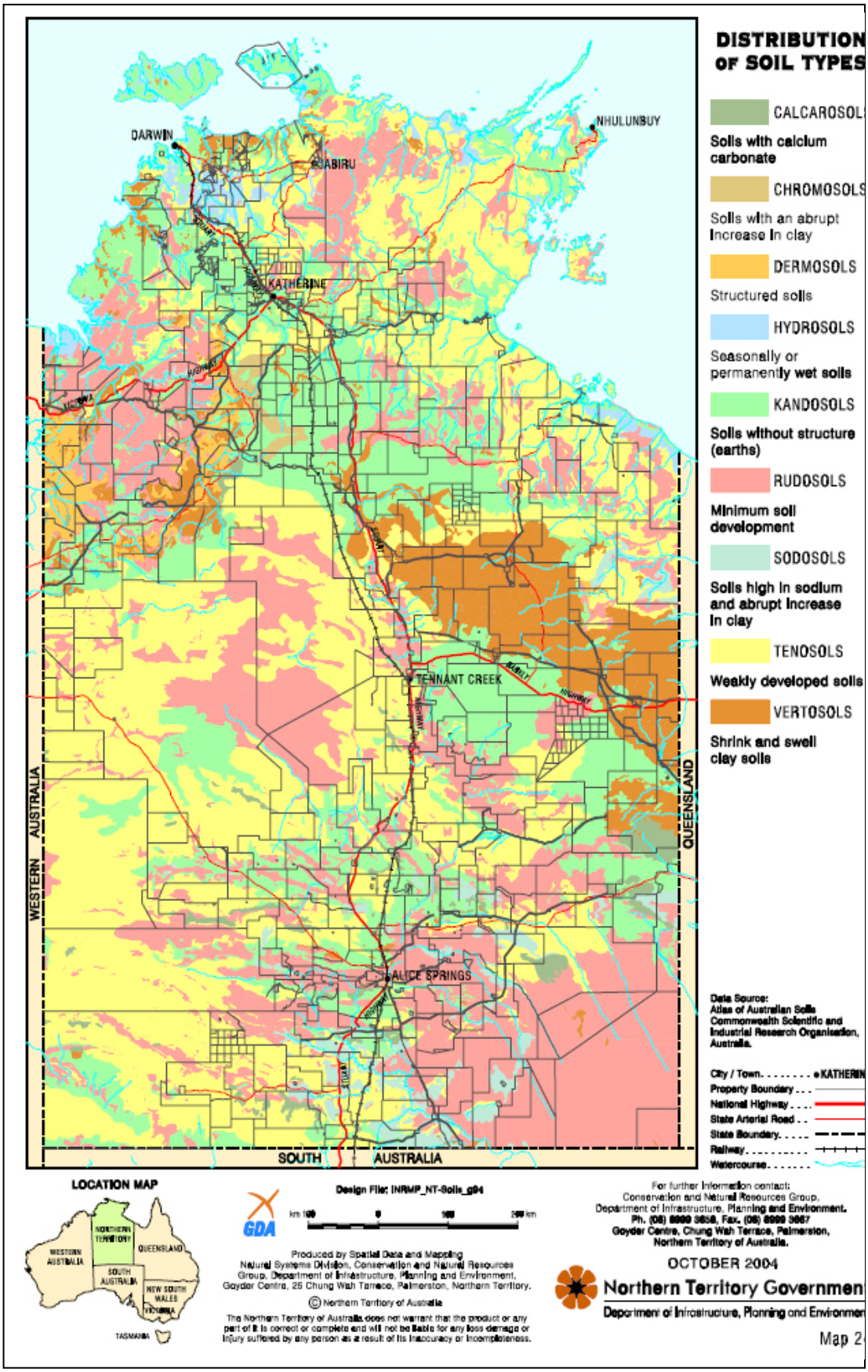


Figure 1.1 Map of the distribution of the major soil groups of the Northern Territory (Northern Territory Government 2004).

Astrebla spp. are tufted rhizomatous perennial grasses occurring mainly on the deep cracking clays of the Barkly Tableland. They have a deep-rooted system which serves to provide protection from drought as does the vertical roots not being overly damaged by the cracking of the clay soils (Milson 2000). It is thought that the cracking effect of the soils is also an important factor in the exclusion (in the main) of trees from the *Astrebla* grasslands (Burrows et al. 1988). Soil surface drying on cracking clay soils will affect secondary root growth in many species of grasses. *Astrebla* spp. have overcome this (and so achieved dominance on the cracking clays) by having a well-developed deep primary root system (Orr 1998).

Further adaptations of *Astrebla* spp. To this soil type are *Astreblas'* starchy rhizomes just beneath the soil surface. These provide a means of regeneration after a fire event (Purcell and Lee 1970). Storage of nutrients means that plants will dry off earlier in the season than will other species, which results in the plant avoiding water loss via transpiration in the hottest time of the year, and the plant will have set and dropped seed ready for a germination opportunity before the end of season. Even in a very wet summer, the total bulk foliage of the grass tends to remain low later in the season as this an adaptation to the low nitrogen levels in the soil of the Barkly Tableland (Milson 2000).

Soil acidification is a problem that can occur in soils above the 500 mm isohyet. The addition of nitrogenous fertilisers (to boost pasture production) in regions of high rainfall in aged soils that lack the natural basic mineral buffers (e.g. carbonates, calcium and magnesium silicates) may result in a drop in the soil pH. Reduced pH in turn affects the capacity of soils to hold basic

cations that are essential to plant nutrition. This increases the probability of aluminium and manganese toxicity occurring in plants in these regions of northern Australia (Williams and Chartres 1991). Consequently, soil chemistry can be changed by pasture management practices which in turn must alter the floristic composition of the plants growing in it, with the native species giving way to more tolerant introduced species.

1.3.4 Erosion

Erosion can be prevented by the establishment of matting vegetation, or it can radically alter a landscape preventing the establishment of any vegetation. The two main causes of erosion in Northern Territory are the results of wind and water. Wind erosion has been occurring on the arid continent for millions of years, leaving a denuded landscape in the centre of Australia (Beale and Fray 1990). Wind separates the soil particles, removing the finer topsoil material (clays, silts and organic matter) leaving behind coarser subsoil particles and gravels (Gunn 2001).

Water is the primary cause of sheet, rill and gully erosion. The energy from the impact of raindrops displaces fine soil particles and damages soil structure. Pores in the soil are blocked, inhibiting water infiltration and causing ponding on the soil surface. Eventually the water will move downhill as run-off and carry soil particles with it. The removal of layers of topsoil is termed ‘sheet erosion’ (Gunn 2001). As water moves with increasing velocity through low points on the soil surface it will begin to carve out small channels or rills. As the rills increase in size, the volume of water moving through increases causing the formation of deep gullies along depressions and drainage lines (Gunn 2001).

Having vegetation cover (in the form of leaf litter or grass tussocks) will increase the infiltration of rainwater and decrease its run-off, preventing the formation of a surface seal or crusting. The vegetation also disperses or blocks the impact from the energy of the raindrops on the soil (Humphries 1991).

Early wet season burning in the top end of Northern Territory will result in the loss of topsoil during the following rains. Burning reduces the cover and leaf litter on the ground, as well as causing deterioration of the soil surface, both inhibit water infiltration and increase run-off water and the removal of soil solids (Grice and Slatter 1996).

The sharp hooves of introduced ruminants have direct effects on vegetation (by trampling) and indirectly upon the soil surface structure (by compaction). Removal of vegetation creates bare patches on the soil surface, and compaction increases their susceptibility to erosion (Humphries 1991). Continual overgrazing of perennial grasses, such as *Astrebla* spp., will lead to surface crusting and reduced infiltration of rainwater. This in turn leads to sheet and rill erosion (Scattini et al. 1988).

1.4 Seed Biology

Seed biology encompasses seed formation, imbibition, germination, and dormancy. Seed formation involves four distinct phases of development. Imbibition is the water uptake of the seed where water acts both as a colloidal solvent and provides pressure to assist in the opening of the seedcoat (Mayer and Poljakoff-Mayber 1989).

Germination (in orthodox seeds) is described as a series of consecutive events that induces a dry, quiescent mature seed, through the imbibition of water and exposure to suitable light and temperature regimes, to demonstrate an increase in its metabolic activity to initiate the elongation of the embryonic axis resulting in the emergence of the seedling from the seed (Mayer and Poljakoff-Mayber 1989; Bewley and Black 1994).

Seed dormancy has been classified into five categories:- imposed, organic, physiological, morphological and cyclic.

1.4.1 Seed Formation

The process of seed formation (for orthodox seeds) undergoes four distinct phases. Histo-differentiation (Phase I) is where the individual cells of the seed develop and form the specialised tissues, such as the embryo and endosperm. It is in maturation (Phase II), in which storage cells are filled with proteins and carbohydrates, that the embryo is fully developed and the seed coat is fused with the pericarp forming the caryopsis wall. Desiccation (Phase III) is when the seed undergoes water loss to a level of 5 -10% moisture content of fresh weight. The cells of the caryopsis wall flatten and become hard and dry. The storage cells in the endosperm collapse as the volume of the carbohydrates is reduced. The embryo itself is seemingly desiccation tolerant, being able to maintain cell respiration and seed viability. Then the stage of quiescence (Phase IV), in which the seed, though at a low degree of moisture, is in a state of suspended animation until all conditions are right for the seed's germination (Mayer and Poljakoff-Mayber 1989; Fujiwara et al. 2002).

Seed storage reserves are mainly composed of carbohydrates (mostly starch), fats and oils (triacylglycerols), proteins and phytin (an insoluble complex of potassium, magnesium and calcium salt of *myo*-inositol hexaphosphoric acid and source of phosphates for ATP production). There are many other components in seeds (phenolic compounds, tannins and flavonoids) that may not be strictly classed as storage components as the seed does not use them for germination or subsequent growth.

1.4.2 Seed Imbibition

The imbibition of water is considered to be an induced flow (the amount of which is known as the hydrodynamic mass) through pores in the seed coat rather than a passive diffusion of water. This assumption is supported by the investigations of (Simon 1984), who recorded negative water potentials to the order of –100 MPa when measuring the water potential of grain seeds such as *Triticum aestivum* (L.) and *Zea mays* (L.).

Water acts as a colloidal solvent, permitting an increase in enzyme synthesis/reactions while the increased pressure caused by the swelling of colloids assists in the rupture of the seed coat. The rate of flow of water is also influenced directly by temperature in that the greater the temperature the less viscous the water and greater the kinetic energy available to processes within the seed (Mayer and Poljakoff-Mayber 1989).

1.4.3 Seed Germination

Germination begins with the imbibition of water by the seed and ends with the emergence of the embryonic axis as the radicle. Imbibition has a triphasic time course, in that Phase I involves the initial rapid absorption of water.

Phase II is a period (which varies considerably from species to species) where the seed water content is relatively constant or only slightly increasing. However, this is still a time of great activity within the seed. And Phase III is where there is a resumption of water uptake in accord with the elongation and growth of the embryo (Welbaum et al. 1998).

These phases are not dependent upon the seed's viability, but rather the seed's colloidal composition, the permeability of the seed coat to water, and the availability of water in the seed's immediate environment (Mayer and Poljakoff-Mayber 1989).

Seeds in which no germination processes are taking place are said to be quiescent, and those seeds that have a mechanism blocking germination are said to be dormant (Bewley and Black 1994).

Whether quiescent or dormant, a series of anatomical, genetic, metabolic and hormonal events take place over time within a seed. Changes in the seed's anatomy include the reduction of protein bodies in embryo axis cells, development of plastids' internal structures, increase in mitochondrial cristae, and an increase in the number of polysomes. Lignification of the scutellum may occur along with an initiation of the embryonic cell division (Jann and Amen 1977).

Genetic changes during germination result in the sequential activation of several previously (prior to quiescence or dormancy) non-active portions of the seed's genome. This hypothesis is supported by the production of differing enzymes and iso-enzymes at various stages of embryonic growth and development (Jann and Amen 1977).

Metabolic changes are heralded by a sharp increase in the rate of respiration when the seed begins to imbibe water. This is followed by a series of chemical changes within the seed – the breakdown of reserve materials, the transport of broken down materials from storage vacuoles to the aleurone layer via the endo-membranes and imbibed water flow, and the synthesis of new materials from the broken-down products (e.g. protein synthesis) (Mayer and Poljakoff-Mayber 1989; Herman and Larkins 1999; Jolliffe et al. 2005). The protein synthesis acts in the growth of the embryonic axis, the synthesis of hydrolytic enzymes, and the cellular mechanisms required for the transport of reserve material (Bradbeer 1988).

Hormones seem to be expressions of specific environmental cues as biochemical messages. They act as switching agents changing the seed from one physiologic state to another either by gene repression or depression, the activation of the translation of genes, or by the alteration of the membrane permeability of cells (Jann and Amen 1977). Hormones operate in the growth and the development of the seed (gibberellins, auxins and cytokinins), its accumulation of storage reserves, the arrest of growth prior to seed maturation (abscisic acids) and the growth and development of extra seminal tissues (Bewley and Black 1994).

The measurement of seed germination of an individual treatment, or soil sample, is usually determined by counting the number of seeds germinated and expressing this number as a percentage of the total number of seeds used in the treatment or soil sample (Bewley and Black 1994).

1.4.4 Seed Dormancy

There are several general types of dormancy:-

(1) Imposed dormancy

(2) Organic dormancy which may be either

a. Endogenous and/or

b. Exogenous (these mechanisms may occur either independent of one another or in a combination).

(3) Physiological dormancy

a. Non-deep dormancy

b. Intermediate physiological dormancy

c. Deep physiological dormancy

(4) Morphological dormancy

(5) Cyclic dormancy

(1) Imposed dormancy is the lack of germination due to imposed environmental conditions, (i.e. lack of water, extreme temperatures, unsuitable red: far red light ratios) (Nikolaeva 1977).

(2) Organic dormancy is related to the properties of the seed that are preventing the germination of the seed (Nikolaeva 1977). Endogenous dormancy is the property of the embryo preventing germination, while in exogenous dormancy some mechanism or property of the seed structure (including endosperm, seed coats or even perisperm) prevents imbibition and germination (Nikolaeva 1977). Endogenous dormancy itself may be caused

by physiological, morphological or morphophysiological conditions within the seed (Baskin and Baskin 1998a).

(3) Physiological dormancy has also been distinguished by Nikolaeva (1977) to have three levels – non-deep, intermediate and deep.

Non-deep dormancy results in the seeds either not germinating at all, or germinating within a very narrow temperature range. It is thought to be caused by the covering structure (a physical restriction) of the seeds restricting oxygen diffusion (a physiological restraint), the release of inhibitors from the seed or by the physical restriction of the seed cover that stops emergence of the embryo (Baskin and Baskin 1998a). Non-deep dormancy may be overcome by use of an after-ripening period (as in *Dichanthium sericeum* (R. Br.) A. Camus), or by the use of chemicals (e.g. thiourea, potassium nitrate, ethylene, gibberellins) (Fesuk and Ashwath 2001).

Intermediate physiological dormancy is indicated when the seeds that have failed to germinate have had their embryos removed with the result that the embryos have developed into normal seedlings (when supplied with substitute food resources such as tissue culture medium). This type of dormancy is not known to occur in tropical grasses.

Deep physiological dormancy differs from intermediate physiological dormancy in that embryos will show a retarded or abnormal growth, and intact seeds require long periods of cold stratification (Nikolaeva 1977).

(4) Morphological dormancy may occur as a result of the embryo being underdeveloped (but differentiated into radicle and cotyledons), or the embryo is undifferentiated (Baskin and Baskin 2001).

In the case of exogenous dormancy characteristic of Australian species, the main physical barriers are permeability barriers acting as gas exchange barriers (Adkins and Bellairs 1999). The hard testa of the grass genera *Aristida, Heteropogon* and *Spinifex* are known to inhibit oxygen uptake (Adkins and Bellairs 1999), as does the mucilaginous coat produced by seeds of *Sporobolus* spp. during imbibition (Bewley and Black 1994; Adkins and Bellairs 1999; Fesuk and Ashwath 2001).

(5) Cyclic dormancy occurs in species of plants that have seeds which undergo a dormancy cycle, i.e. they gradually change from a dormant to a non-dormant state and back again in response to environmental conditions (Baskin and Baskin 2001). In northern Australia the main two environmental conditions are rainfall (resulting in very sharply delineated growing seasons) and temperature with some sites having recorded soil surface temperatures with a mean maximum in excess of 65 ^{0}C for up to four months of the year (Mott 1972).

1.5 Seed Storage

For purposes of research, rehabilitation or agriculture, seeds will be stored in artificial seed stores, or seedbanks. In nature, seeds are preserved somewhat in natural seedbanks such as topsoil or organic mulch.

Seeds, however, are subject to ageing from a range of biochemical processes under either storage regime that result in loss of vigour. These

processes have been a focus of proteomics and enzyme analysis of stored seed. Scanning electron microscopy is also now being utilised when the degradation to the seed is external, in that it affects the seed husk or seedcoat.

1.5.1 Artificial Seedbanks

Artificial seedbanks range in sophistication from those whose seeds are stored in insect-proof containers in controlled temperature environments to those in which the seeds are bulk-stored in bags in sheds where temperatures may reach in excess of 50 ^{0}C and seeds are exposed to vermin and fungal infestations. It is known that temperature affects the rate of maturing, or after-ripening of seed as are also the duration (or onset) of dormancy cycles (Roberts 1972; Ashwath et al. 2000; Desai 2004).

The term “artificial seedbanks” also includes the use of soil dumps from the saving of topsoil at mine sites prior to mining operations where the top layer of soil is heaped into large piles to be spread out again once the mine pit has been refilled. The disadvantage of this is that seeds are relocated within the soil and they may no longer be in optimal germinating conditions (e.g. correct ratios of red: far red light) or, once germinated, be placed too deep within the soil for their shoots to emerge to the surface (Bellairs and Koch 1999).

1.5.2 Natural Seedbanks

Natural seedbanks are dynamic resources that will vary seasonally in both the density of the seed present in the bank and in its species composition. In northern regions, the maximum seed densities usually occur in summer and autumn following the northern monsoons (Bellairs and Koch 1999).

The floristic composition of a site is not always indicative of the species present above-ground as the topsoil seedbank retains seeds of all species. Furthermore not all the seeds in the seedbank will be germinable due to the effect of residual seed dormancy and seeds of some species requiring a series of wetting cycles before germination will occur. Alternatively, some seeds require different environmental conditions to stimulate germination (e.g. heat, smoke products, light ratios or microbial breakdown of seedcoats) (Orr 1999).

1.5.3 Seed Ageing and Loss of Vigour

A phenomenon observed in many Australian native plant species is that some species have seeds that undergo an after-ripening process, a time of maturation during which the seed generally will not germinate (Fesuk and Ashwath 2001). The ability of a seed to survive for long periods of time in storage is known as seed longevity which may also be described as the period taken for (usually) 50% of the stored seed to die (Roberts 1972).

Loss of vigour in seeds is as a result of both physiological and biochemical changes. In terms of physiological changes, there are indications of the occurrence of chemical reactions in seeds of low moisture content, where it is recognised that enzymic metabolism and respiration ceases when the seed moisture content is below 15-20% (Vertucci and Farrant 1995). Under these conditions there is a decrease in catalase activity and expression and/or an increase in hydrogen peroxide (H_2O_2) content in an inverse relation to water content (Bailly et al. 2005), as well as leaking of metabolites and ions from dehydrated cell membranes (Maguire 1977; Mayer 1977). Biochemical

changes in seeds, with loss of vigour, include decreased protein (including enzymes) and nucleic acid biosynthesis (Roberts and Ellis 1982).

The biochemical processes involved in the loss of vigour and longevity are still not clear, however, recent research in seed desiccation tolerance using comparative proteomics of heat stable proteins and metabolite profiling analysis have indicated that seeds will only survive if all the protective molecules such as late embryogenesis abundant (LEA) proteins (highly hydrophilic proteins synthesised at the onset of seed desiccation) and non-reducing sugars are present to help maintain macromolecular stability during and after the drying process. The regulation of metabolism seems to have a dual role in preventing oxidative damage, and the provision of energy to synthesise protective molecules (Garay-Arroyo et al. 2000; Buitink 2005).

Ageing and some environmental factors may also result in the production of free-radicals (such as H_2O_2 or reactive oxygen species) which reduce seed longevity by degrading the lipid membranes of the seed cells (Salisbury and Ross 1992; Bernal-lugo et al. 2000). The production and presence of peroxidase can prevent the production or build-up of free radicals and this will enhance seed storage and viability (Nkang and Ashwath 2004), and assist in the seeds' resistance to fungal infection (Bernal-lugo et al. 2000; Tavakkol-Afshari 2005).

Deterioration of proteins has been found to occur via sugar hydrolysis and subsequent Maillard reactions on the seed proteins. As dry seeds undergo very little metabolic activity, they are unable to repair themselves and the progressive accumulation of Maillard products can eventually cause seed death (Roberts 1972; Berlett and Stadtman 1997; Murthy and Sun 2000).

As seed banks require the storage of seeds for many years, it is important that the response of a seed to various forms of storage be studied in order to establish protocols for optimal long-term and species-specific seed storage (Nkang and Ashwath 2004). The use of techniques such as sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) allows a visual analysis of the proteins that are present in the tissues being examined. By comparing with control tissue and known protein molecular markers, the protein composition (and degradation), presence of isozymes, effectiveness of cell/tissue fractionation changes in gene expression during germination may confidently and accurately be determined (Weber and Osborn 1969; Caprette 1996).

1.5.4 Scanning Electron Microscopy (SEM) and Seed Ageing

The use of scanning electron microscope is known to be of value in determining taxonomic differences between closely related plants (Watanabe et al. 1999). Mature dried seeds may be mounted directly onto stubs and splutter coated with no drying preparation required (Garnock-Jones 1991). However, as yet little work has been published on using SEM to examine the effects of ageing on seed coats and husks.

It is known that tissues which envelope seeds also function in a dual capacity as dormancy mechanisms (both physical and chemical)(Tran and Cavanagh 1984; Hopkins 1996). Seed imbibition occurs at different rates throughout different tissues in the seed and that the permeability of the seed coat will be greater in some areas than others (Mayer and Poljakoff-Mayber 1989). Impermeability of a seed coat to water is a known form of seed-coat-imposed dormancy (Adkins et al. 2001).

Triodia species are recognised to have a seed coat imposed dormancy (Wells et al. 1999), as trials showed increased germination of *T. basedowii* when pericarp in the vicinity of embryo is punctured/pierced. Trials have also shown that *T. bitextura* has increased germination response over time that varies between different storage conditions (Fesuk and Ashwath 2004). However, no external SEM examination of the seed coat surface has been undertaken for any of the Australian native grass seeds undergoing storage treatments.

However, change in the glume structure of *Themeda triandra* was discovered from an SEM examination of seeds exposed to heat and smoke. Micrographs clearly showed the dissolved wax plugs in the glume pores (Paterson et al. 2001). In this case, it was heat more so than smoke that unblocked the glume pores, which is relevant in the event of moisture imbibition or gaseous exchange.

Scanning electron microscope examination of seed coats has been undertaken both for taxonomical purposes and to study the effect of scarification and other germination treatments. Seed coat impermeability is linked to the palisade layer of the seed coat, the thickness of which will vary over different parts of the seed coat. Research is yet to be carried out on the role that the external effects of storage conditions will have on the morphological features of the seed coat.

1.6 Aims and Objectives

1.6.1 Rationale

More than 75 genera of native grasses have been reported to occur in tropical Australia. In addition, several genera have been introduced for pasture production. Studies on seed germination have concentrated on introduced species and only in the last five or so years, focus has been placed on understanding the uses of and the biology of the seeds of native species. The limited research illustrates that the native grasses require a variety of treatments to obtain satisfactory germination, or to break seed dormancy mechanisms (Campbell et al. 1994). There are a number of financial and ecological gains to be made by the use of native species for grazing or restoration of disturbed sites. Native species are well adapted to survive in the harsh climate of northern Australia, but the procedures of establishing them in the field are not well developed. The study of the phenology of three native species during experimentation of batter stabilisation has given insight into the effectiveness of these species for rehabilitation work.

Other studies carried out (such as germination and storage trials by the Native Plant Seedbank at Central Queensland University which used more than 500 native plant seedlots) suggest that the native plants have very low germination percentage (32%) compared to introduced species (90%) (Ashwath et al. 2000). Thus, any investigation that leads to improved seed germination will make a significant contribution to the successful establishment of native grasses in the field. An understanding of the seed

biology of native species will also assist in managing these grasses in the field along with the introduced species.

Native grasses require species-specific treatments to break seed dormancy. These treatments will include both storage and germination treatments. Limited studies suggest that the treating of seeds with external agents such as heat, hormones, smoke water and light (Bell 1994) could induce germination. Identification of treatments that are practical and easy to implement is the key requirement for the use of native grasses by pastoralists.

Effects of seed priming (soaking the seed in water and slow drying in shade, seed treatment with glycine-betaine), partial germination (Campbell et al. 1994) and seed coating on field germination of native and introduced grasses and other pasture species need to be developed to assist the pastoralists in the establishment of a wide range of pasture species.

1.6.2 Aim

The aim of this project is to study germination and storage requirements of a selected range of Australian tropical native grass species, with the view to promoting the use of native grasses in land rehabilitation and stabilisation.

1.6.3 Objectives

The objectives are to:

1. Observe the effectiveness of the planting (by GANT) of native grass species in a range of field sites across the Northern Territory, with respect to edaphic, climatic and habitat alteration as a result of human activity (e.g. mining, dredging or grazing)

2. Test the effects of various storage methods and smoke germination treatment for each of the selected grass species used in the study.
3. Assess the establishment and stabilisation potential of three native grass species (*Brachyachne convergens, Chloris pectinata* and *Iseilema vaginiflorum* selected by GANT for their potential both as fodder and batter stabilisers) and compare their performance to a commonly used grass (*Chloris gayana*) for slope stabilisation in locations such as railway embankments or landfill batters. Stabilisation of these structures will minimise their impact on the surrounding natural (or rehabilitated) environment.
4. Examine the effects of various storage treatments via sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) on the composition of proteins in the seeds of *Triodia bitextura* (selected for its response to storage treatments and its importance in desert rehabilitation).
5. Test, via SEM imagery, the effects of various storage treatments on the seedcoat (testa) of species of native grasses.

These objectives are addressed in Chapters 2 to 6 respectfully, inaddition to a chapter of general discussion (Chapter 7).

Chapter 2
Soil Seedbanks of Selected Revegetated Sites in Northern Territory

2.1 Abstract

A number of rehabilitated sites across Northern Territory were examined with a view to selecting native grass species suitable for a wide range of rehabilitation scenarios. During the course of the monitoring, three species of native grasses, (*Chrysopogon elongatus* (R. Br.) Benth.*, Alloteropsis semialata* (R. Br.) Hitchcock and *Dichanthium fecundum* S.T. Blake*)* demonstrated the ability to adapt to a range of environmentally difficult conditions (as a result of over-stocking, mining or weed infestation) and are now under commercial cultivation. A fourth species, *Brachyachne convergens*, is under continued trials to develop genotypes that become less susceptible to invasion by more aggressive grasses.

2.2 Introduction

The Northern Territory consists of tropical rainforests, extensive coastal wetlands, river system floodplains, tropical savannah and arid lands established on ancient weathered, leached and denuded soils. Rainfall varies from ~200 mm in the southern arid region to 3,000 mm in the northern

tropics. Since the 1860's European habitation has resulted in the clearing and over-utilisation of the land for grazing and mining purposes (Sullivan and Kraatz 2001). This in turn has led to massive erosion of the delicate skeletal soils, invasion by a plethora of exotic plants and the loss of the use of thousands of hectares of once arable land.

A lack of understanding of the relationship between the vegetation and the soils on which they grow, the impact of introducing exotic species of plants and animals by government agencies and primary producers, notably during the 1950's and 60's in response to drought, and the unsustainable expectation placed on native/indigenous pastures, has created a need for large-scale rehabilitation and sustainability programs based on research into the ecology of our indigenous plant and animal species. Good ecological management must include the maintenance of (or endeavour to re-establish) the integrity of the soil resources. This will focus on the use of indigenous grasses, legumes, forbs and shrubs that have evolved to exist in nutrient-poor skeletal soils as found in the Northern Territory, rather than maintain an ecologically-unsound reliance on exotic pastures which eventually results in further degradation of the environment (Burrows 1991; NT Government 2005).

Management will also involve control measures (using native grasses) to reduce the spread of, and assist in the eradication of invasive exotic species that are either Declared Weeds such as *Jatropha gossypifolia* (L.) (Bellyache bush) and *Andropogon gayanus* (Kunth) (Gamba grass) or species such as *Cenchrus cilliarus* (L.) (Buffel grass) that have replaced native species, reducing the pastoral productivity and ecological integrity of the Northern

Territory Rangelands (NT Government 2005). Some control measures are labour-intensive, e.g. manual removal of plants, and others chemical-dependent, the use of which may also damage native vegetation. Greening Australia Northern Territory (GANT) has experimented with biological measures utilising indigenous plant species in a bid to out-compete invasive exotic species and for the dicotyledonous weed *Jatropha gossypifolia* (Bellyache bush), they have used indigenous monocots to create a fuel load that provides the opportunity to apply a fire regime to reduce the recruitment of the dicotyledonous weeds following spraying.

In order to select the correct grass species (for potential commercialisation) for different soil types and climatic conditions around Northern Territory, GANT began long-term monitoring trials in what they categorised as severely degraded locations.

GANT's Katherine Rangeland Revegetation Centre Rangeland Officer identified ten sites at which a selection of indigenous species of grasses was sown. Depending on the availability of seed stock, eight species were sown at each trial site. At each site, two to four native species not indigenous to the immediate area were trialled to determine the suitability of developing a range of native grasses as "improved pasture species" for the Northern Territory pastoral industry, to lessen the reliance on exotic species. The trials were initially set up in 2001, with monitoring continuing to 2004. This chapter records the results of two years of monitoring these sites by assessing the presence of seeds of the sown species in the soil seed bank (and thus the probable establishment of the species), their presence as mature plants at

the site, and their germination from soil samples placed in glasshouse trials at Central Queensland University (CQU), Rockhampton, Queensland.

The purpose of this trial was to identify native grasses that may be suitable for establishment as commercial crops in Katherine. The seed stock should meet industry demands for indigenous species, to provide improved pasture species for the pastoral industry, for revegetation activities within the mining and construction industries, as biological control agents and for specific environmental projects funded by the Northern Territory Government with a requirement to source stocks of native grass seed. These observations also led to testing the potential of native grass species for revegetation in competition with exotic species of grasses. This resultant study is presented as Chapter Four of this thesis.

2.3 Materials and Methods

2.3.1 Trial Sites

The data presented for this chapter is presented quantitatively when referring to the presence of plant species at various sites, or in soil seed bank glass house trials. Table 2.1 introduces the site locations, the main plant species native to the location, the target species that were planted, in addition to the emergent species that were identified in the glasshouse trials at CQU Rockhampton. Table 2.2 summarises the soil analysis data (CSBP Limited 2005) and lists the management concerns as identified by the GANT Rangelands Officer (Shaw 2006a). Together the sites form a representative sample of the range of land management issues across Northern Territory.

The ratio of monocotyledonous seeds to dicotyledonous seed in soil samples (Figure 2.6) and seed densities per m^2 for each site (Figure 2.8), are presented quantitatively having been extrapolated (for seed densities) from actual sample counts and then the following formula was used to calculate the number of seeds per m^2 from the 300 g samples. The density of the soil is used to convert 300 g to volume (in mL) of soil. This is expressed as a fraction of 500 mL which is the volume of a sample of 20 cm long x 10 cm wide x 2.5 cm deep and surface area $0.2m^2$.

$$N = \left(\frac{Sx1m^2}{300gx0.02m^2} \right) x \left(\frac{1}{d \times 500mL} \right)$$

N = number of seeds per m^2
d = soil density g/mL
S = number seeds per 300 g top soil

2.3.2 Soil seedbank sampling and seed extraction protocol

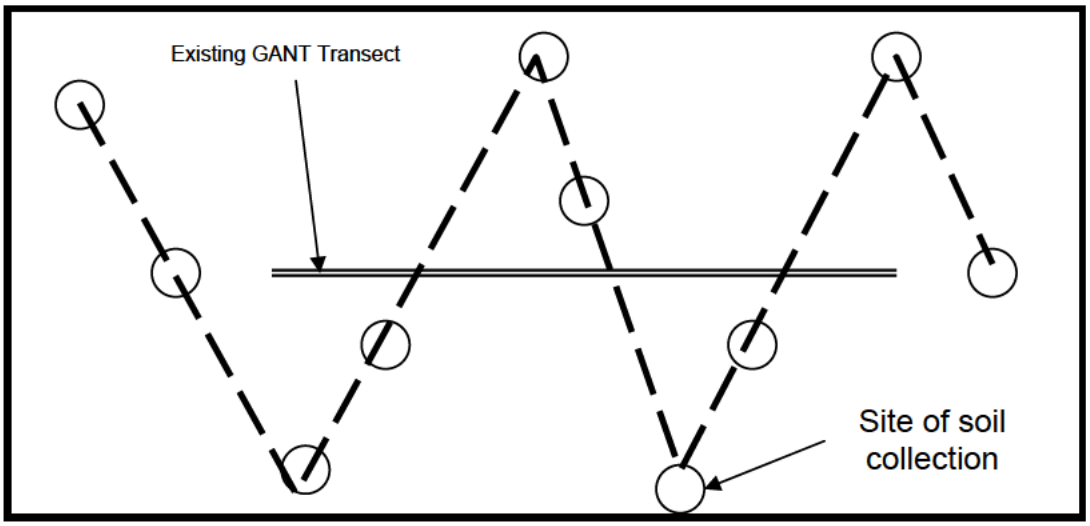


Figure 2.1 Soil collection pattern for determining soil seedbank

Ten samples from each site were collected evenly along the 10 m Greening Australia Northern Territory (GANT) transect in June 2002. In June 2003 samples were collected using a zigzag pattern (in order to test fresh non-sampled soil) over the GANT test site area (Figure 2.1).

Samples consisted of ten 20 cm x 10 cm quadrats dug out to a depth of 2.5 cm (Ashwath et al. 1999). The samples for each site were thoroughly mixed together, and then quartered. Two quarters diagonally opposite each other were then combined, bagged and labelled. Upon their return to CQU, the samples were sieved through a 5 mm sieve, after crushing in a mortar and pestle to break up aggregates. The >5 mm fraction (comprised of leaves, sticks, stones and occasionally bone) was checked for seeds before being discarded. Sub-samples of approximately 300 g of the remaining fraction were sieved through 2 mm and 0.1 mm sieves and the 2.0-0.1 mm fraction retained, the majority of seeds in soil seed banks falling within this fraction (Murphy and Lonergan 1999). The <0.1 mm “fines” were then placed in a Petri dish, moistened and placed in a controlled temperature room and examined periodically for germinants.

Seeds from the 300 g fraction were extracted by the floatation of the organic fraction in a 10% salt (domestic table salt) solution, and detected and removed using a binocular microscope.

Two litres of the soil remaining from the <5 mm fraction was measured out and weighed. It was then spread over a bed of sterile sand in germination trays and kept in a glasshouse utilising ambient light and temperature. Soil samples were approximately 2.5 cm deep. The sample trays were placed in a shallow tank of water with paper wicks dipping into the water to maintain a constant supply of moisture to the soil contained in the tray.

2.3.3 Monitoring of emergent seedlings

Germination trays were checked daily for emergence of seedlings and recorded as being either dicotyledonous or monocotyledonous species. Representative samples of each new species were transplanted into pots and allowed to grow to maturity before being photographed and identified.

Table 2.1 - Biogeographical and vegetative data for field sites. Source (NT Government 2005) and GANT records. Average annual rainfall figures were from Bureau of Meteorology data collected between 1961-1990.

Site	Biogeo-graphical Region	Average Rainfall p.a. (mm)	Vegetation type	Main Plant Species (GANT Records)	Planted Target Species (GANT Records)	Soil Seedbank Species (CQU)
Benmara	Gulf Fall and Upland	<600	Low open woodlands with grass understorey	*Acacia* spp*, Eucalyptus* sp*., Eragrostis tenuellula, Fimbristylis* sp*., Indigofera linifolia, Triodia* sp.	*Astrebla pectinata* and *Astrebla squarrosa*	None present
Bing Bong 1 - Fines	Gulf Coastal	<1200	Sparse shrublands	*Acacia* sp*, Aristida holothera, Astrebla* sp*., Brachyachne convergens, Calotropis procera, Chloris barbata, Dactyloctenium radulans, Iseilema sp., Neptunia* sp*. Panicum decompositum, Passioflora foetida, Pterocaulon* sp*,* Samphire*, Sorghum bicolour, Trichodesma zeylanicum, Xerochloa berbis*	*Brachyachne convergens, Chrysopogon elongatus* (both seed and plugs), *Heteropogon contortus, Sarga timorense, Sarga plumosum*	*Brachyachne convergens Chrysopogon elongatus*
Bing Bong 2 -Heavy texture	Gulf Coastal	<1200	Sparse shrublands	*Ectrosia leporina*, *Cyperus* sp., and halophytic forbs	As for Bing Bong 1	*Chrysopogon elongatus*
Hayfield	Mitchell Grass Downs	<1000	Mixed species low open woodlands with grass understorey	*Lysophyllum* sp*, Eucalyptus* sp*., Aristida inaequiglumis, Brachiaria* sp*, Brachyachne convergens, Cleome viscosa, Dactyloctenium radulans, Digitaria bicornis, Echinochloa colona, Enneapogon pallidus, Eragrostis tenellula, Indigofera* sp*, Perotis rara,* Portulaceae*,*	*Brachyachne convergens, Chloris pectinata, Dichanthium fecundum, Heteropogon contortus, Iseilema vaginiflorum, Setaria apiculata, Vacoparis laxiflorum, Themeda triandra*	None present

				Pseudoraphis spinescens, Sporobolus australasicus, Tragus australianus, Whitechloa airoides, Gomphrena canescens, Grewia sp.		
Katherine High School	Daly Basin	<1600	Woodlands	*Acacia* sp*, Eucalyptus* sp*., Pennisetum pedicellatum, Themeda triandra, Heteropogon contortus, Brachyachne convergens, Chrysopogon latifolius, Aristida inaequiglumis, Bothriochloa pertusa, Cynodon dactylon, Hyptis* sp*., Iseilema vaginiflorum, Sehima nervosum, Sarga intrans* and *Sarga timorense*	*Brachyachne convergens, Dactyloctenium radulans, Eragrostis tenellula, Iseilema vaginiflorum, Sarga intrans* and *Sarga timorense*	*Brachyachne convergens*
Limbunya	Ord Victoria Plain	<800	Woodlands with grass understorey	*Eucalyptus* sp*., Aristida* sp*., Chloris* sp*., Chrysopogon fallax, Digitaria bicornis, Stylosanthes* sp.	*Brachyachne convergens, Chloris pectinata, Chrysopogon fallax, Dactyloctenium radulans, Iseilema vaginiflorum, Vacoparis laxiflorum*	*Iseilema vaginiflorum*
Mainoru	Gulf Fall and Upland	<1600	Cleared woodlands	*Brachyachne convergens, Chrysopogon latifolius, Iseilema vaginiflorum, Setaria apiculata, Vacoparis laxiflorum, Themeda triandra*	*Brachyachne convergens, Chloris pectinata, Dichanthium fecundum, Heteropogon contortus, Iseilema vaginiflorum, Setaria apiculata, Vacoparis laxiflorum, Themeda triandra*	*Brachyachne convergens, Chloris pectinata*
Maud Creek	Daly Basin	<1600	Woodlands/ mine spoil	*Brachyachne convergens, Alysicarpus ovalifolius, Chrysopogon fallax, Echinochloa colona, Hyptis*	*Pseudopogonatherum contortum, Sarga plumosum, Iseilema vaginiflorum, Brachyachne convergens,*	*Brachyachne convergens*

				suaveolens, Sarga plumosum and a range of forbs.	*Sarga timorense, Astrebla pectinata, Astrebla squarrosa, Alloteropsis semialata*	
Mount Todd	Daly Basin	<1600	Woodlands/ mine spoil	*Aristida* spp*., Brachyachne convergens, Melinis* sp*., Cenchrus* sp*., Eragrostis, Acacia* spp.	*Sarga plumosum, Iseilema vaginiflorum, Brachyachne convergens, Sarga timorense, Setaria apiculata, Aristida holathera, Aristida, inaequiglumis*	*Aristida* sp.
Willaroo	Ord Victoria Plain	<1000	Miscellaneous shrublands	*Eucalyptus* sp*. Lysiphyllum* sp., *Heteropogon contortus, Jatropha gossypifolia, Martynia annua, Neptunia dimorphantha, Sarga timorense, Urochloa* sp.	*Dichanthium aristatum and Vacoparis laxiflorum*	None present
Control Site 1	Mitchell Grass Downs	<600	Grasslands	*Aristida* spp*., Eulalia aurea, Sporobolus* sp*., Acacia* spp*, Grevillea striata.*	Not applicable	Not applicable
Control Site 2	Ord Victoria Plain	<1000	Low woodlands (cleared)	*Eucalyptus* sp*, Heteropogon contortus, Chrysopogon* sp*, Sporobolus* sp*, Brachychiton* sp.	Not applicable	Not applicable
Control Site 3	Gulf Fall and Upland	<1600	Mixed species – low open woodland	*Eucalyptus* sp*, Lysiphyllum* sp*, Carissa lanceolata, Cymbopogon* sp*, Chrysopogon* sp*., Gomphrena* sp.	Not applicable	Not applicable

Table 2.2 – Management concerns of Northern Territory sites as identified by GANT Rangelands Officer (Shaw 2006a) and Soil analysis data (CSBP Limited 2005).

Site	Soil type	pH (1:5 H_2O)	Conductivity (1:5 H_2O) (µS/cm)	Cause for rehabilitation	Management concerns	Other
Benmara	Tenosols	8.3	36	Stock impact / drought	Establishing pasture grasses on low nutrient and weakly developed soils. Site is continuing to be grazed with no opportunity for rest.	Very low NPK values
Bing Bong 1 - Fine	Spoil from dredging sea bottom (fine textured)	8.0	695	Dredge dump	Still has relatively high salinity; however this has been counteracted with salt-tolerant species such as *Chrysopogon elongatus.* Important to note that salts include high concentrations of sodium, magnesium and other metal based salts associated with sea water.	Mainly sand and shell particles Cl 108 mg/Kg and S 548 mg/kg
Bing Bong 2 -Heavy	Spoil from dredging sea bottom (coarse textured)	8.3	84	Dredge dump	Inability to hold water and lack of soil or fine particles for root establishment. No subsurface sediment/soils for a depth of 600 mm.	Built into levees that have had salts leached out by high rainfall
Hayfield	Vertosols	7.4	223	Erosion / stock impact	Soil susceptible to rill and sheet erosion once soil crust is gone. Hard clay pan remains and water penetration is much reduced.	Brown/grey clay thinly capped with hard crust
Katherine High School	Kandosols	7.6	81	Cleared due to urban development	Fire lighting by locals burns out a high percentage of regrowth.	Red soils no horizon.
Limbunya	Rudosols	-	-	Stock impact on loose soils	Continues to be heavily stocked holding paddock.	Very sandy, non-structured soil.

Mainoru	Kandosols	7.4	568	Clearing, erosion and impact of stock	Salinity levels of soil quite high, non-structured soil under hard crust and very susceptible to erosion if integrity of crust is compromised.	Yellow soils, Cl 696 mg/kg and Na 277 mg/kg
Maud Creek	Kandosols with some mine spoil	7.4	199	Mine spoil and stock impact	Continued impact of stock and possible resumption of mining. Revegetation is designed to accommodate continued stock impact as the lessee overstocks paddocks to a severe level.	Brown soils, clayey, heavy cattle traffic area with S 67 mg/kg
Mount Todd	Tenosols and mine spoil	6.9	82	Mine spoil dumps	Little top soil, steep slopes on dump faces, higher concentrations of Cu and Zn may limit performance of plants.	High spoil dumps, soil sparse cover over rocks. Cu 7.97 mg/kg and Zn 3.19 mg/kg
Willaroo	Tenosols	7.9	14	Stock impact on loose soils and infestation of *Jatropha gossypifolia*	Control of stocking rates.	Heavy traffic from cattle, grey clayey soil
Control Site 1	Vertosols	6.4	78	Not applicable	Not applicable	Brown cracking clay soils
Control Site 2	Vertosols	6.5	4	Not applicable	Not applicable	Shallow stony soil on hillside
Control Site 3	Tenosols	6.2	24	Not applicable	Not applicable	Siltstone cap on soil

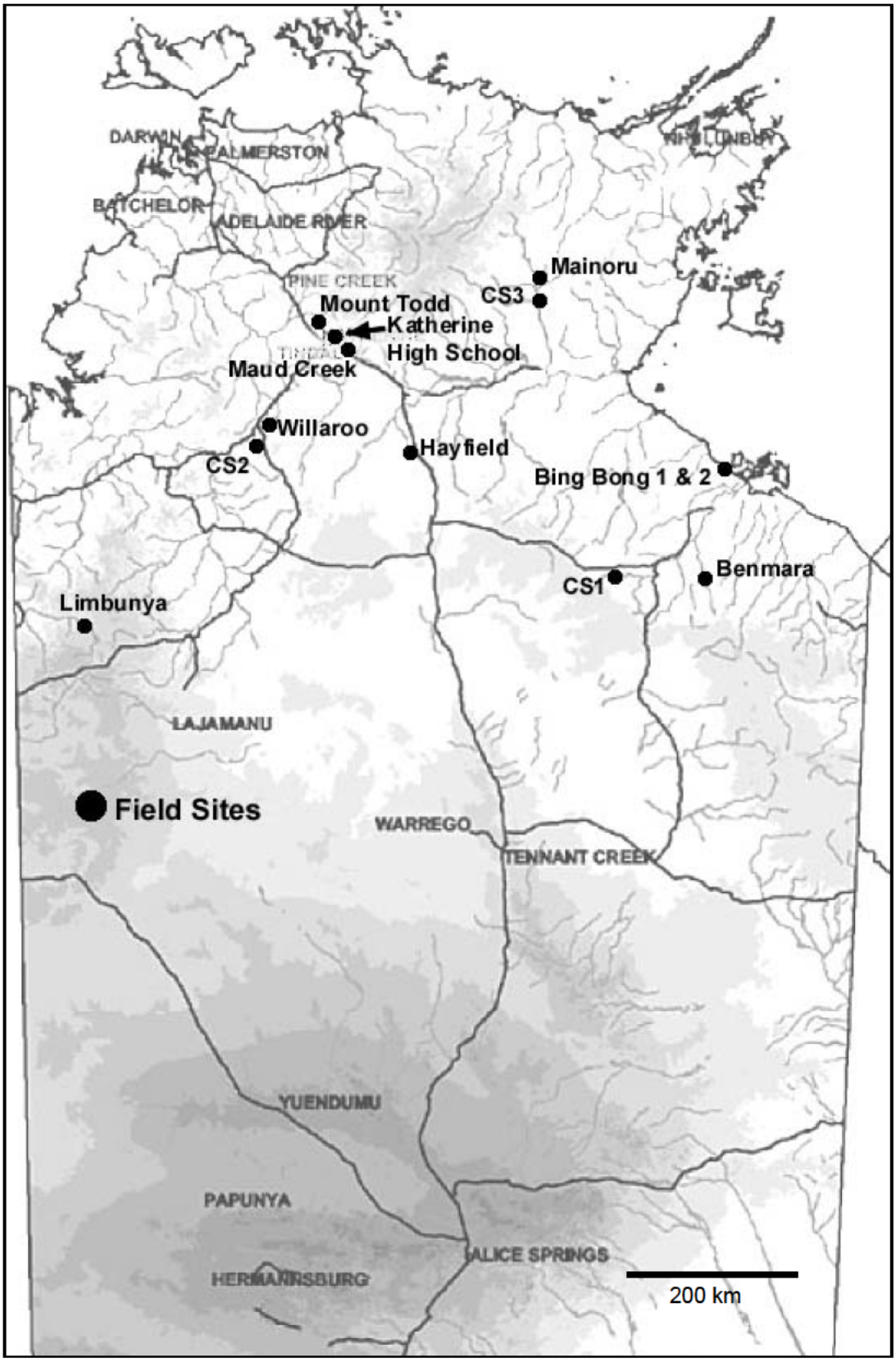


Figure 2.2 Field site locations in Northern Territory. Source – (Commonwealth of Australia 2004)

2.4 Results

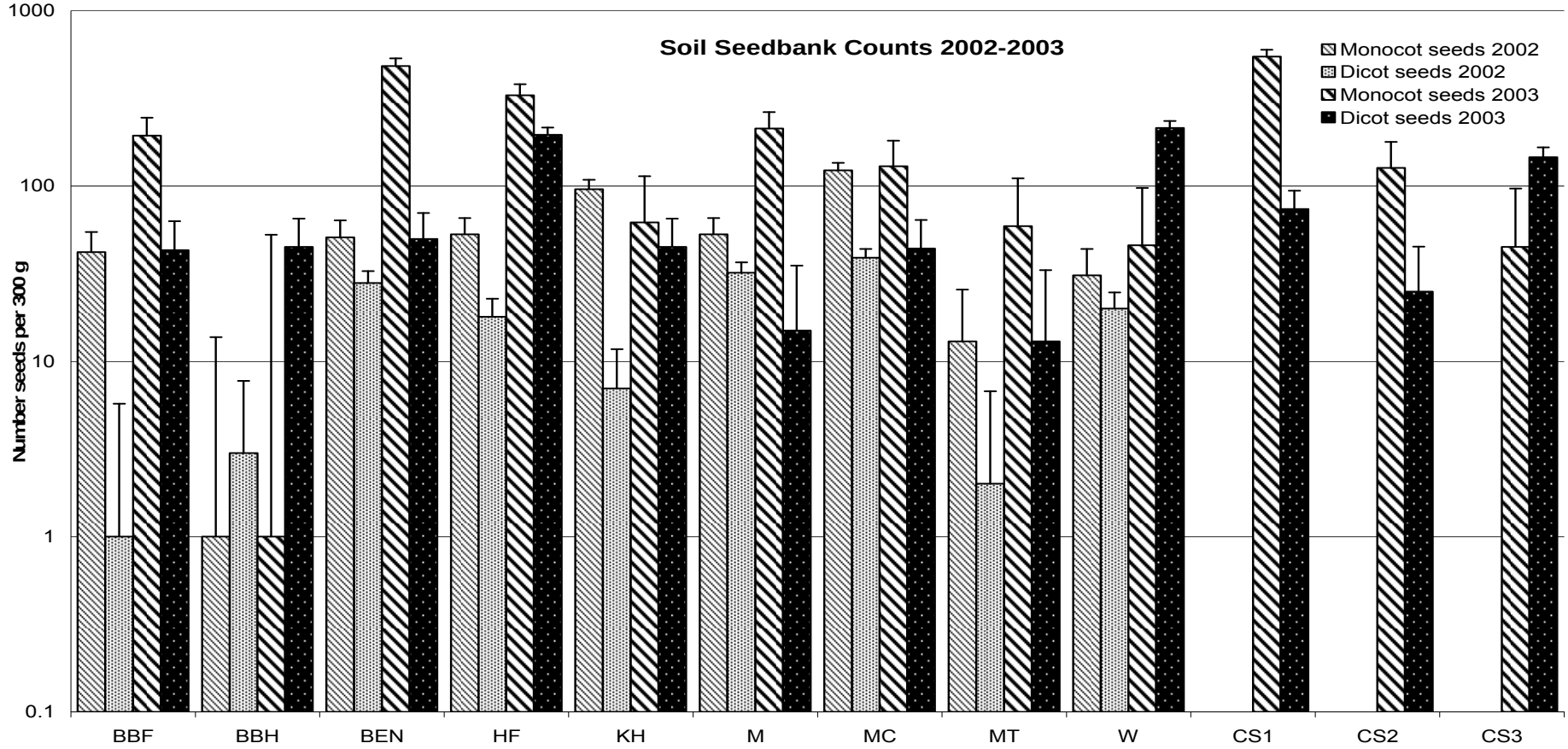


Figure 2.3 Comparison of monocotyledonous seeds vs. dicotyledonous seeds (per 300 g of soil) found in the soil seedbank sampled in 2002 and 2003. (BBF-Bing Bong Fines; BBH - Bing Bong Heavy; BEN – Benmara; HF – Hayfield; KH – Katherine High School; M – Mainoru; MC – Maud Creek; MT – Mt Todd; W – Willaroo; CS – Control Site). Error bars represent variation between the means of the representative samples. Note that the CS undisturbed sites were sampled only in 2003.

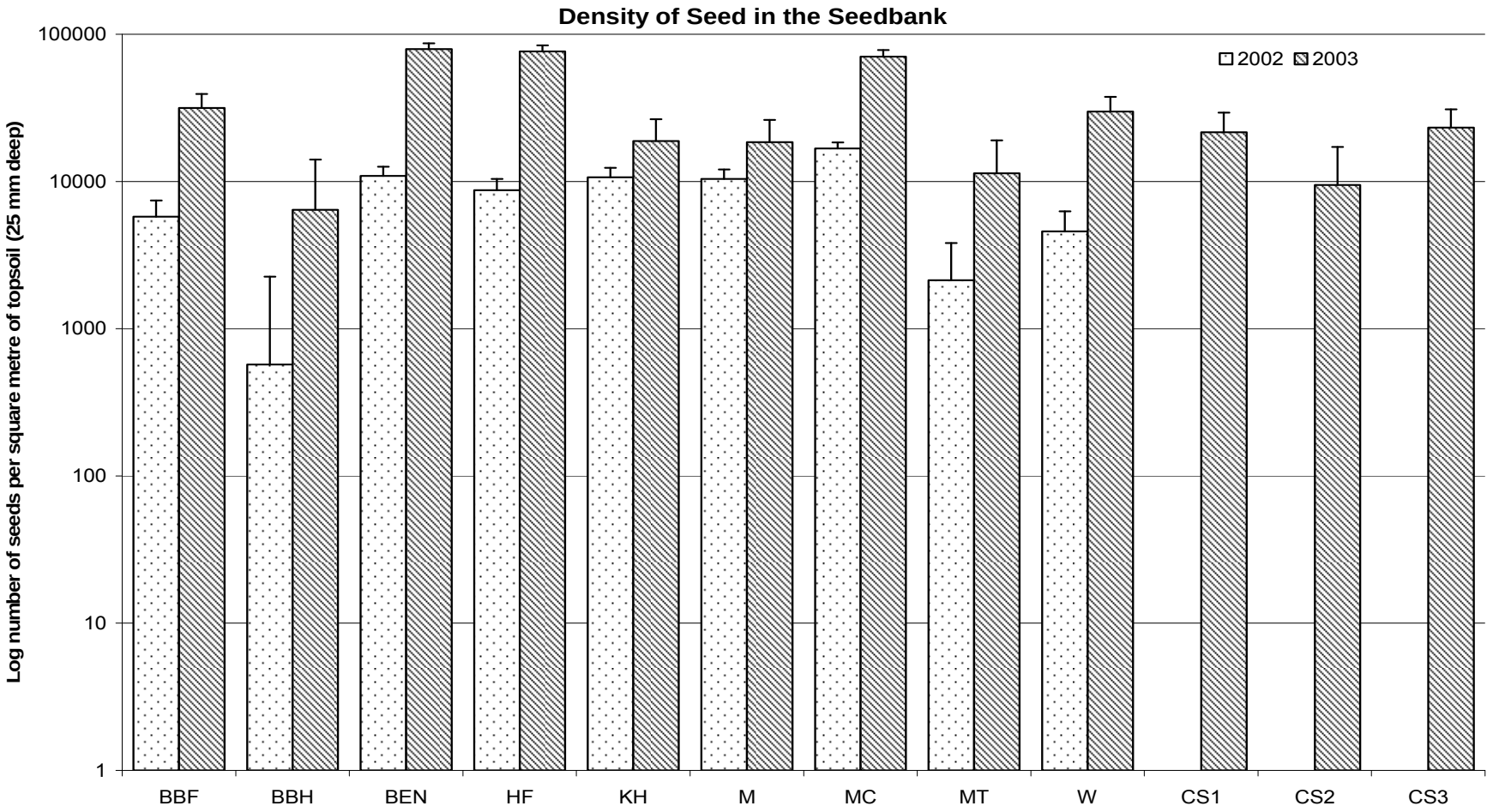


Figure 2.4 Variation in the density of seeds present in the soil seedbanks for each site using a logarithmic scale. Error bars represent variation between representative samples. No 2002 values available for the Control Sites.

2.5 Discussion

Soil seedbanks contain future generations of plant communities and are essentially physical and biochemical fortresses against the ravages of time, heat, moisture, UV light and predation. The seeds may be of species that have annual or perennial life cycles, and represent either monocotyledonous or dicotyledonous species. Annual seeds may exist in the soil for one to five years, viability declining rapidly after four years for some species (such as *Heteropogon contortus* (L.) Roemer and Schultes) while annual seeds may remain quiescent for longer periods (Fenner and Thompson 2005).

The soil seedbank is a dynamic resource dependant on seasonal fluctuations in temperature, rainfall, topography, floristic composition, annual:perennial ratio, fire regimes (where applicable) and human activity. The density of the soil seedbank will vary from year to year and season to season (Bellairs and Koch 1999; Thompson 2000; Maia et al. 2004). Indeed, the data presented in Figure 2.4 demonstrate this, for in the 12 months from June 2002 to June 2003, the seed densities in some cases increased one hundred-fold, the reasons for which are both general (climatic variation), and specific (human interaction) to both the site and the morphological and physiological traits of the species involved.

Factors responsible for this could include seed residual dormancy, position in the dormancy cycle from deep to non-deep dormancy, onset of drought and/or lack of seasonal rains, higher temperature due to drought, chemical inhibition as a result of excess or lack of critical soil elements, or even the destruction of habitat (Olaitan and Lombin 1984; Bellairs and Koch 1999; Orr 1999; Fenner and Thompson 2005). Having stated this, noted that the northern tropics of the Northern Territory experienced record rainfall during the months of January 2003 to March 2003. Following a prolonged period of drought (thought to be initiated by several years of El Nino effect (Conlan and Service

2000)), the response of seeds which had lain dormant was impressive and the replenishing of the diminishing soil seedbank even more so (Williams 2003).

With this in mind, the occurrence of the target species will be discussed on a site-by-site basis, as each site had its own particular set of conditions (soil composition, topography, human interference) that affect the long-term viability of their soil seedbanks.

2.5.1 Benmara

Benmara is situated in the northern region of the Barkley Tableland, close to the NT-QLD border (see Figure 2.2). Its soil is generally comprised of tenosols - weakly developed brown-grey sandy soils that have poor water retention and are significantly at risk of erosion (NT Government 2005).

In an attempt to improve the quality of the soil, prevent erosion and grow a palatable and high protein pasture crop, GANT test sowed *Astrebla pectinata* ((Lindl.) ex Muell. ex Benth.) and *A. squarrosa* (C.E. Hubbard) at the rate of 15 kg ha^{-1}. It was discovered later that the seed had been stored incorrectly and was no longer viable. However, Benmara did experience an increase in its soil seed bank, with the majority of the seed being monocotyledonous *Fimbristylis* sp. (which is not a suitable fodder plant).

Soils can be improved, and the actions of GANT are in accordance with (Sullivan and Kraatz 2001) who have published a decision-tree in *"Pastoral Land Rehabilitation"* that deals with degraded black/grey soils. Selection of species is important as local wildlife, such as the Gouldian Finch, rely on native grains as a major food source. Species must be adaptable to a hot wet season and produce seed that is able to lay in poorly drained soil for much of the year. In essence, reduced stock impact on fragile areas and establishment of selected species (such as a wide range of indigenous grasses and legumes that are able to fix atmospheric nitrogen) will improve the arability of the soil. It can be speculated that *Brachyachne convergens* should grow well at this site (due to the

alkaline pH range of 7.1 – 8.3) and has proven to be competent at providing a dense vegetation cover over soil, thus minimising erosion.

Figure 2.5 Benmara site showing sparse soil cover with scattered shrubs and trees of *Acacia* and *Eucalyptus* spp.

2.5.2 Bing Bong

Bing Bong Port, situated near the Sir Edward Pellew Group of islands (Figure 2.1) on the lower western part of the Gulf of Carpentaria, was selected as the shipping port for the nearby zinc mine, managed by McArthur River Mining, part of the X-Strata Group. In 1995, to enable deep-water ships to access the port, and to preserve the extensive sea-grass beds, a channel was dredged out into the gulf and the dredge spoil dumped inland of the port (Visible in Figure 2.6). At the behest of the Federal Government and in co-operation with GANT, McArthur River Mining began to actively work at rehabilitating the dumping grounds (Browning 2003).

The spoil was broken into five sections and the revegetation is being monitored on the basis of the nature of the material, e.g. fines material, coarse or heavy dredge spoil (Sites Bing Bong Fines (BBF) and Bing Bong Heavy (BBH) respectively in Figure 2.6). Taking seasonal rainfall into account, the dredge was separated into coarse and fine sediments, with the coarse material formed into protective levee banks, and the fine material spread out. A replanting program of the levees was initiated, however, the coarse material lacked the ability to hold water, or provide sufficient soil to facilitate the anchoring of plants and their interactions with the soil environment.

The fines material was spread out over the tidal flats and scattered over chenopod low scrubland. Environmental reports from the early 1990's indicate that these areas were composed mainly of calcareous sands and yellow podzols that were alkaline in nature with electrical conductivity (1:5 H_2O) of up to 5.4 dS/m (Browning 2003; Walker 2004).

After eight years, little vegetation had established on the levees, although certainly the soil analysis with an electrical conductivity of 0.695 dS/m indicates that the majority of sea salts have leached out of the system. This coincides with McArthur Rivers' goals which include the acceleration of the leaching process to promote the establishment of a stable vegetation platform and to promote the process of natural revegetation on the fine spoil material i.e. BBF site (Browning 2003).

The soil analysis also revealed high quantities of sulphur (548 mg/kg), moderate levels of iron (5.89 mg/kg) (in comparison to other sites in the study) and low quantities of manganese (2.02 mg/kg). This is indicative of the original soils being acid sulphate soils, which are common on the low-lying coastal regions around Australia. Iron sulphates are usually present in inundated saline soils, present in both the tidal flats and the sea floor, with a lack of oxygen preserving the sulphates in the soil structure. In times of drought (or displacement by dredging), exposure to air of these soils results in the oxidation of the

iron with the production of acidic sulphates leaching into the surrounding area (Sammut 2000).

In the case of the Bing Bong Rehabilitation plan, a profile was established that facilitated the leaching of salts from the dredge spoil to allow the establishment of vegetation on the spoil. An unplanned yet beneficial additional effect of this on the BBF site was the draining of the acid sulphate soils, allowing the oxidation of the sulphates and in turn the reduction of the alkalinity of the sea salts present in the soil. At the time of this sampling the soil had shown a reduction in soil EC, a reduction in the pH from an average of 9.5 – 8 down to 8 – 7.5, a stripping of iron and manganese from the soil, (which is indicative of oxidised acid sulphates soils (Sammut 2000)), and a reduction of soil salinity.

The BBH site, being situated on the raised levees, has undergone leaching at a greater rate than does the BBF site and so has far lower values of S (28.8 mg/kg), Fe (4.95 mg/kg) and Cl (12 mg/kg). As vegetative matter is very sparse, organic carbon levels are quite low (0.09%). This site still offers a harsh environment for plants, being composed of shell and stone rubble with little water-holding capacity, exposure to salt-laden winds, full sunshine and a pH range of 7.2 – 8.3. Successful short-term revegetation of this site will require the addition of topsoil to provide a suitable growth environment for a range of species.

Of the target species planted only *Brachyachne convergens* and *Chrysopogon elongatus* have survived to place their seed into the soil seedbank. *Chrysopogon elongatus* was selected on the basis of its ability to tolerate low levels of salinity, as well as for its spreading growth habit (Figure 2.9) in addition to the setting and dropping of seed.

Figure 2.6 Satellite Image of Bing Bong Port Facility and Field Sites BBH and BBF (DigitalGlobe 2006).

Figure 2.7 Bing Bong Fines Site (BBF) with *Brachyachne convergens* in the foreground and tall clumps of *Chrysopogon elongatus* in the background.

Figure 2.8 Bing Bong Heavy Site (BBH) showing very little established vegetation in 2003.

Figure 2.9 *Chrysopogon elongatus* spreading via rhizomes at BBF.

Salinity does affect water uptake, germination (by default as well as by ionic interference) and poor development of root systems. The salt also affects the seed physiologically during the initial stage of water intake when the osmotic priming potential of the seed is somewhat greater than that of the surrounding soil and salts are taken into the membranes of the embryonic tissue, affecting the colloidal properties of proteins and so the synthesis of enzymes essential for germination or for continued plant growth (Marcar 2001; Fenner and Thompson 2005).

The pH of soil will also affect imbibition by seeds during wetting. Survival of the seedling will depend on the species' ability to survive in either a low or high pH environment. The precise mechanisms by which this occurs is still a topic of investigation (Baskin and Baskin 1998a).

Brachyachne convergens is recognised as growing in association with *Iseilema membranaceum* (Lindl.) Domin which grows on soils with a pH of 7.0 or slightly higher (Skerman and Riveros 1990). An interesting observation is that *Brachyachne* was not reported (in the current study) as occurring at any site where the soil pH was below 7.0.

Other studies have reported that *Brachyachne* will undergo an after-ripening period of up to 2 years (Shaw and Fesuk 2003). It is also known that while many plants have a tolerance for saline conditions (seawater being approximately 3.3% salt or approximately 58 dS/m), they are unable to germinate and must utilise a "window of opportunity" that is offered during times of seasonal rain – in this case during the summer monsoon season (Baskin and Baskin 2001; Walker 2004; Fenner and Thompson 2005).

The soil samples were collected in June of 2002 and 2003; the soil analysis conducted using the 2003 samples. By this time the monsoon season had finished and *Brachyachne* (an annual) would have germinated, set and dropped seeds within 12 weeks from germination, and been laying down as a protective mulch over the seeds to shield them

while slowly breaking down to provides nutrients for the coming season (See Chapter 4 for a phenology of *Brachyachne*). The seed drop for this species is prodigious, and a ground cover will be laid down over the next few seasons providing an organic base for plants that will germinate as the salt concentrations decrease over time.

2.5.3 Hayfield

Hayfield is a station divided by the Stuart Highway just to the north of the township of Elliott (Figure 2.2). The land on the western side of the highway is well-watered, and has soils mainly comprised of vertosols which are basically cracking clay soils, common in arid regions and capable of supporting good grazing (NT Government 2005). However, this area has formed a hard, crust-like covering over the brown soil. When this covering is broken, particularly in one of the many small dry water courses present at this site, the soil is very susceptible to erosion. In areas where erosion has occurred there is an accumulation of fine reddish-brown sands in the run-off channels.

None of the GANT targeted species sown at this site germinated in the CQU glasshouse trials with soil collected from Hayfield (Table 2.1), and the only target species present at the site was *Brachyachne convergens*. If this species does have an after-ripening period (which is also indicated by its germination results in Chapter 3) then seed that had been dropped during the 2002/3 wet season would be present in the seedbank (which they were) but ready for germination in the 2003/4 wet season.

The other target species - *Chloris pectinata, Dichanthium fecundum, Heteropogon contortus, Iseilema vaginiflorum, Setaria apiculata* (Scribn. & Merr.)(K.Schumn.)*, Vacoparis laxiflorum* (F.M.Bailey)(Spangler) *and Themeda triandra* were not seen at the site in 2003.

Figure 2.10 Hayfield Site with covered areas of *Brachyachne convergens*.

Compared with other test sites, the soil at Hayfield is high in N (67 mg/kg), K (315 mg/kg) and slightly higher in P (13 mg/kg) than other sites. This would suggest that at some point in the past there was an attempt to improve these pastures and yet these levels are not proving to be detrimental for the other species that exist there.

The major factor in the lack of germination of targeted species therefore, would be the hard crust on the surface of the soil that did not provide microsites for the lodgement of seed until it rained (Peart 1984). At the onset of rain, seeds that had survived predation by ants (large populations observed at the site) and other granivores, such as finches (also present in abundance), exposure to the weather, fire and trampling by livestock. They would be positioned to drill into the softened soil using their hygroscopic awns (a morphological characteristic that all the missing species possess) (Wheeler 1992). *Brachyachne convergens* does not have long awns, its primary advantage having a much

smaller seed than the other species, and being able to utilise smaller niches than the species with bigger seeds.

Though *Brachyachne convergens* does layover at maturation providing mulch cover over the soil surface, it can be prone to invasion by more aggressive grass species. Therefore, one recommendation for rehabilitation of this site would be to sow using a protective covering over the seeds (such as jute mats or hydromulch which was trialled in batter stabilisation (see Chapter 4), or attempt to sow seed mixed with chaff (to provide buffer for the seeds during rainfall) immediately preceding summer rains.

2.5.4 Katherine High School

Located on the south-eastern outskirts of Katherine (Figures 2.2 and 2.11), this site is outside the school's boundary in eucalypt woodlands with grass understorey. The soil is an unstructured red kandosol that is high (with respect to other sites) in both Mn (47.44 mg/kg) and Fe (917 mg/kg) and no apparent deficiencies in the other main elements.

Of the six target species planted, four have successfully integrated into the site (*Iseilema vaginiflorum, Sarga intrans* (F.Muell.) Spangler*, Sarga timorense* (Kunth) Spangler, and *Brachyachne convergens*). Of these species only *Brachyachne convergens* emerged in the CQU glasshouse trials. The two other species not seen were *Dactyloctenium radulans* and *Eragrostis tenellula* (Kunth) Steud. Both will readily vegetate damaged or denuded nitrate rich areas (such as stockyards and gateways) or along calcium rich floodplains (Shaw and Fesuk 2003). This site has neither, and already had a healthy selection of indigenous species that populate the niche that these two species would have taken. It is also noted that if *Dactyloctenium radulans* (a short-lived annual species) had been present, then its distinctive seeds would have been present in the soil seed bank (Figure 2.12).

Figure 2.11 Katherine High School Site

As this soil type is prevalent around Katherine, it was deemed prudent to GANT trial alternative species to those already growing locally in the event that the market for native seed will increase. Trials such as this serve to increase the database of knowledge with respect to adaptability of these species to iron-rich environments.

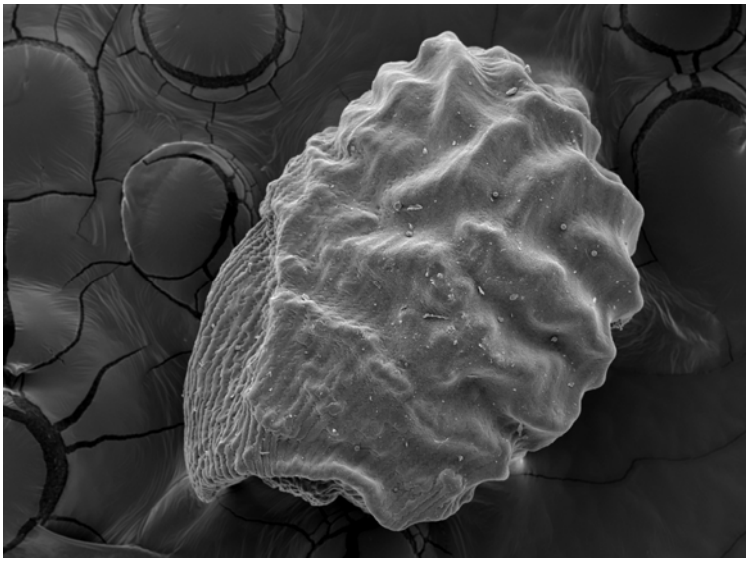


Figure 2.12 SEM image of *Dactyloctenium radulans* seed (x100 Magnification)

2.5.5 Limbunya

Limbunya is situated on the southern edge of the Eastern Kimberley, just above the Tanami Desert Shield (Figure 2.2). The soils are grey, very sandy, and non-textured soil and are subject to intensive trampling and grazing by livestock.

Under natural conditions this area would be rehabilitated to open woodland with grass understorey; however, being in a holding paddock exposes it to over-utilisation in terms of grazing and traffic. Of the target species planted there, both to trial rehabilitation and provide stock feed, only *Chloris* sp., *Chrysopogon fallax* (S.T.Blake) and *Iseilema vaginiflorum* (Table 2.1) were found established at the site and only *Iseilema vaginiflorum* seed germinated in the glasshouse trials (in 2002 only, access to the site was not available in 2003 due to station mustering commitments). *Iseilema vaginiflorum* has a fragile structure, with stems and inflorescences easily broken off, a feature discovered in landfill batter trials (Chapter 4). This makes it somewhat susceptible to damage by stock trampling, however, it also permits the release of the seed which are enclosed within a spathe (Figure 2.13) and are offered some protection when driven into the soft sandy soil.

Chrysopogon fallax, while proving to be resistant to grazing and displaying an ability to establish itself on pasture soils ranging from red kandosols to clays and now sandy vertolsols failed to germinate in the glasshouse trials. GANT trials have revealed that it requires a two year after-ripening period before field sowing (Shaw and Fesuk 2003).

Figure 2.13 *Iseilema vaginiflorum* seed is enclosed in a spathe-like inflorescence.

Restoration strategy for this site will primarily be to rest and replant this paddock and only permit moderate grazing access when the planted species have stabilised the soil surface.

2.5.6 Mainoru

Mainoru borders on the southern edge of Arnhem Land (Figure 2.2); the site is situated on a cleared area of 700 ha that was once woodland. The site is watered via underground springs that feed into the Mainoru River system. The soils are yellow kandosols that have a crusted surface (in areas further west a hard siltstone cap) that are

moderately high in iron (1103 mg/kg) and chlorine (696 mg/kg) with a conductivity of 0.568 dS/m, which is little lower than 0.695 dS/m recorded from the Bing Bong Fines sample (Table 2.2). Underlying soil layers are prone to erosion when the hard soil cap is compromised by machinery or hoofed animals.

No visible stunting of vegetation or crusting of salts (which should not occur at 0.695 dS/m) was noticeable at this site, however, it could be speculated that the clearing of the 700 ha of woodland has permitted a rise in the water table. The high level of Cl^- ions, however, is cause for concern as chloride is a main subsoil factor that affects plant growth and fruit yields by affecting plants ability to take up water (Grewal et al. 2004; Dalal 2006).

Figure 2.14 Mainoru site with the hard soil cap clearly illustrated.

Of the target species planted (Table 2.1), four species (*Brachyachne convergens, Iseilema vaginiflorum, Vacoparis laxiflorum* and *Themeda triandra*) were noted at the site both in 2002 and 2003. However, only *Brachyachne convergens* and *Chloris pectinata* germinated during the glasshouse trial, which means that although plant specimens of *Chloris* were not present, certainly its seed had persisted in the soil seedbank. That these species were able to establish in a soil that has moderately high chloride levels increases the viability of these grasses as commercial pasture plants.

2.5.7 Maud Creek Site

In 2000, the Katherine Mining NL, in association with AngloGold Australasia, conducted a six month open-cut mining operation to remove gold from the sub-surface oxide layers at Maud Creek, (approximately 20 km north-east of Katherine, NT) (Figure 2.1). The pit did not exceed a depth of 40 m as beneath this level (below a water impermeable cap) were layers rich in gold sulphide ores, the mining of which could prove detrimental to the local water supplies affecting urban and agricultural water supplies. Mine waste was stockpiled to a height of ten metres with slopes battered to approximately 14^0 (Figures 3.15 and 3.16). A bund wall surrounds the mine pit except where it receives run-off water from the waste dump (Katherine Mining N.L. 2000).

The GANT revegetation site is situated in the run-off zone between the waste dumps and mine pit. This site (Figure 2.15) has soil moderately rich in both iron and calcium and of the target species planted; only *Brachyachne convergens* and *Sarga plumosum* (R.Br.) Spangler were found in the samples collected.

Of the two species, *Brachyachne* was the most successful in establishing itself in the area sown (Figure 2.15). The site consists of brown kandosols over a water table which is above an impermeable rock layer under which lie gold mineral ores high in sulphides. Soil analysis showed nominal sulphur levels of 67 mg/kg, however the main concern of

the management of this site is the impact of cattle and water buffalo both on the soil surface and over-grazing. At the time of capturing this photograph stock had been withheld from this paddock for six to eight months, allowing the establishment of the *Brachyachne convergens.*

In March 2006, the NT Department of Primary Industry, Fisheries and Mines (NTDPIFM) produced a paper listing potential mining developments in Northern Territory. GBS Gold International Inc had acquired rights to the Maud Creek mine and intends to resume oxide mining in mid 2007, with a view to sulphide mining as well (NT Government 2006).

When metal sulphides are exposed in quantity to water or the atmosphere the oxidisation results in the release of acid and metal ions. Low pH and high metal concentrations adversely affect water quality and aquatic biota in terms of plant growth, microbial function and seed germination (US EPA 2000; Renault et al. 2002). Studies from Jabiru mine site at Kakadu National Park have shown that elevated levels of magnesium sulphate severely decreases the germination rates of the majority of native plants tested (Malden et al. 1994).

The formulation of an effective rehabilitation program, with reference to GANT data and utilisation of species such as *Brachyachne convergens*, should be an integral part of the company's Environmental Impact Statement.

Figure 2.15 Maud Creek Site. A thick covering of *Brachyachne convergens* is in the foreground. The bare slope to the right is the run-off from the waste dumps.

Figure 2.16 Satellite Image of Maud Creek Site (DigitalGlobe 2006).

2.5.8 Mt Todd

Mt. Todd (Figure 2.2) was opened as a mine in 1994. Due to falling gold prices operations were halted in 1997 before being reopened in 1999. The mine closed operations again in 2000 with an estimated rehabilitation cost of $20 million. The site poses an environmental risk with leakage from its decomposing cyanide holding tanks and leaking overflow bunds threatening to enter the catchment system of Katherine's water supply (ECNT 2000). A press release on February 28th 2006 announced the sale of the mine to Vista Gold Corp., whose immediate task is the stabilisation of the toxic chemical tanks and rehabilitation of the mine site before further mining is carried out (Vista Gold Corp 2006).

Figure 2.17 Mt Todd Site with *Aristida* sp. in the foreground and the natural vegetation on good soil in the background.

The GANT site is situated on mine spoil dumps on the southern side of Mt Todd mine. The dumps are in excess of 50 m high and are covered with a thin layer of stony soil

(Figure 2.17). High annual rainfall resulted in the surface soil analysis not revealing any abnormally high concentrations of irons or sulphides, although levels of copper and zinc are slightly higher here than at other sites and the pH ranges from 6.4 - 6.9.

As a rehabilitation site without adequate topsoil or access to water, and exposure to the north-western sun, the mine spoil dumps are extreme environments in terms of aridity, heat and lack of soil substrate. The only planted species able to establish themselves were *Aristida* sp. (Figure 2.17) and *Brachyachne convergens*.

One of the reasons for these species being established could be in the structure of the seed of each of the species. Aristida (Figure 2.18) is an extremely slender seed with a sharp pointed and barbed callus. The seed has three long twisted hygroscopic awns that are extremely effective at drilling the seed into soil, or any gaps in the stony substrate. Brachyachne is a tiny seed that is very flat and protected by thin overlapping glumes. This feature allows the seed to occupy niches that would be unavailable for seeds of other species. Once safely in the micro-environment of a niche, the seed would be exposed to higher moisture levels and sheltered from extreme heat and sunlight (Peart 1981).

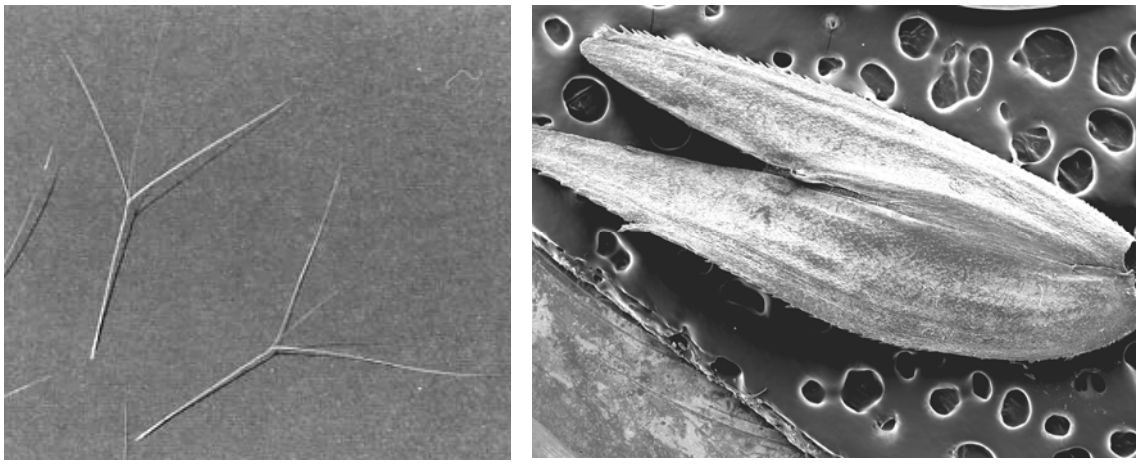


Figure 2.18 Seed structures of *Aristida* sp. and *Brachyachne convergens.*

Due to the extreme conditions existing at present, if a range of species is to be established in a short period of time then the dump slopes must be spread with topsoil before further seeding is undertaken.

2.5.9 Willaroo

Willaroo is a station situated south-west of Katherine (Figures 3.2) in an area that has a high density of *Jatropha gossypifolia* (Belly-ache Bush), a declared weed.

As a control measure for the eradication of *Jatropha*, *Dichanthium aristatum* (Poir.) C.E. Hubb. and *Vacoparis laxiflorum* were planted to provide a vigorous growth and produce high fuel loads to attempt to burn out *Jatropha*. The trial was successful as no *Jatropha* was present during the 2003 inspection of the site (Figure 2.19).

At this stage the grasses had not yet established themselves long term in the soil seedbank for there were none present growing and none emerged from the soil seedbank glasshouse trials. Curiously enough, *Brachyachne convergens* did germinate in the glasshouse, not having been recorded at the site in either 2002 or 2003, indicating that seed had been present for some years.

After the 2003 trial, neither was there any *Dichanthium* or *Vacoparis* present, for after the eradication of *Jatropha,* the grazier reopened the paddock to his stock and the newly germinated grasses were grazed out.

Willaroo is an example of a site where the use of a fire regime can be utilised to manage the eradication of exotic species by exploiting the growth habits of indigenous grasses in association with grazing practices and sowing of selected grass species. The selective use of fire is an effective tool to stimulate plant growth, seed germination and to destroy or reduce animal and plant pests, however, it is important to have an integrated management system comprising not just fire, but stocking rates, erosion control and replanting as well (Anderson et al. 1988).

One of the factors in successful fire management is the control of stocking in the areas to be managed. Moderate grazing will allow the increase of fuel loads for late season burning. This increases fire intensity to combat the invasion by exotic plants (Ash et al. 1997). The overstocking of pastures severely reduces the use of fire to control woody shrub populations by limiting the amount of litter left by the die off of annual grasses (Dyer et al. 1996).

The timing and the regularity of burning is also a factor that needs careful consideration. Spring burning is useful in promoting the occurrence of *Heteropogon contortus* at the expense of *Aristida ramose* (R.Br.)(Campbell 1995). In central Australia, winter fires (wet season) are easily controlled and maintain or improve palatable grasses. Summer fires (dry season) reduce palatable grasses, so rainfall also proves to be a major factor in the intensity of fires used in combination with seasonal fuel loads (Griffin and Friedel 1984).

Plants can be more susceptible to fire if they experience it at infrequent intervals, under adverse conditions (e.g. prolonged drought) or at times when they are physiologically active either as mature plants or as seedlings or juveniles (Campbell 1995). In many instances, regular burning off is needed to maintain a balance between grass and woody plants (Dyer et al. 1996). In the case of annual grasses, such as *Vacoparis* and *Sarga* (Sorghums), burning can interrupt seed set of these plants, yet seed will be left in soil at the end of the growing season (i.e. end of the monsoon season in the northern tropics) but only in the short term.

Continued burning after seedling emergence will eliminate species such as *Sarga intrans* as this type of annual does not establish seed in the long term in the soil seedbank (Mott and Andrew 1985). Annual dry season or biennial dry season burning has no effect on abundance of sorghums, although annual burning has been recorded as reducing the

abundance of *Themeda triandra*, which is also a desirable fodder species (Lazarides et al. 1991).

Figure 2.19 Willaroo Site with a predominant stand of *Heteropogon contortus*.

A thorough knowledge of the growth habits of desirable species is an integral part of any burning regime, another example of this being the use of fire as a management tool for rubber vine (*Cryptostegia grandiflora* (Roxb. ex.R.Br.)). This method can be effective yet care is required so as not to decimate native species such as sandalwood (*Eremophila mitchellii*)(Benth.), stands of which died out after two years of consecutive burning (Noble 1996).

2.5.10 Control Sites 1, 2 and 3

Figure 2.20 Control Site 1

Figure 2.21 Control Site 2

Figure 2.22 Control Site 3

These sites (Figure 2.2) were sampled in order to provide a natural check on the species that exist across the Northern Territory without being part of the GANT monitoring and revegetation scheme. CS1 (Figure 2.20) was sampled from grasslands on the Barkly

Tableland while en route to Benmara. From a site whose grasses were predominantly *Eulalia aurea, Aristida* spp., and *Sporobolus* spp., *Sporobolus* was the only grass genus to germinate in the CQU glasshouse trials, besides the normal collection of *Digitaria bicornis* and other monocots such as *Cyperus* and *Fimbristylis*.

CS2 (Figure 2.21) was situated close to Willaroo, and the floristic composition consisted predominantly of *Heteropogon contortus, Chrysopogon* sp. and *Sporobolus* spp., none of which were present in the CQU glasshouse germination trials which only resulted in the germination of *Sarga* sp. and forbs. CS3 (Figure 2.22) which had predominant stands of *Cymbopogon* sp*, Chrysopogon* sp. in the CQU germination trials produced *Sarga* sp*., Eragrostis* sp. and *Sporobolus* sp. in addition to the ever-present *Digitaria.*

2.6 Conclusion

This chapter summarises the results that were part of trials being conducted by GANT personnel over a number of consecutive years over many sites across the northern half of Northern Territory. An examination of species being planted in areas outside of their known natural range has given insight into the adaptability of those species and their usefulness in specific rangeland rehabilitation scenarios.

Accordingly, three species (*Chrysopogon elongatus, Alloteropsis semialata* and *Dichanthium fecundum)* are now under commercial cultivation for seed supply on properties located near Katherine with the intent to develop new cultivars.

Brachyachne convergens has proven to be a resilient, persistent and ecologically useful restoration plant able to establish itself in harsh environmental conditions. However, unless sown at high densities, it is susceptible to invasion by more aggressive plant colonisers it is therefore difficult to establish pure stands of this grass. Research options to enable weeds in *Brachyachne* stands to be eradicated, yet leaving a pure monoculture, would include attempting to produce a cultivar of this species that is more

aggressive in its spread, offering denser ground coverage (and further increasing its usefulness in batter stabilisation) or genetically modify it to be herbicide resistant (an action fraught with potential environmental liabilities and one which must be researched with utmost diligence and lack of bias).

Chapter 3
Germination and Storage of Selected Tropical Grasses of Northern Territory

3.1 Abstract

Seeds of 25 native grass species were collected from sites across the Northern Territory and were placed into four long-term storage treatments (Cool Room, Air-conditioning, Freezer and Shed storage). Stored seeds were subjected to an initial germination trial followed by two more sets (over the following three years) of germination storage trials that tested the seeds response to smoke water and control germination treatments. Seeds of sixteen native grass species, that had little or no germination response in an initial germination trial, were subjected to tetrazolium testing using non-imbibed seeds as controls. Seedlots whose seeds were deemed to be metabolically dead were taken out of the trials. Results showed that the majority of the NT grass species trialled maintain better viability in cold storage, and several species demonstrated an adverse response to smoke treatments.

3.2 Introduction

Native grasses play a vital role in landscape stability and functioning, especially in the tropics where frequent bushfires and intense rainfalls occur. Native grasses are particularly sought after in ecological restoration (Ashwath et al 1994; Ashwath 1995), sustainable grazing and land management (Shaw and Fesuk 2003). However, native

grass seeds are expensive to purchase and often have low seed viability (Ashwath et al 1994). Prior to this study little was known of the germination requirements of the majority of Australian native grasses, although it is known that the germination requirements and dormancy vary between different ecotypes of the same species, and that desert or arid species of grasses are more prone to long-term dormancies than their sub-tropical relatives (Groves et al. 1982; McBurnie 2002). Only those grasses in past decades that were considered to be of economic or having pastoral significance had funding available for research into their germination requirements (Campbell et al. 1994).

Research so far has focussed on developing techniques for seed collection, seed germination (Merritt et al. 1999; Tieu et al. 1999), and plant establishment in the field (Shaw and Fesuk 2003), with little attention being paid to optimising seed storage conditions. The latter is crucial for promoting the use of native grasses, as seed from native grasses usually has to be collected over several seasons and stands as their seed production is unpredictable, the costs of procurement high, and large quantities of seeds are required for revegetation (both due to a naturally low germination rate and the large areas requiring seeding).

As experimentation continues to close the gap between ignorance and understanding, it has become obvious that there is a need to be able to assess seed quality, in terms of matching seed viability (the potential of a seed to germinate) with germinability (the capacity of a seed to germinate). This is done in order to determine whether or not the treatments being used to stimulate seed germination are suitable for that species, the seed is in dormancy, at some early stage of maturation, or, if in fact, the seed is non-viable and will not germinate under any conditions (Dixon and Meney 1994).

It is known that seed dormancy itself is not as a result of any one condition. Often dormancy is caused by a combination of mechanisms, as well as, or in conjunction with

ecotype variability (Groves et al. 1982; Van Rensburg et al. 1999). Assessing seed viability by means of seed inspection, cut-test or with1,3,5 triphenyl tetrazolium chloride (TTZ), therefore, becomes an essential procedure in understanding the species-specific germination requirements (Mortlock 1999).

The TTZ test involves the treatment of seeds with a solution of tetrazolium salts to stain metabolically active tissue that is present in a viable embryo. As the TTZ solution is imbibed into the embryo tissue, it is reduced by NADPH dehyrogenase to formazan, by an enzyme found in viable metabolic tissue, which is a red, stable and non-diffusible compound (MacKay 1972; Thompson et al. 2001). The viability of the seed can then be assessed by cutting the seed open to expose the embryo, and then observing the areas of the seed that are deeply stained (red), partially stained (pink) and not stained (no change in seed colour).

This study tested the responses of a selection of tropical native grasses to smoke treatment and storage conditions over a period of three years with the view to optimising conditions for extending the viability of seeds in storage. The TTZ testing was carried out to assess the viability of stored seedlots before making the decision to continue them in the storage trials.

3.3 Materials and Methods

3.3.1 Germination and Storage

Seeds of 25 tropical native grass species were collected from different parts of Northern Territory and the Kimberley region mostly during early 2001 (Provenance of each seedlot is listed in Appendix 9.2). The seeds were stored in a large, ventilated room at Katherine for 1-2 months before being despatched to Central Queensland University (except *Astrebla squarrosa* and *A. pectinata* which were kept in an unventilated railway storage container for six and seven years respectively, and the *Dichanthium sericeum* (2000)

seedlot which was harvested in 2000 and stored in a dry ventilated storage room for 12 months). Upon receipt at CQU, the seeds were air-dried (at ambient temperature in large ventilated room) to moisture levels ranging from 4% to 8% and then split equally into four lots (where possible, some seed lots had insufficient seed for four treatments and were split into three lots with the exemption of the Air-condition storage treatment) and placed in either paper (for fine seed) or cotton bags (for chaffy seed). The bags were transferred to tin containers with air-tight lids and placed in a (i) garden shed (8-60 ^{0}C), (ii) air conditioned laboratory (25 ^{0}C), (iii) cool room (8 ^{0}C), and (iv) a chest freezer (-18 ^{0}C). The seeds were tested for germination three times, first at the time of transferring to the storage treatment conditions (in 2001), and after one year (2002) and three years (2004) of storage.

For the germination tests, uniform and fully-filled seeds were manually selected and placed in Petri plates containing filter papers resting on moist vermiculite. Four replicates were used, with each Petri plate containing 25 seeds. The Petri plates were placed in a controlled environment room maintained at 33/23 ^{0}C day/night temperature respectively, light intensity of 480 µmol/s/cm^2 on a 12-hour day/night cycle was provided by fluorescent and incandescent lights. Seed germination was monitored daily for 35 days, and distilled water was added to Petri plates as required. Germinated seeds were counted and removed from the Petri plates at each count. Germination was defined as the emergence of the radicle to a length of 1 mm for seeds less than 1 mm in diameter, and 2 mm for larger seeds.

Two germination treatments were used, the first being a ‘Control’ in which the seed was left intact within its palea and lemma (husk) (except the *Astrebla* species which do not contain a palea and lemma). The second treatment was ‘Smoke’ treatment in which dry seeds, with husk intact, were exposed to aerosol smoke (Lloyd and Dixon 2000) for an hour (other periods of time could be considered for later studies) and then immediately

placed into germination conditions. Germination trials were conducted upon receipt of seeds (results published in Fesuk and Ashwath 2001) and at twice more at18 month intervals.

3.3.2 Data Analysis

Data was collated and converted to percentage germinated for each replicate. These were expressed as a decimal fraction, and, as there were many values of 0 or less than 0.1, both arcsine and square root transformations were employed (Gomez and Gomez 1984). Replicates with the majority of values less than 0.1 were transformed by the formula SQRT(X + 0.5) and all other replicates were transformed using ARCSINE(SQRT X). Both values were then converted to radians by use of the formula 180/π (X). The data was then analysed using a one-way ANOVA with a p value of 0.05 and LSD post-hoc test.

3.3.3 Tetrazolium (TTZ) Test

Prior to the 2002 trials, ten clean and undamaged seeds were counted out for each of three treatments (control, cut-treatment and TTZ test) for each of the eight species of native grasses that had shown little or no germination response from the earlier trials (Table 3.1). The seeds were sourced from seedlots in current cool room storage trials (with the exception of *Alloteropsis semialata, Aristida inaequiglumis* and *Astrebla squarrosa* all of which were drawn from Garden shed storage treatments). The species were selected on the basis of their failure to germinate under optimal control, or specific treatment conditions. As the seeds were small and lack woody coats, no scarification was required of any of the treatments to ensure efficient imbibition by the cut-treatment and TTZ seeds for 24 hours prior to treatment.

The control for these treatments was prepared by cutting the dry seeds in half to both examine the condition of the embryo, ensure the seed was full and to allow a basis for comparison with the other treatments. The cut seeds were then photographed.

The cut-treatment involved the imbibing of the seeds for 24 hours in distilled water, then, longitudinally bisecting the seed to photograph the exposed embryo. A seed could be considered alive and healthy if the seed tissue was not deformed or pale and soft, and there was a mature embryo present (Thompson et al. 2001).

The TTZ test involved the pre-soaking of the seeds in distilled water for 24 hours. The seeds were then immersed in a solution of 0.5% 1,3,5 triphenyl tetrazolium chloride covered and placed in a dark cupboard for 12-14 hours with an ambient temperature of 20-25^{0}C. The seeds were then removed from the solution, rinsed and precisely cut to expose the embryo, and then photographed (Thompson et al. 2001).

Viability of the seeds was assessed on the colour pattern produced in the seed embryos. If the embryos were stained red in the TTZ treatment, then the seeds were considered viable. The seed was considered to be potentially viable if the shades ranged from red to pink. Seeds that contained no distinct staining were considered non-viable (Thompson et al. 2001).

The longitudinally bisected seeds from each treatment were photographed using a Nikon digital camera mounted on a dissecting microscope. A representative specimen from each treatment was placed in a composite image to compare for both the embryo condition and colour resulting from the treatments.

3.4 Results

The results of the seed treatments for 16 of the species are presented in Figure 2.1. The other nine species originally in the trial in 2001 have been found through the 12 month

TTZ test to have lost their viability over the storage period and so were removed from the trials.

3.4.1 Effects of Storage Treatments

Storage in cool room for three years did not affect seed germination (with respect to the germination rates) of the other storage treatments in most species. The exceptions were *Astrebla squarrosa* and *Brachyachne convergens* where germination in cool room treatment were much lower than those stored in freezer and in a garden shed respectively. Cool room storage resulted in the highest germination rates for *Chloris pectinata* and *Triodia bitextura.*

Seeds of some species failed to germinate in any treatment even after 3½ years of storage. For *Paraneurachne muelleri* (S.T.Blake) the seeds were somewhat metabolically active, although TTZ tests later showed a decline in viability of the seed between the 2002 and 2004 trials. Storage in a large garden shed had a detrimental effect on the viability of five of the tested species (*Alloteropsis semialata, Aristida inaequiglumis* (Domin)*, Astrebla squarrosa, Themeda triandra* and *Triodia longiceps* (J.M.Black)*)*, and in contrast, this storage had a definite positive effect on the germinability of *Brachyachne convergens*. In all other species, storage in a garden shed either had no, or only little, effect.

Storage in an air conditioned room was as effective as other best storage conditions for *Triodia bitextura, Chrysopogon fallax* and *Dichanthium sericeum* 2001*,* and slightly reduced the germination of *Brachyachne convergens* and *Triodia inutilis* (N.T.Burb.)*.*

Freezer storage improved germination in *Astrebla squarrosa* and it markedly negative effect on the germination of *Brachyachne convergens* and *Sorghum macrospermum* (E.D.Garber). *Triodia basedowii* seed germination was completely inhibited by freezer

storage. In *Alloteropsis semialata* the seeds lost viability after 1 year in other storage conditions, however, remained viable in the freezer treatment.

3.4.2 Response to Smoke Treatment

Overall, smoke treatment had very little effect on the germination of these tropical grasses. However, it improved germination in a few species, but only in certain storage conditions (e.g. *Triodia bitextura* in freezer storage). Smoke treatment also inhibited germination in some species under certain storage conditions (e.g. *Eulalia aurea* in freezer storage).

3.4.3 Response to TTZ Treatment

Examination of the control and cut treatments gave no indication of any abnormality or fungal rot present in any of the grass seeds tested.

Table 3.1 Results of TTZ testing and germination trials for selected grass species

Species	Storage Treatment	% Germ.	% Red stain	% Pink stain	% Nil stain
Grass species recording little or no viability from TTZ testing					
Alloteropsis semialata	Shed	0	10	0	90
Aristida inaequiglumis	Shed	0-4	0	0	100
Astrebla pectinata	Cool Room	0	0	0	100
Dactyloctenium radulans	Cool Room	0	0	0	100
Enneapogon polyphyllus	Cool Room	0	0	0	100
Panicum decompositum	Cool Room	1	0	0	100
Panicum trachyrhachis	Cool Room	0	0	0	100
Sporobolus mitchellii	Cool Room	0	0	0	100
Grass species recording limited viability from TTZ testing					
Astrebla squarrosa	Shed	0	20	10	70
Sporobolus actinocladus	Cool Room	0	20	0	80
Triodia basedowii	Cool Room	0	20	20	60
Triodia inutilis	Cool Room	8	10	20	70
Triodia longiceps	Cool Room	3	10	10	80
Grass species recording high viability from TTZ testing					
Dichanthium sericeum (2000)	Cool Room	33	50	30	20
Paraneurachne muelleri	Cool Room	1	40	30	30
Themeda triandra	Cool Room	1	90	10	0

Table 3.1 summarises the TTZ results with the actual germination percentages achieved by each of the species during the 2002 germination trials. The total of the percentages for red and pink stains indicate the potential viability of the seeds for each of the grass species listed. The species listed were selected due to their poor germination response during the first storage trial.

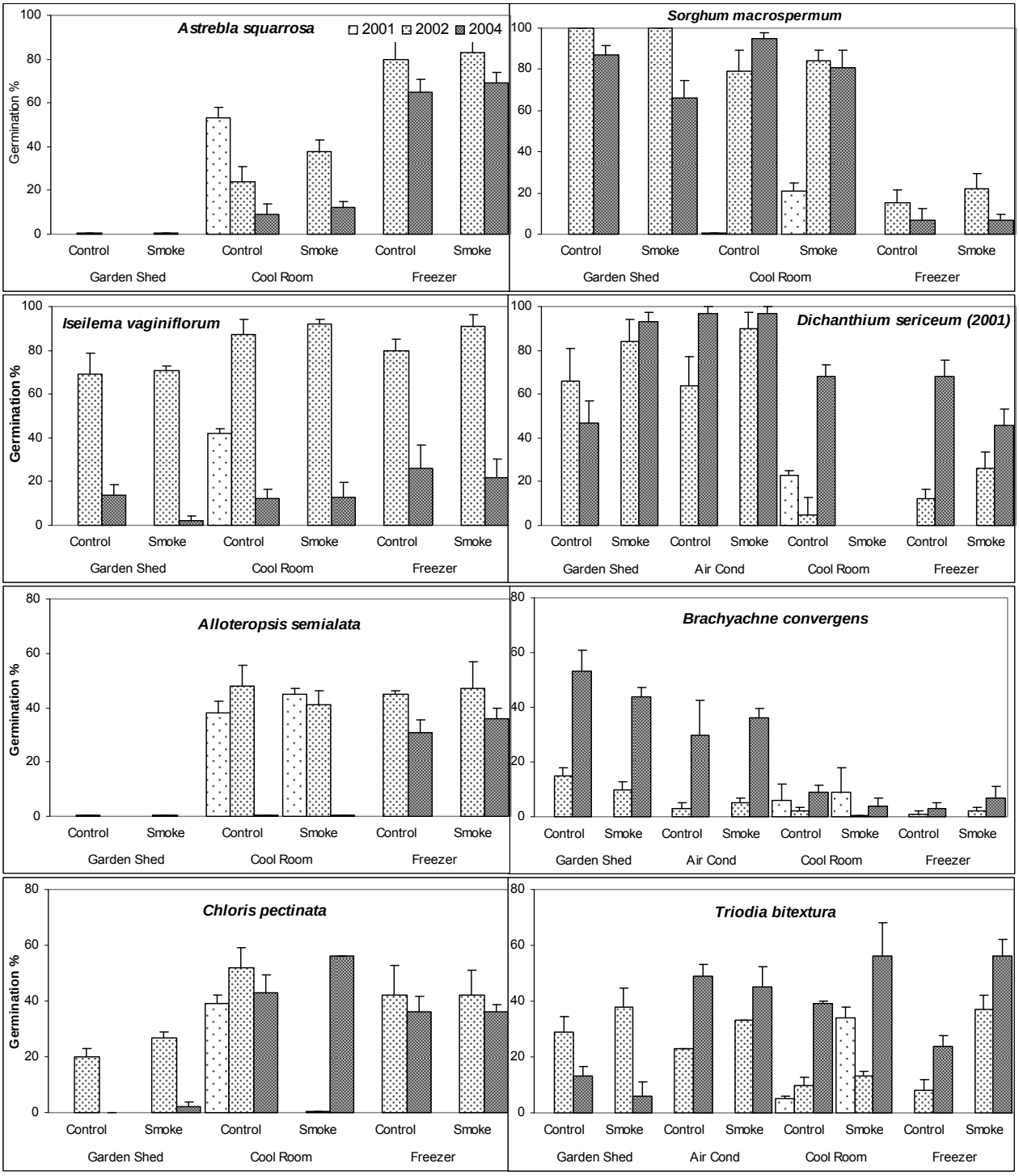


Figure 3.1 The effects of seed treatment and storage conditions on germination of 15 tropical grasses - soon after collection ⬚ , one year ⬚ and three years ⬛ of storage under four storage conditions. ⌶ bars represent standard error.

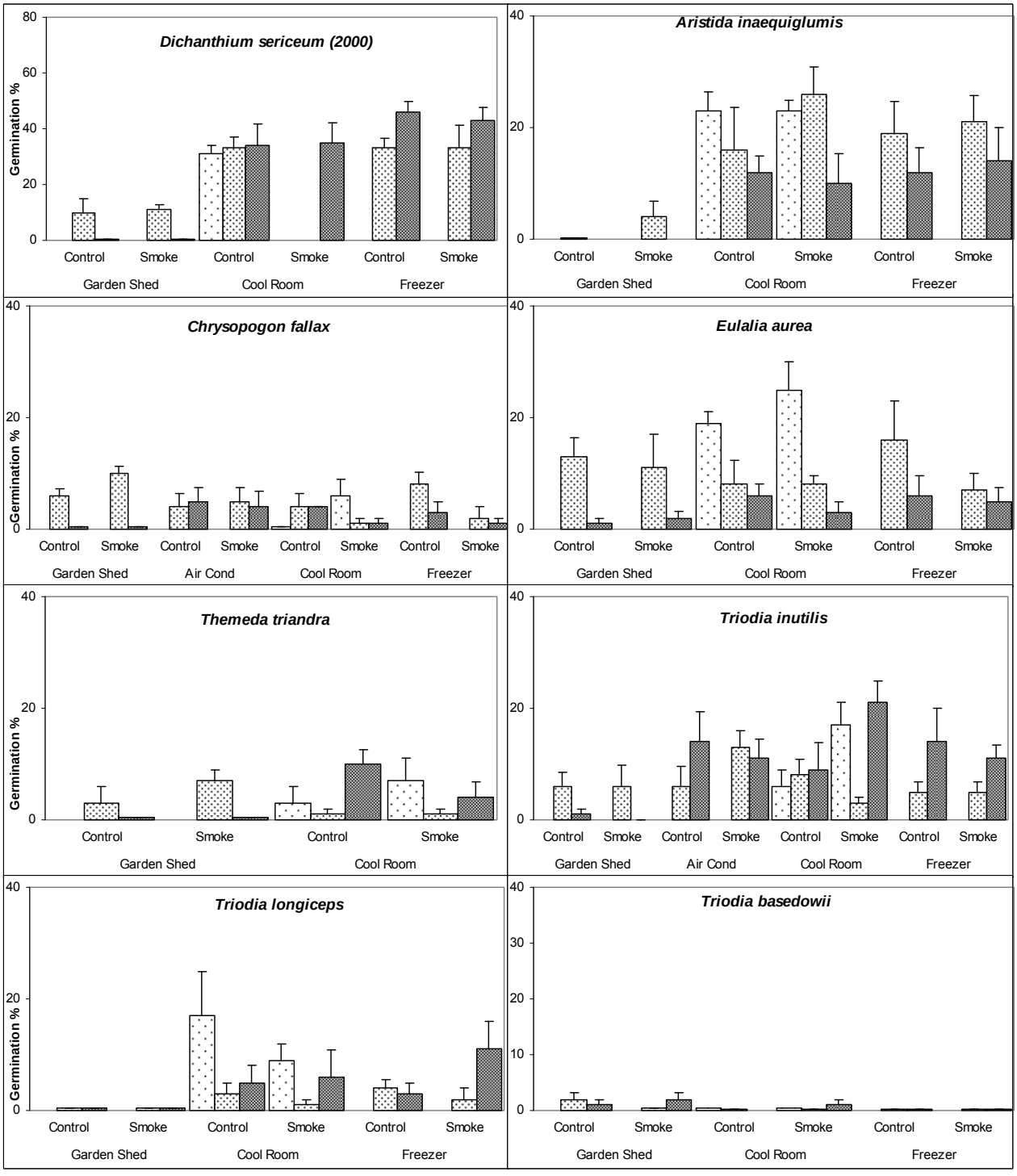


Figure 3.1 continued

Eight of the sixteen species (Table 3.1) indicated seed viability of 10% or less. This validity of this result was reinforced by the recording of actual germination values of 4% or less for each of these eight species.

Five species exhibited low germination rates as well as low viability potentials. The remaining three species recorded germination rates far below their apparent potential, based upon the TTZ test.

3.5 Discussion

For *Astrebla squarrosa,* freezer storage made a large difference in maintaining seed viability. Lane (1999) reported 80% germinability with fresh seed and virtually no germinability after twelve months of ambient temperature storage although their TTZ tests indicated 60-90% viability. The seeds of this current study were harvested in 1995, stored in a rail container and tested in 2001 before being placed in the storage treatments. The freezer storage maintained viability of the seed more so than the other storage treatments. Other studies in the population dynamics of *Astrebla* species have indicated that rainfall (not smoke or fire) is one of the major stimulants for the germination of this species (Orr 1986) and that *A. squarrosa* is a species whose range has spread from the more southern temperate climes into the tropics (Figure 3.2).

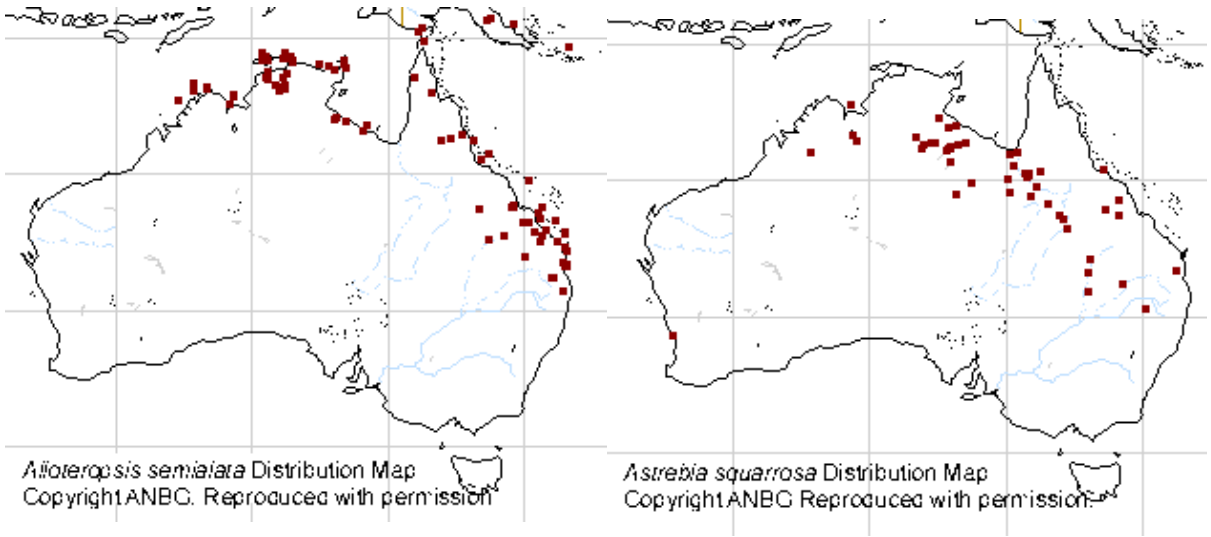


Figure 3.2 Distribution maps of *Alloteropsis semialata* and *Astrebla squarrosa*.

Astrebla squarrosa, Iseilema vaginiflorum, Dichanthium sericeum, Alloteropsis semialata and *Triodia bitextura* are species that responded positively (>50% germination) to freezer

storage (Figure 3.2) have distribution ranges that extend either far inland, or into the temperate southern regions of Australia (Shaw and Fesuk 2003). These species are adapted to cold climate conditions and not just the tropics where they are found. In contrast, *Sorghum macrospermum* is restricted to the tropics and its germination was markedly reduced by the freezer storage. *Brachyachne convergens,* in contrast to *A. squarrosa*, has spread to southern coastal areas of eastern Australia (Figure 3.3) from the warm tropic regions, possibility through human intervention, and still responds unfavourably to the freezing process.

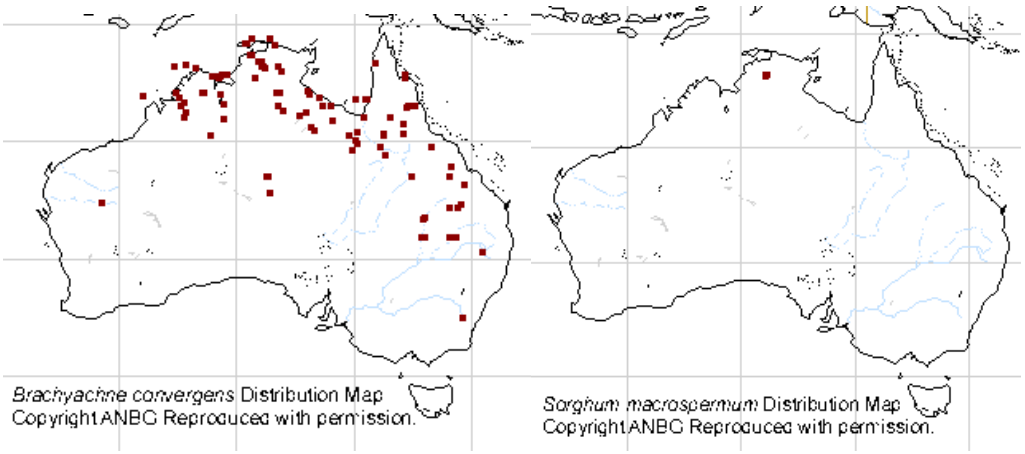


Figure 3.3 Distribution Maps of *Brachyachne convergens* and *Sorghum macrospermum*

The germination results for the two provenances of *Dichanthium sericeum* are very indicative of a dormancy cycle (after a period of after-ripening), and this cycle is influenced by smoke treatment. It is known that seeds of some species cycle through periods of dormancy and non-dormancy on an annual basis (Baskin and Baskin 2001) and the length of time that the seeds do this is at least partially dependent on the seeds remaining viable under conditions in which they are being stored (either in natural surroundings or in artificially controlled environments).

Non-deep dormancy seeds can go in cycles from dormant (only germinate under a narrow range of favourable conditions) to conditional dormant (environmental conditions

promote dormancy loss) to non-dormant (will germinate under a wide variety of conditions – generally) (Baskin and Baskin 2001). Grasses tend to go dormant, non-dormant, and then exhibit the secondary dormancy again which appears deeper than the non-deep type (Baskin and Baskin 2001).

Mott and Groves (1981) reiterate from earlier research that storage at a higher temperature will hasten or shorten the after-ripening period of harvested seed. This was evident in the responses of the 2000 harvest of *Dichanthium sericeum.*

The phenomenon of savannah grasses coming out of dormancy in time for the wet has been documented for *Andropogon brevifolius* (Sw.)*, Aristida capillacea* (Cav.) and *Microchloa indica* (L.f.) P.Beauv. (Baskin and Baskin 2001). Therefore, it could be said that the species on trial in this study that are apparently undergoing dormancy cycles should be expected to be in a non-deep dormant state in late spring and summer, which is the normal monsoonal rain period that they would experience in their natural habitats. Further research is necessary to fully qualify the assumptions made for the results from this experiment.

Vleeshouwers (1996), in Obendorf (1996) asserts that seeds do not passively wait for the correct environmental conditions to germinate. Rather seeds are constantly adjusting their degree of dormancy as a reaction to the environment that they are both experiencing and have experienced during seed development and maturation on the mother plant. Hence, the importance of knowing the origin of seeds that are being tested, for this may give indications as to the degree, or depth of dormancy that a seed is undergoing.

Since 1990, smoke treatment has been recognised as a form of germination stimulant for many plant species (Vigilante *et al* 1998), and its importance in the germination of *Triodia* species and desert plants has been recorded in other studies (Davidson and Adkins

1997; Koch and Dixon 1999). However, the current trial with a large number of tropical grasses that were stored for various times under different conditions show that smoke treatment is of little value in these grass species.

Seeds have varied germination requirements, both between species and with different eco-types within a species. The environment that the plant has developed in, especially those taxa that occur over a large geographical area, influences the degree of dormancy where a seed develops, its rate of maturity and after-ripening (Koch and Dixon 1999). The duration of a seed's dormancy is dependant on either seasonal or climax changes in environmental conditions that will initiate physiological or metabolic changes resulting in the germination of the seed (Hopkins et al. 1999).

Laboratory trials attempt to simulate typically favourable conditions in order to release the seed from its dormancy. However, since many species are specific in their germination requirements, there is a need to assess the seeds' viability and germinability at the beginning of the trials (Gravina and Bellairs 1999).

The TTZ test is the standard viability test that is recommended by the International Seed Testing Association (ISTA) and is used worldwide. Staining patterns may be very distinct and easy to interpret (Gravina and Bellairs 1999) as were the majority of the current seedlots, however, one species (*Aristida inaequiglumis*) produced an indistinct pattern whereby no significant staining could be recognised in any of the seeds. Eight grass species (in Table 3.1), achieved a staining pattern of less than 10%, and these may be considered metabolically inactive, therefore non-viable or "dead".

3.6 Conclusion

Seeds of most tropical native grasses require refrigerated storage, and some species store well in freezers. There are some exceptions such as *Brachyachne convergens* which performed better after storage in warm and dry or ambient conditions.

Response to smoke treatment varied with the species storage conditions and age of the seed and this experiment demonstrates limited benefit of treating tropical grasses with smoke.

Trials such as these should not be considered a definitive end unto themselves, rather as a stimulus to conduct further experiments with respect to the biochemistry of seeds, and the subsequent species response in establishing itself in a new environment under adverse conditions.

Chapter 4
Comparative Performance of Native Grasses Over an Exotic Species on Landfill Batters at Rockhampton, Australia.

4.1 Abstract

A trial was conducted to compare the effectiveness of three species of native grasses (*Brachyachne convergens* (F.Muell.) Stapf, *Iseilema vaginiflorum* Domin. and *Chloris pectinata* Benth.) for slope stabilisation, germination, drought tolerance, soil seedbank establishment and revegetation. A commonly used exotic species, *Chloris gayana* Kunth was also established as a control.

Although *Chloris gayana* is a perennial species and efficient in spreading over large areas through its stoloniferous habit, it has high fuel loads, grows up to 1.5 metres in height, is susceptible to drought and is highly invasive. Of the annuals, *Iseilema vaginiflorum* is vigorous in growth, flowers and seeds within three months. However, it has a clumping habit and is less effective than grasses with stolons in halting soil erosion on slopes, particularly in its first season of growth. Both *Brachyachne convergens* and *Chloris pectinata* are stoloniferous with *Brachyachne convergens* more prolific in the number of stolons, culms and branches produced. *Brachyachne convergens* is opportunistic, and produces and drops large amounts of seed which is covered by a thick thatch of previous season's culms. These virtues, as well as the suitability of

Brachyachne convergens as a pasture crop (and its non-invasive habit), results in *Brachyachne convergens* being recommended as being suitable for slope stabilisation.

4.2 Introduction

Native grasses, with few exceptions, have long been overlooked in the role they play in landscape stability and rehabilitation mainly due to a lack of understanding of their germination requirements, their unavailability in substantial quantity, quality and purity as well as the lack of knowledge of their effectiveness in soil binding or slope stabilisation. In the mid-1990's ecological studies had only been carried out on a limited number of species (Campbell et al. 1994), despite some native grasses being known to be quickly maturing annuals with a fibrous root system combined with a dense foliage cover, both of which are excellent in soil stabilisation and weed control (Stafford 1990). Native grasses are adapted to the Australian climate and have an ability to grow in soils with low fertility and poor structure. Native grasses, unlike exotic perennials such as *Chloris gayana*, tend to be non-invasive in respect to neighbouring plant communities (Whalley 1997).

Australia is made up of a diverse range of ecosystems, and, in turn, has large numbers of native grass species many of which are restricted to specific soil types or geographic conditions. Thus, the habit, habitat and genotype of grasses need to be examined for their suitability for use on a revegetation site (Huxtable and Waters 2001). This study examines the suitability of three native grass species (*Brachyachne convergens*, *Iseilema vaginiflorum* and *Chloris pectinata*) for batter slope stabilisations on a landfill site in comparison with an exotic species Rhodes grass (*Chloris gayana*), which has been extensively used in the tropics in revegetation of road sides, railway batters (Gyasi-Agyei et al. 2001) and mine sites (Ashwath et al. 1994; Huxtable and Waters 2001).

Brachyachne convergens germinates and establishes quickly and requires little or no maintenance. It has demonstrated suitability for revegetation of disturbed sites, and has

been useful in erosion control and soil stabilisation in the Northern Territory (Shaw and Fesuk 2003). *Chloris pectinata* has been used in revegetation of red clays and gravels and responds quickly to rain, and is able to colonise disturbed areas. *Iseilema vaginiflorum* germinates readily and establishes quickly and is able to tolerate harsh soil conditions (Fesuk et al. 2002).

This study examines the initial germination response after sowing (using hydro mulching techniques) and the mean number of plants after 120 days, the percentage coverage achieved by each species and the rate at which that coverage was achieved, the habit and phenology of the plants, and the response of the plants to the onset of the following wet season.

4.3 Materials and Methods

4.3.1 Site conditions, species selection and sowing rates

The trial site at the Rockhampton City Council landfill is composed of two elevated areas of alluvial soil, each 50 m in length and 25 m in width with slopes approximately 1 m in length. This gave a perimeter of 150 m per area. Each perimeter was divided into twelve sections of 12.5 m each. This permitted six replicates for each species. The slope of the raised areas was shaped to approximately 35^0 upwards from the horizontal by use of a template as this was the steepest slope that was able to be shaped due to the dry, unstable soil that was used.

Three native tropical species (*Brachyachne convergens, Chloris pectinata* and *Iseilema vaginiflorum*) were selected for revegetation/stabilisation of batters surrounding the landfill site at Rockhampton. An exotic species *Chloris gayana*, already in use by the mining and transport industries, was selected as a comparison/Control species.

Seeds of the native species were supplied by the GANT seed stores, Katherine and *Chloris gayana* seed was provided by Selected Seeds, Rockhampton. The native seed sowing rate was calculated by the weight of seed required to sow at a rate of two seeds germinating per 25 cm^2 (800 viable seeds per m^2 or 10 000 seeds per plot) (Table 4.1). Pre-trials conducted in a glasshouse using bare soil and hydromulch gave indications as to the germinability of the native seeds - *Brachyachne convergens* - 12%*, Chloris pectinata* – 4% and *Iseilema vaginiflorum* – 23%. *Chloris gayana* was certified to have a germination rate of 47%. The sowing rate for each species was calculated by the formula; seed (g) per rep = $10000 \times s^{-1} \times p^{-1}$ where *p* = the germination percentage expressed as a decimal fraction and *s* = seeds per gram

4.3.2 Application of seed

In November 2003, the six replicates per species were allocated at random and then sown using a hydromulch technique devised by *Lanyonscapes* (Gladstone) as per the application method illustrated in Figure 4.1. Seeds for six plots each species were weighed out (Table 4.1), mixed with two 20 kg bales of hydromulch (mix of sugar cane, recycled paper and guar gum) and 10000 L water and applied at the rate of 5000 kg/ha evenly over the six plots per species.

Due to extreme hot and dry weather conditions, the plots were watered twice daily (except for rainy days) either by hand-held hose or by Rockhampton City Council (RCC) water truck for eight weeks.

Table 4.1 – Species sowing rates.

Species	Germination %	Seeds / gram	Grams / plot (12.5 m^2)	Grams / species (75 m^2)
Brachyachne convergens	12	1 400	60.0	360
Chloris gayana	47	2 000	10.6	64
Chloris pectinata	4	1 620	154.3	926
Iseilema vaginiflorum	23	170 (seed + spathe)	256	1536

Figure 4.1 Sowing seed using LanyonScapes Hydromulching equipment (1) Graded bare batters (2) Mixing grass seed, sugar cane mulch, glue and water (3 & 4) Applying seed/mulch mix using water cannon (5) Seeded and mulched batters (6) Close-up view of hydro-mulched surface.

4.3.3 Monitoring germination and ground cover

Initial germination rates were determined by counting the germinants in ten fixed and marked 30 cm x 30 cm quadrats in each plot every seven to ten days from the time of the

emergence of the first seedlings. This process was continued for seven weeks. In April 2004, the same quadrats were used to determine the number of mature plants per m^2, as at this time the annual plants were senescing.

Figure 4.2 Calculating percentage cover by the use of photographs taken for each quadrat.

Ground cover was determined by photographing of either ten 0.75 m x 0.75 m or ten 0.9 m x 0.42 m quadrats per plot and determining percentage coverage of the target species (both green and dead culms), weeds and bare ground estimated visually (Figure 4.2).

4.3.4 Seedhead phenology

Figure 4.3 Monitoring seedhead development for *Iseilema vaginiflorum* at pre-flower stage.

Phenology of the grass seedhead was established by tagging ten randomly selected plants per plot and recording the time taken (in days) for the development of the seedhead from the point of swelling through emergence of the inflorescence, to full maturation as a seedhead (Figure 4.3).

4.3.4 Soil seedbank

The composition (i.e. proportion of target species seed compared to those of other species) of the soil seed bank after the first growing season (April 2004) was established by the collection of soil from five randomly selected quadrats (10 cm wide x 20 cm long x 2.5 cm deep) from each plot, although not within 1 m of the plot boundaries to avoid cross-contamination. The samples for each plot were mixed, crushed with a mortar and pestle and sieved through a 5 mm sieve. The >5 mm fraction was checked for seeds before being discarded. Sub-samples of 300 g of the remaining fraction were sieved through 2 mm and 0.1 mm sieves and the 2.0-0.1 mm fraction retained. Seeds from this 2.0-0.1 mm fraction were extracted by the floatation of the organic fraction in a 10% salt

solution and then seeds were separated from the organic matter by hand according to (Ashwath et al. 1999). The following formula was used to calculate the number of seeds per m^2 from the 300 g samples by using the soil density to convert 300 g to a volume of 500 mL, the samples of which had a surface area of 0.2 m (at a depth of 2.5 cm).

$$N = \left(\frac{Sx1m^2}{300gx0.02m^2} \right) x \left(\frac{1}{d \times 500mL} \right)$$

N = number of seeds per m^2
d = soil density g/mL
S = number seeds per 300 g top soil

This gives an indication of the number of seeds available for germination at the beginning of the next growing season.

4.3.5 Maintenance

The experimental plots were weeded periodically for the first year, and then the species were left to establish themselves in the second generation. The three native species are all annuals and a majority of the plant populations undergo die off (senescence and death of the plant after maturation), with the resultant falling over of stems to produce a ground cover. *Chloris gayana* is a perennial and its vigorous growth created dense stands, which in turn made recording of observations by means of laying down quadrats quite difficult.

4.3.6 Data analysis

Statistical analysis (with p -value of 0.05) was carried out using *GenStat v8.0* or *SPSS v13.0* to perform one–way ANOVAs and graphs were constructed using *GenStat v8.0* or *Excel* (LSD and SE figures calculated by *GenStat* and presented in Tables 9.2 and 9.3). Dunnett's T3 was used for a Post-Hoc test as the variances were observed to be unequal between the replicates and species. Tukey's honestly significant difference test was used for pair-wise comparison of the results for germination and ground coverage. Meteorological data was downloaded from the Australian Bureau of Meteorology website (Australian Bureau of Meteorology 2006).

4.4 Results

Initial germination counts indicated a rapid germination response from the exotic *Chloris gayana*; however, this figure may have been exaggerated due to the similarity in appearance of the *Chloris gayana* seedlings to that of *Echinochloa colona*, also an exotic grass species. A one–way ANOVA demonstrated a significant difference between the germination response of *Chloris gayana* and *Brachyachne convergens* over that of *Chloris pectinata* and *Iseilema vaginiflorum* (Section 9.3).

4.4.1 Monitoring germination rates and ground cover

The initial seedling emergence counts indicated a rapid germination response by the exotic species *Chloris gayana*, followed by *Brachyachne convergens*, *Iseilema vaginiflorum* and *Chloris pectinata* (Figure 4.4). A large proportion of the earlier emerged seedlings died due to hot and dry weather conditions that followed sowing. Seedling emergence in *Chloris pectinata* and *Iseilema vaginiflorum* was slow yet increased steadily for up to 28 days. After 43 days*, Iseilema vaginiflorum* consisted of close to 60 plants/m^2, whereas the other grasses had 20-30 plants/m^2.

Hot, dry weather conditions and black, friable soil shaped into a mound lacking any sub-surface moisture, created a situation in which artificial watering twice a day was unable to sustain seedling development and many seedlings died off and were subsequently replaced by new germinations following periods of heavy rainfall (as shown in Figure 4.4).

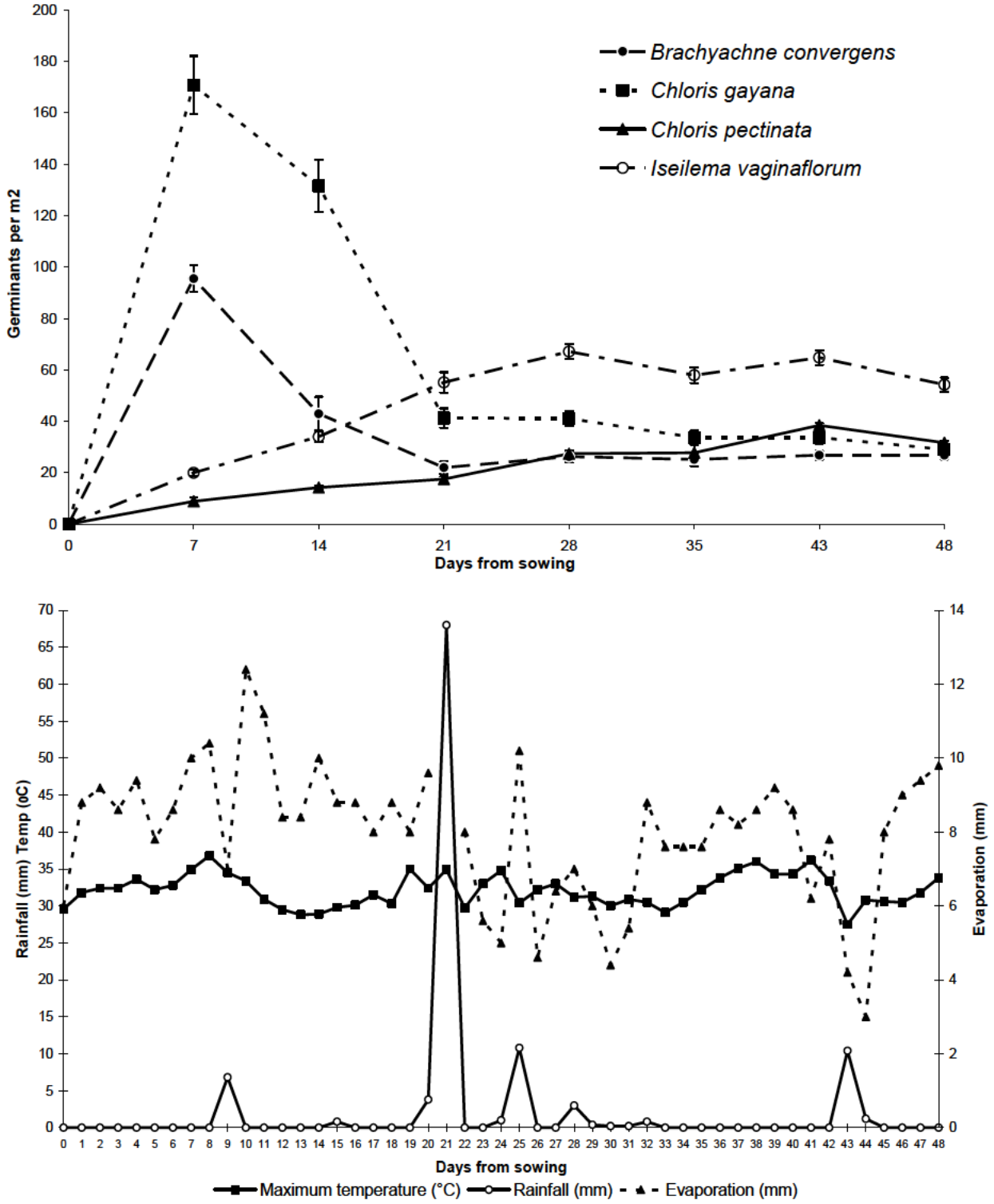


Figure 4.4 Weather conditions and the initial germination results per m^2 of the four grass species following sowing in 2003 (Bars represent standard error). Note the decline in seedling numbers of *Chloris gayana* due to hot and dry weather.

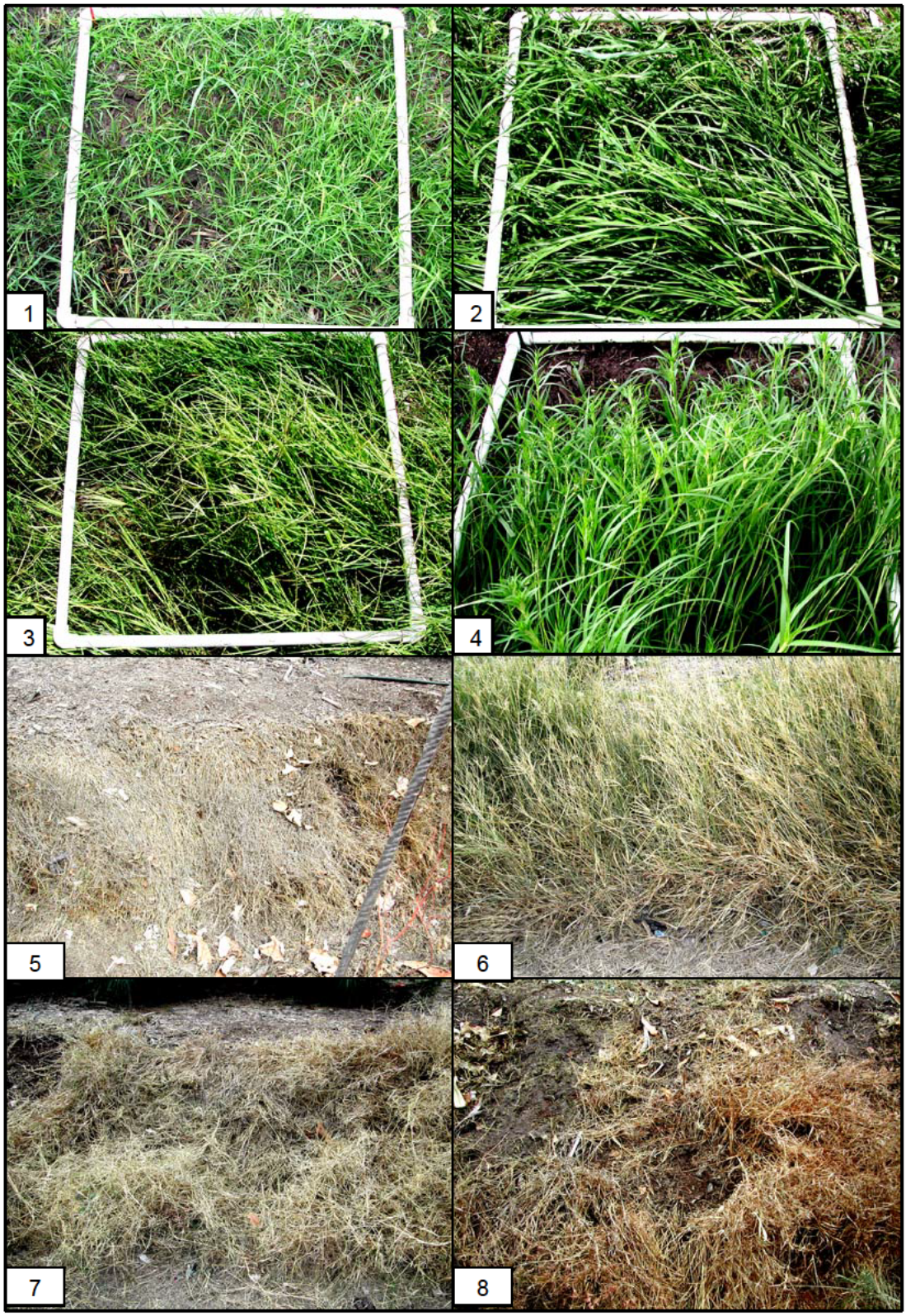


Figure 4.5 Images 1 – 4 are quadrat photographs of *Brachyachne convergens, Chloris gayana, Chloris pectinata* and *Iseilema vaginiflorum* respectively, taken 60 days after sowing. Images 5 – 8 are images of the same plots taken 231 after sowing. Note the thick thatch formation (5) of *Brachyachne convergens* and the ground cover and fuel load of (6) *Chloris gayana*.

Total ground coverage from January 2004 and December 2004 (December cover data includes newly emerged grasses and the old grass from last season's culms) is shown in Figure 4.7.

The results indicate that while *Chloris gayana* maintained a high ground cover with no winter die back, the annual grasses, in the space of 17 weeks, have achieved good coverage of the batter. This new grass cover and the remnant plant cover from previous growth provide up to 70% cover in the second year.

4.4.2 Phenology of the four grass species

The established grasses grew well producing good ground cover (Figures 4.5 to 4.7 and Table 4.2) at the end of the first growing season. *Chloris gayana* produced the highest number of culms per plant (Figure 4.6). The native grasses matured and died out around June/July 2004 whereas *Chloris gayana* continued to grow and produce a thick ground cover (Figure 4.7).

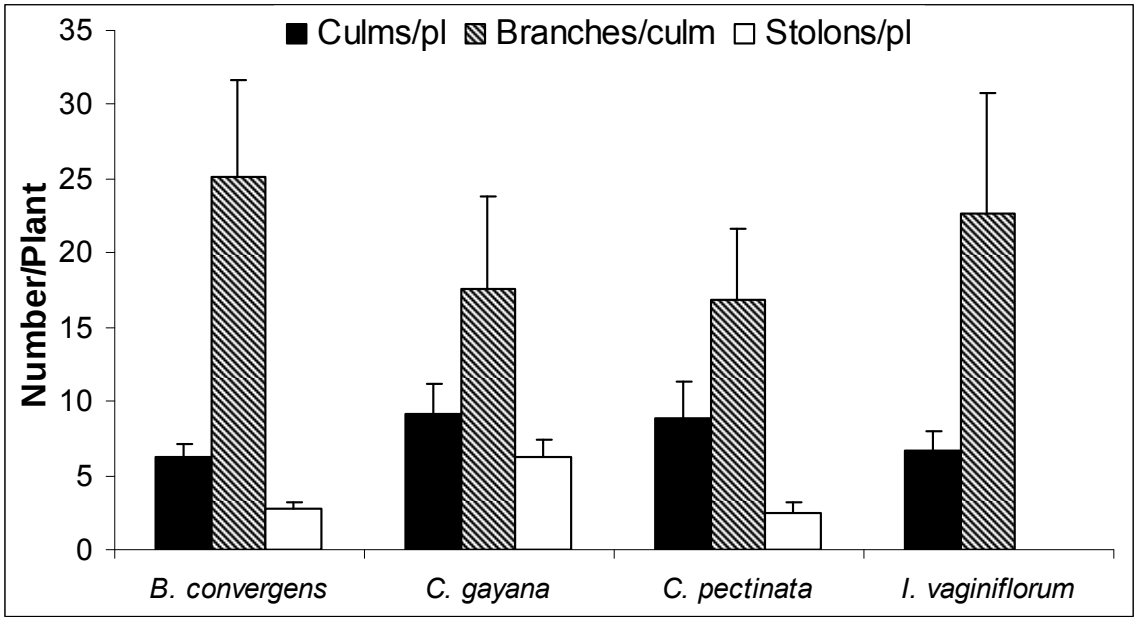


Figure 4.6 Mean number of culms per plant, branches per culm, and mean number of stolons produced per plant (bars denote standard deviation). Note that *Iseilema vaginiflorum* does not produce stolons.

Regeneration of the annuals occurred after 45 mm of rain in October of 2004. Total ground coverage (which includes cover of ground via grasses and the thatch from last season's culms) is shown in Figure 4.7. Standard error values were calculated by one–way ANOVA. The chart indicates that while *Chloris gayana* maintained a 100% ground cover with no winter die back, the annual plants, in the space of 7 weeks, achieved good coverage of the batter.

Table 4.2 Qualitative phenology data

Date	Days	*Brachyachne convergens*	*Chloris gayana*	*Chloris pectinata*	*Iseilema vaginiflorum*
15/11/2003	0	Sown in hot dry conditions	Sown in hot dry conditions	Sown in hot dry conditions	Sown in hot dry conditions
22/11/2003	7	First germination	Strong initial germination	Low germination numbers	Very few germinants
08/12/2003	23	Some washing out of reps and drying (die off) out of some plots	Some dying/drying out of plots	Wash out of 3 plots	Increase in germinations in Rep 2 Plot 6
22/12/2003	37				First inflorescence
05/01/2004	51	Flowering with new germination 	Establishment of tillers 	Flowering with new germination 	All plots now germinating 
13/01/2004	59	Development of tillers <0.1m long, continued flowering. Strong tillering, networks across soil surface 	Strong tillering, many per plant, more new germinations plus a dense covering of quadrats 	Abundant seed drop from first inflorescences, some tillering 	Drying out of mature seedheads, new germinations still occurring, continued development of inflorescences and many culms per single clump, but no tillering 
31/01/2004	77	Strong branching, new flowers and tillers <0.5 m 	First appearance of new inflorescences 	Seed dropping, new inflorescences, well-developed tillers 	Some plants senescence, Rep 1 Plot 8 has dense inflorescence on short clumps 

Date	Days	*Brachyachne convergens*	*Chloris gayana*	*Chloris pectinata*	*Iseilema vaginiflorum*
13/03/2004	119	Abundant seed drop, senescence of older, fruited plants 	Tillers/stolons >2 m long Abundant flowering 		Dropping seed as older culms/inflorescence begin to turn red. Stunted adventitious roots on culm joints. New germinations still occurring. 
12/04/2004	149	Senescence of plants, laying down in direction of wind to form thatch cover 	Senescence of old culms and invasion into other species plots such as *Iseilema vaginiflorum* 	Senescence 	Senescence of mature fruited culms and invasion from *Chloris gayana* 
02/07/2004	230	Thick thatch as surface cover 		Thatching over plots 	Some laying down of culms to form cover over plots 
18/10/2004	338	Rain 47.4 mm over 4 days	Rain 47.4 mm over 4 days	Rain 47.4 mm over 4 days	Rain 47.4 mm over 4 days

Date	Days	*Brachyachne convergens*	*Chloris gayana*	*Chloris pectinata*	*Iseilema vaginiflorum*
23/10/2004	343	New germinants 		New germinants 	New germinants 
31/10/2004	351	New germinants 	Few new germinants, as plots still densely covered by this perennial 	New germinants 	New germinants 
08/12/2004	389	Severe die off due to hot dry conditions interspersed by periods of light rainfall 		Severe die off due to hot dry conditions interspersed by periods of light rainfall 	Severe die off due to hot dry conditions interspersed by periods of light rainfall 

4.4.3 Soil seedbank

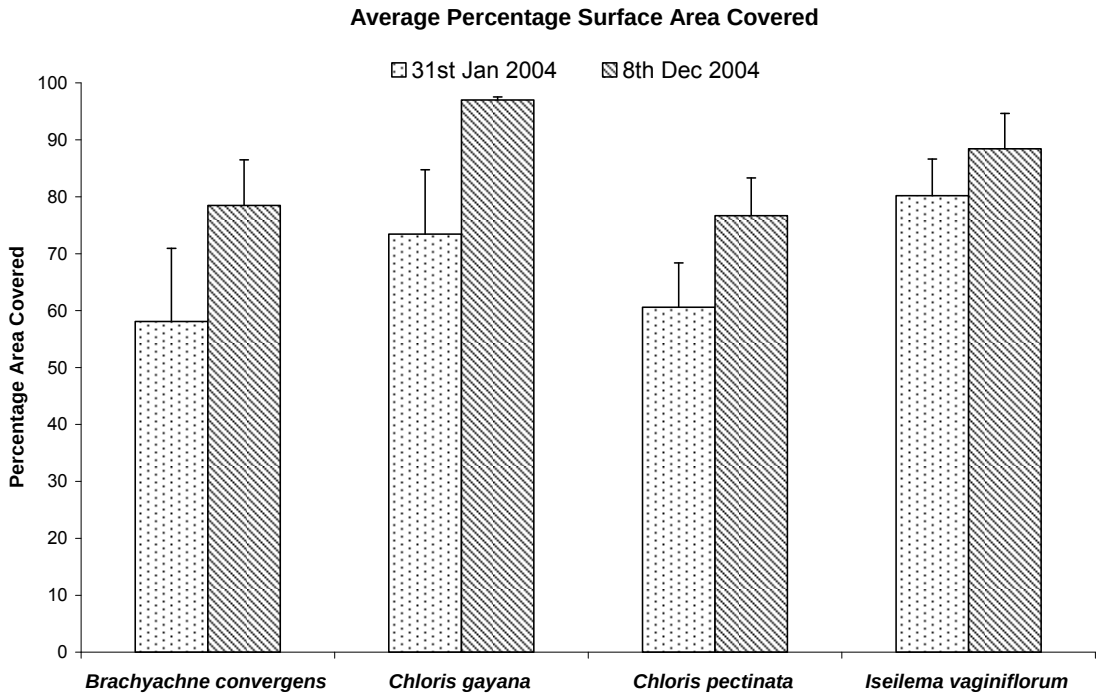


Figure 4.7 Percentage surface area covered

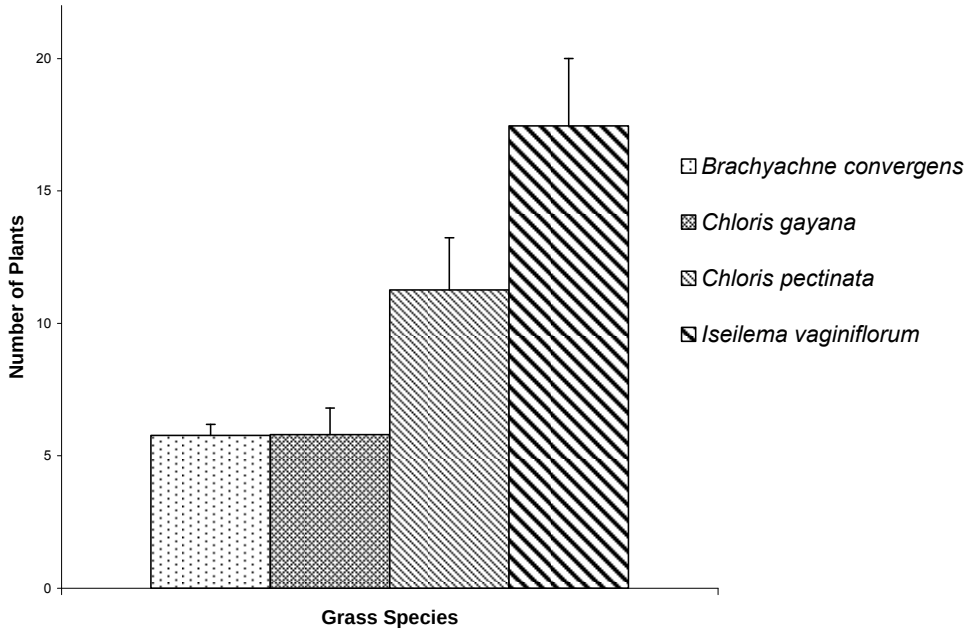


Figure 4.8 Mean number of plants per m^2 (with error bars).

Figure 4.8 shows the mean number of plants per m^2 at 120 days after sowing. By this time the original plants were mature and many individual plants had seeded several times. *Iseilema vaginiflorum* had the highest number of individual plants per m^2 due

to its tufted habit. *Chloris gayana* had the lowest number of individual plants, it also showed the most prolific growth. There was little difference between the number of plants per m^2 for *Brachyachne convergens* and *Chloris gayana* both having a strong spreading habit.

4.5 Discussion

The purpose of this trial was to determine which, if any, of the three annual indigenous species of grasses could germinate quickly, spread out over a bare soil surface and provide a dense, yet short (hence not requiring slashing) cover on a stabilised batter slope of the landfill cover.

Figure 4.4 demonstrates the trait of the native species to respond to a wetting stimulus with staggered germinations, in as much that only a small proportion of seed (*Brachyachne convergens* responded with a larger portion than the other two native species) germinated in response to initial stimuli. Rather there was a slow response curve as these species are all from the Northern Territory, where monsoonal conditions are similar to Rockhampton in that intermittent rains between times of high temperatures precede the normal monsoonal rains. In this current experiment, *Chloris gayana* responded to the wetting and showed quick germination, only to die off under the hot and dry conditions. *Brachyachne convergens* also demonstrated a quick response, though not so dramatic as *Chloris gayana,* and experienced withering. *Iseilema vaginiflorum* showed the most stable response to wetting, achieving the highest number of plants per m^2.

Also important is the production of stolons and the ability of the plant to spread vegetatively. Figure 4.9 shows the stoloniferous habit of *Chloris gayana*.

Figure 4.9 *Chloris gayana* spreads itself effectively using stolons

As a perennial, *Chloris gayana* does not die off annually, and its habit produces a dense, tall cover over the batters. Light levels are so low at the soil surface, that germination does not occur unless the top has been clipped and light is permitted to reach the ground. This offers the species a large seedbank of seeds ready to germinate should the parent plants be lost in a fire climax event, or have been over-grazed. However, in terms of vegetative propagation, *Chloris gayana* is the most efficient of the four species trialled. There was no significant difference the number of stolons produced between *Chloris gayana*, *Brachyachne convergens* and *Chloris pectinata* (Figure 4.6). It was noted that *Brachyachne convergens* formed a network across the soil surface (Figure 4.11), whereas *Chloris pectinata* did not produce this same surface-stabilising mesh.

Figure 4.10 (a) growth habit of *Iseilema vaginiflorum* - note the small amount of erosion around the base of the plant and (b) cover provided by *Chloris pectinata* (1)(foreground) and *Iseilema vaginiflorum* (2)(mid-ground)

Figure 4.11 Surface cover by *Brachyachne convergens*

Iseilema vaginiflorum did produce a significantly greater number of branches per culm than did the other species, which resulted in its forming a dense cover over the batters in its plots. However, as Figure 4.10 shows, *Iseilema vaginiflorum* is a tufted grass and offers no immediate erosion protection in the event of heavy rainfall.

In terms of maturation and seed drop, Table 4.2 records the development of each species, and it is interesting to note the speed at which *Chloris pectinata* matured to its seed drop phase. This seed would then be available for a second generation of germination should optimal conditions present themselves, otherwise, the seed will enter a cycle of dormancy. This is, however, speculation for at this stage studies such as this need to be conducted for the majority of our native species.

It is clear in Table 4.2 that *Chloris gayana* was very slow in developing and maturing seedheads. This was noticed during data collection on the development of seedheads, that the seedheads of *Chloris gayana* that formed within the dense canopy took a much longer time to develop and mature before dropping seed. This is in agreement with findings by other researchers that seedheads that developed in shadow of the canopy took longer to reach maturity (Adkins and Armstrong 2005).

The three indigenous species are annuals, and died off in autumn of 2004, covering the batter surface with a layer of vegetative matter that resembled roofing thatch. This offered protection both to the slope and to seeds buried beneath the thatch. It has been mentioned in Chapters 2 and 3 that *Brachyachne convergens* does have an after-ripening period, so subsequent germinations later in the season should have been the result of the sown seed rather than the seed dropped in and around mid-March (Table 4.2). An efficient thatched cover will protect the seeds while they undergo their after-ripening period.

When rainfall began again in October 2004 there were abundant new germinants recorded for the indigenous species compared with the few counted in the (still) densely covered *Chloris gayana* plots. Once again this can be due to the seeds needing to respond to a change in light stimulus and respond if the competition from the parent plants is removed.

The traits that these grasses have demonstrated are important in determining their use for long-term stabilisation management. *Brachyachne convergens'* stoloniferous habit and fibrous roots make it ideal for surface stabilisation and the reduction of erosion. The thick thatch produced by the laying over of senescing culms provides safe shelter for seeds as they after-ripen and the same time acts as a mulch to prevent the germination of competing species.

Chloris gayana also has these traits, however, its height and fuel loads will make it expensive to maintain. Its extraction of extra nutrients from the soil to maintain its growth habit will also add to costs. These are serious drawbacks for its use in mixed species sowing, whereby grasses are sown with trees and shrubs in order to quickly stabilise the soil while the larger species have time to grow (Tekle and Bekele 2000). Attempts to utilise *Chloris gayana* in this manner would result in the non-germination of seeds of the other species in the mix, the competition for soil nutrients, and the high fuel levels resulting in hotter fires with a higher scorch line, effectively killing off the other species that may have managed to establish themselves (Griffin and Friedel 1984; Mott and Andrew 1985; Grice and Slatter 1996).

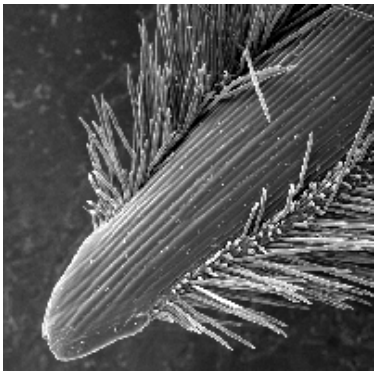
4.6 Conclusion

In the final analysis, it is desirable to utilise indigenous species of grasses for any rehabilitation work, be it stabilisation of batters or restoring denuded pasture lands. This is particularly so when mixed seed combinations of trees, shrubs and grasses are used. Based on the photographic evidence from the trial, *Chloris gayana* was too aggressive for the smaller grasses trialled. Of the indigenous species, *Iseilema vaginiflorum* proved to be unsuitable for slope stabilisation, at least in the early stages of growth, and this is directly due to its clumping habit. *Chloris pectinata* and *Brachyachne convergens* produced roughly the same amount of stolons, however,

Brachyachne convergens has the advantage in that its stolons formed a network mesh over the soil surface.

Having demonstrated its ability to establish itself in extreme conditions (see Chapters 2 and 5), *Brachyachne convergens* would be the recommended species for slope stabilisation of batters. It is important that initial establishment steps be modified (such as increasing the sowing rate to provide an earlier and more dense cover) to prevent other species dominating it and, in turn, ensure build up of the soil seed bank.

Chapter 5
Scanning Electron Microscopy to Determine the Effects of Seed Ageing upon Tropical Australian Grass Species under Varied Storage Conditions



5.1 Abstract

Eight seedlots comprised of seven different species (two seedlots of one species) stored in four storage conditions (Cool Room, Air-conditioning, Freezer and Shed storage) were examined under a scanning electron microscope for any external morphological differences caused by different storage treatments. The results showed no clear differences between the storage treatments, indicating that the effects are internal and so biochemical in nature; however, the study has provided a database of images pertinent to taxonomical or morphological studies.

5.2 Introduction

Unaided, the human eye has a resolution, the ability to define distinct objects, of around 75 *μm*, or roughly the thickness of a strand of human hair (Jenkins and White 1976). Objects or features smaller than this will appear indistinct or blurred together, and give the impression of a smooth surface. Magnification, via the use of lenses, enables the viewing of objects with a greater definition of detail, as the object, to the viewer, is enlarged (Watt 1997). An ordinary magnifying glass (x10 magnification) is

a simple microscope; when various shaped prisms, lenses and mirrors are organised into a compound microscope, a far greater degree of magnification (x400+) of an object is achieved (Brown 1978).

Nevertheless, compound microscopes do have limitations as to the degree of magnification they can produce, as the resolution of an object is dependent on the wavelengths of light reflected from the surface of, or transmitted through, an object or specimen. Visible light has a wavelength range of approximately 400 *ηm* to 700 *ηm*, and so a compound light microscope will have a resolving power of approximately 200 *ηm*. This means that small viruses, cell membrane structures and cell surface features cannot be visualised using a light microscope as a resolving power of at least 50 *ηm* (or x20,000 magnification) is required (Flegler et al. 1993).

Thompson in 1897 discovered that negatively charged particles (electrons) with a tiny mass could be discharged from an incandescent metallic filament and be directed through a small hole by the use of a positively charged anode and suitably arranged electric and magnetic fields. The higher the voltage used to produce the electron, the higher the electron's energy and so, the higher its velocity (Watt 1997). In 1924, de Brolgie discovered that electrons display a dual nature and could be considered as a moving charged particle, or, be associated with wavelength. Once again, the higher the voltage used to discharge the electron the greater the energy and the shorter the wavelength. An electron produced with a 100 keV supply will have a wavelength of 0.0037 *ηm* (Watt 1997).

Transmission electron microscopes (TEM) utilise these features and, when using a 1.0 MV voltage accelerator, can direct (using magnetic and electrostatic lenses) a stream of electrons through a sample in a vacuum chamber with a resolution of 0.10 *ηm* (Flegler et al. 1993). The TEM will produce high-resolution images of cell

membranes structure, viruses, phages, DNA, or localise elements within a sample (such as proteins or enzymes), however, the samples must be specially and carefully prepared so that in the preparation of the sample there will be no alteration of the structures being examined. Samples are sliced into ultra-thin sections of 0.02 *μm* - 1 *μm* thickness and stained in solutions containing heavy metals that are absorbed into specialised tissues and have a high-electron-scattering capability thus allowing clearer specimen detail (Watt 1997).

Scanning Electron Microscopes (SEM) work on a slightly different principle whereby the width of the electron beam being directed onto the sample surface is the most important factor in the determination of the viewing resolution (Flegler et al. 1993). Basically, in an SEM, an electron gun produces a stream of electrons that are attracted by an anode and pass through a double condenser lens (electromagnetic) system and are focussed onto the surface of the sample specimen by an electromagnetic objective lens. A set of scan coils within the objective lens are provided with a set sequence of varying voltage which, in turn, causes the electron stream to cycle over the surface of the sample. At the same time, the same variable voltage is being supplied to a cathode-ray tube (CRT) which imitates the scanning pattern. When the electron stream strikes the sample, the electrons will penetrate a short distance into the sample as the individual electrons interact with the atoms in the sample. As the electrons are deflected they will have both lost energy and have changed direction. An array of collectors in the SEM detect the energy and angle of the scattered electrons, the information of which is transferred to the CRT where a three dimensional image is produced (Flegler et al. 1993; Watt 1997).

Samples for examination are mounted on stubs (either aluminium or carbon), usually up to 12.5 *mm* in diameter with the sample being up to 3 *mm* thick. A sample of this

size will be able to have a 360^0 horizontal rotation with 75^0 to 90^0 tilt from the vertical axis. The size of samples to be scanned is limited by the size of the specimen chamber, and the degree of rotation needed for specimens' examination (Watt 1997).

When the electron beam is focussed on the sample, some of the electrons are scattered from the sample and collected. The remaining electrons need to be able to leak through the sample and stub, lest the specimen build up charge, as does a capacitor. To facilitate efficient electrical discharge of non-conductive specimens, samples are coated (2 *ηm* - 14 *ηm* thick) with a metal such as gold or palladium. Not only will this coating discharge effectively, but will also reflect electrons more efficiently, allowing for an enhanced imaging of the sample surface features (Watt 1997).

With the exception of "wet" SEM mounts (Barshack et al. 2004) which examine fully hydrated samples at atmospheric pressure, SEM samples need to be dehydrated and devoid of solvents or other materials that could vaporise in the vacuum in the specimen chamber and so contaminate the column, or interfere with the emission and collection of electrons. Drying procedures for biological samples include air-drying, freeze-drying or dehydration using alcohols or acetones, followed by critical-point drying in a liquid CO_2 chamber (Flegler et al. 1993).

The use of SEM is known to be of value in determining taxonomic differences for identification of closely related plants (Watanabe et al. 1999) as mature dried seeds may be mounted directly onto stubs and splutter coated with no drying preparation required (Garnock-Jones 1991). However, aside from data presented by Tieu that compares the effects of acids and enzymes that have been applied to seeds with respect to natural seed ageing effects, little research has been published on the use of SEM to examine the effects of ageing on seed coats and husks (Tieu, 2001).

Seeds develop from a fertilised ovule and have an outer coat called a testa and an inner coat called a tegument both of which are formed from ovular integuments (Tran and Cavanagh 1984). Therefore, the seed coat is composed primarily of the layers of the integument surrounding the embryo. However, layers of the nucellus and cells of the endosperm can become part of the seed coat composition.

The fruit of grasses of the family Poaceae are classed as a caryopsis, which is an indehiscent dry fruit in which the layers of the integument and the pericarp are fused together to form the seed coat (Whalley 1987; Fahn 1990). The embryo, which is small relative to the seed size, is usually laterally positioned to one side of the seed in deference to a large starchy endosperm (Baskin and Baskin 1998a). The seed will be laterally compressed with the hilum (the scar on the seed where the ovule was attached via the funiculus to the parent plant) on the opposite side to the embryo. Some seeds may have differently shaped or aligned hilums (Degano et al. 1997; Wheeler et al. 2002).

The seed surface may be smooth or granular, and the seed will be enclosed within a floret made up of an overlapping palea and lemma. The florets in turn may be enclosed in glumes. Both florets and glumes may be thick, keeled, ridged, ciliated, fringed or have awns (Wheeler et al. 2002), or possess unicellular or multi-cellular hairs and wax granules (Degano et al. 1997).

Seed imbibition occurs at different rates throughout different tissues in the seed and the permeability of the seed coat will be greater in some areas than others (Mayer and Poljakoff-Mayber 1989). It is known that changes in the thickness of the seed coat can also be noticed in the flattening of ridges or surface features on the seeds of species such as *Cicer reticulatum* (Ladiz.)(Murray 1984).

Impermeability of a seed coat to water is a known form of seed coat imposed dormancy (Adkins et al. 2001). *Triodia* species are recognised to have a seed coat imposed dormancy (Wells et al. 1999) as trials showed increased germination in *T basedowii* when the pericarp in the vicinity of the embryo was punctured/pierced. Trials have also shown that germination response of *Triodia bitextura* increased over time and varied between different storage conditions (Fesuk and Ashwath 2004); however, to date no external SEM examination of the seed coat surface has been undertaken for any native grass seeds undergoing storage treatments to explain treatment differences in germinability.

Some research has been undertaken to determine that seed death due to ageing does occur (Baskin and Baskin 1998a). It has also been shown that the tissues which envelope seeds may also function in the parallel capacity as mechanisms conditioning dormancy (both physical and chemical)(Tran and Cavanagh 1984; Hopkins 1996).

Chaffy seeds have the added protection of a husk (lemma, palea and glumes) that buffers against variable climatic conditions. For example, the presence of the husk inhibits water access to the caryopsis if the seed is exposed to a shower of rain followed by dry weather. The advantage of this is the caryopsis will only begin imbibition if the rainfall is substantial. The callus hairs on these chaffy seeds have been observed to act as straw siphons bringing moisture at a controlled rate through to the caryopsis (Paterson et al. 2001).

The removal of the palea and lemma resulted in a positive germination response of *Echinochloa turneriana* (Domin) J.M.Black*,* yet the storage life of the seed is extended when the caryopsis is left within the husk. This is an indication that the husk inhibits germination of the seed (Whalley 1987). In other trials it was found that

oxygen uptake by glumellae, as in *Hordeum vulgare* (L.) also resulted in seeds remaining dormant (Khan 1977; Mayer and Poljakoff-Mayber 1989; Rodriguez et al. 2001).

A change in the glume structure of *Themeda triandra* was discovered from an SEM examination of seeds exposed to heat and smoke. Micrographs clearly showed the dissolved wax plugs in the glume pores following such treatment (Paterson et al. 2001). In this case it was heat more so than smoke that unblocked the glume pores, which is relevant in the event of moisture imbibition or gaseous exchange.

In summary, SEM examination of seed coats has been undertaken both for taxonomical purposes and to study the effect of scarification and other germination treatments. Seed coat impermeability is linked to the palisade layer of the seed coat the thickness of which will vary over different parts of the seed coat. Research is yet to be published on the effects that different storage regimes will have on the external morphological features of the seed coat.

5.3 Materials and Methods

Aristida inaequiglumis, Astrebla squarrosa, Brachyachne convergens, Chloris pectinata, Dichanthium sericeum (2000 and 2001 seedlots), *Vacoparis macrospermum* and *Triodia bitextura* seeds were mounted on aluminium stubs and then sputter-coated using a Fisions VG Microtech Polaron SEM Coating System (Model SC515) which was evacuated (using a Edwards High Vacuum pump Model E2M5) for three minutes with a current of 15 mA, voltage of 1.1 kV at a pressure of 0.15 mbar/Pa, to a cover depth of approximately 15 *ηm*.

The seeds had been in 4 storage conditions for 4 years (with the exception of *Dichanthium sericeum* 2000 and *Astrebla squarrosa* which had been in prior ambient temperature storage for 5 years and 9 years respectively).

After coating, the seeds were examined using a JEOL JSM-6360LA analytical scanning electron microscope. Surface features such as the embryo region, embryo region surface cells, funicular and general surface cell areas were examined for each species at similar magnifications that ranged from 30x to 2 000x.

5.4 Results

Observations are presented separately for each species.

Aristida inaequiglumis: - No visible surface variations were observed due to seeds being tightly encased by the outer glumes (Figure 5.1).

Astrebla squarrosa:- A distinct difference in the desiccation of the embryonic testa cells was evident when compared with the relatively smooth testa cells covering the rest of the seed. This is an indication that imbibition could initially occur in the embryonic region of the seed. Shed storage samples had large amount of fungi present on seed, Cool room treatment had some fungal growth present and Freezer storage very little fungal growth evident at all. No other differences between the storage samples were evident (Figure 5.2).

Brachyachne convergens: - No distinct differences in the appearance of the testa between any of the storage treatments were noted (Figures 5.3 and 5.4).

Chloris pectinata:- Again, no distinct differences in the appearance of the testa between any of the storage treatments were noted (Figure 5.5).

Dichanthium sericeum (2000 and 2001 seedlots):- Neither the caryopsis nor the palea/lemma showed any differences either between storage treatments or between

the 2000 and the 2001 seedlots. Pores present on the glume surfaces in both seedlots were intact, for all treatments had evidence of slight cracking of the seed surface over the embryo; however, this may have also been due to handling of the seed while removing the palea and lemma (Figures 5.6 and 5.7).

Triodia bitextura:- Seeds of this species had a thick layer covering the outer testa cells; this was particularly evident with the Freezer storage specimens which showed underlying desiccated cells. These seeds also showed intact pores on the glume surfaces. (Figures 5.8 – 5.10).

Vacoparis macrospermum:- The Shed storage treatment had fungal infestation with the presence of hyphae networked across the seed surface. This feature was not observed on seeds of the other storage treatments (Figure 5.11).

5.5 Discussion

Seed dormancy may be as a result of either chemical influence (in seed tissue surrounding the embryo, in the seed coat cells or as a result of chemicals present within the embryo itself) or due to mechanical influences. Mechanical or coat-imposed dormancy occurs as a result of the seed coat inhibiting water or gas uptake, modifying light that could stimulate the embryo, or merely acting as a mechanical restraint. Or, indeed, dormancy could be, and often is a combination of these various factors (Bewley and Black 1994; Desai 2004).

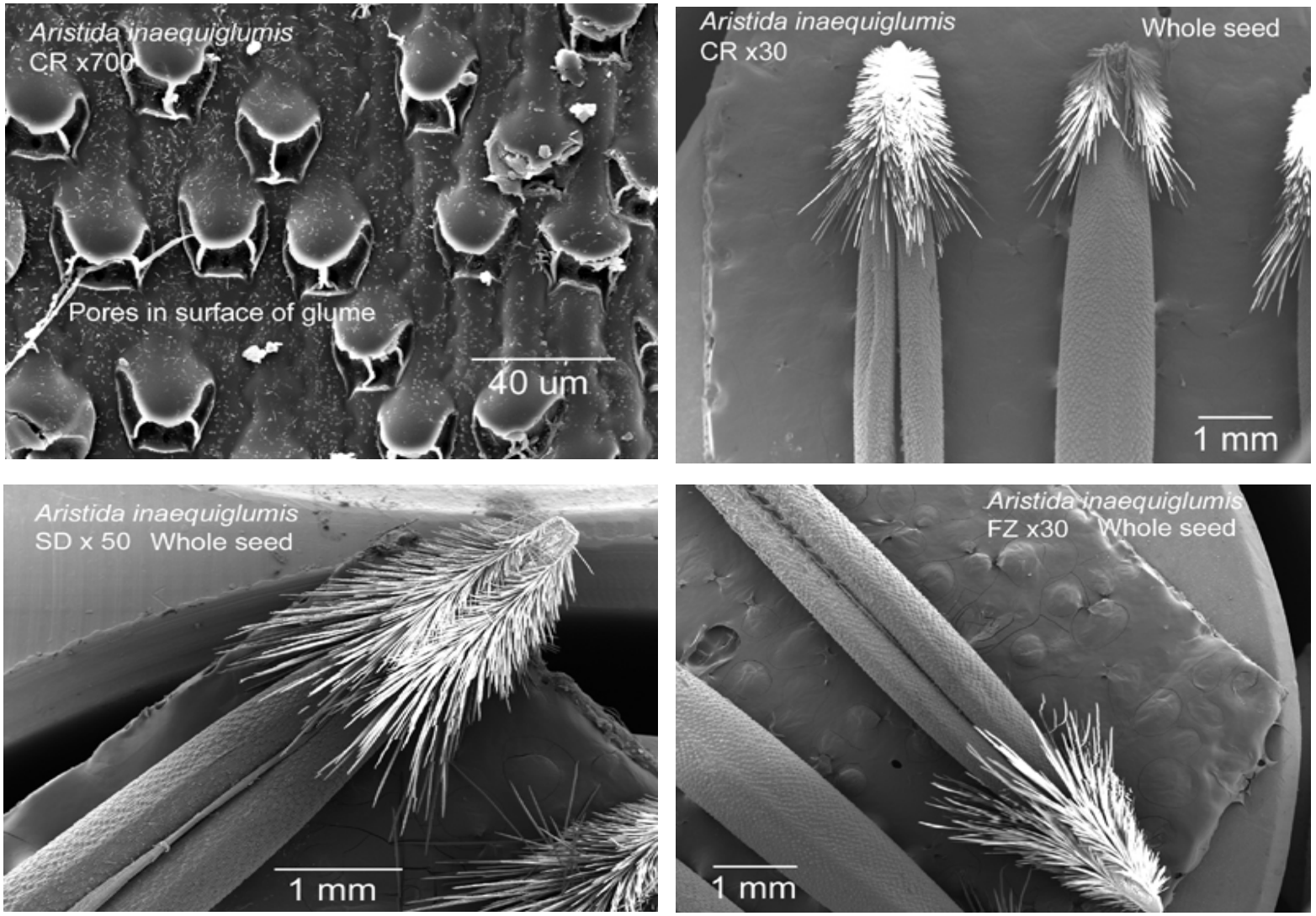


Figure 5.1 Comparison of whole seed of *Aristida inaequiglumis* and micrograph of surface pores present in *Aristida* glumes. Note in all micrographs CR = Cool Room, AC = Air-conditioning, FZ = Freezer and SD = Shed storage treatments.

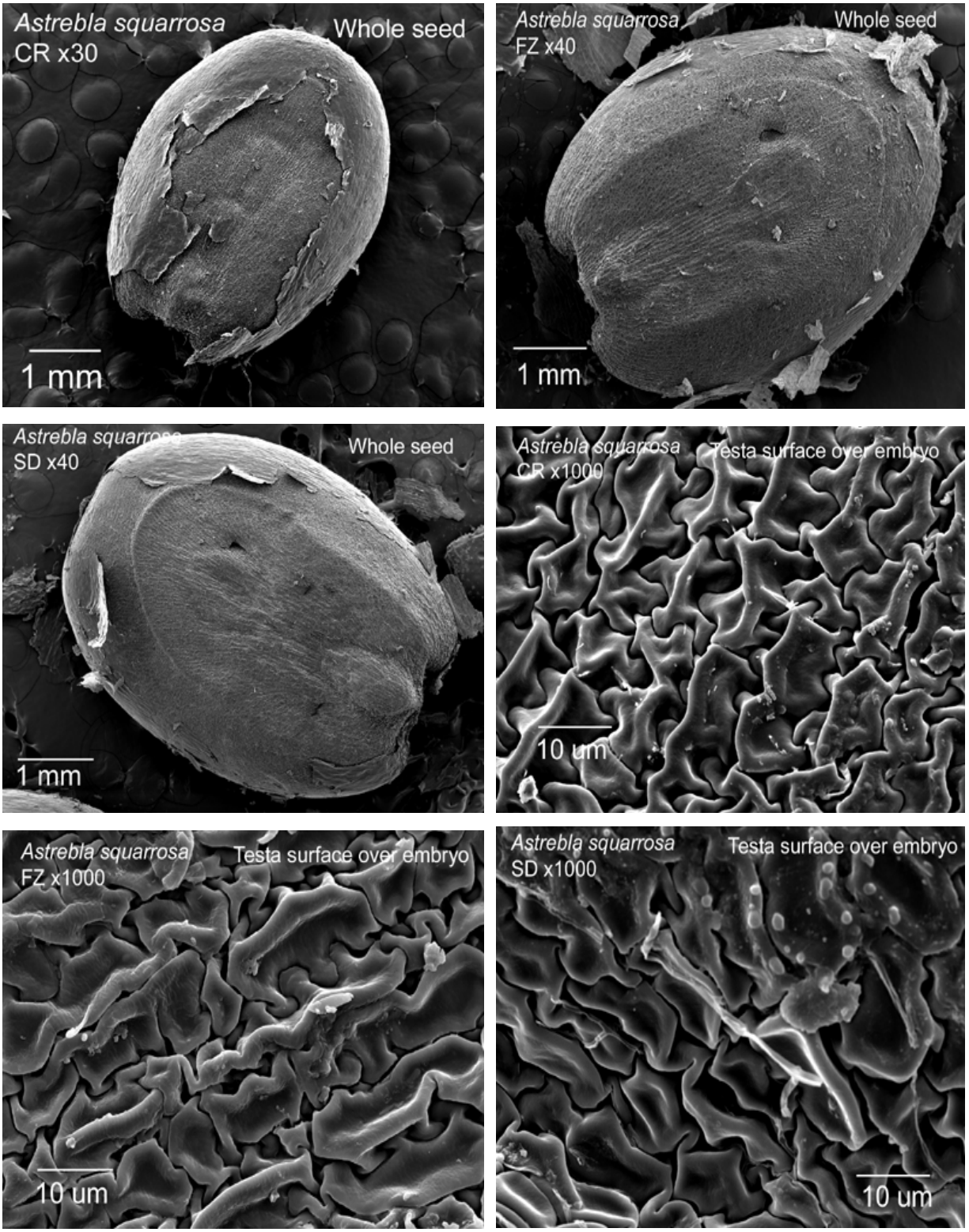


Figure 5.2 Micrographs of the whole caryopsis of *Astrebla squarrosa*. Note that the peeled membrane occurred on removal of caryopsis from husk. Close view of reticulated cellular pattern. Although there are no structural differences there is variation in the degree of fungal infestation.

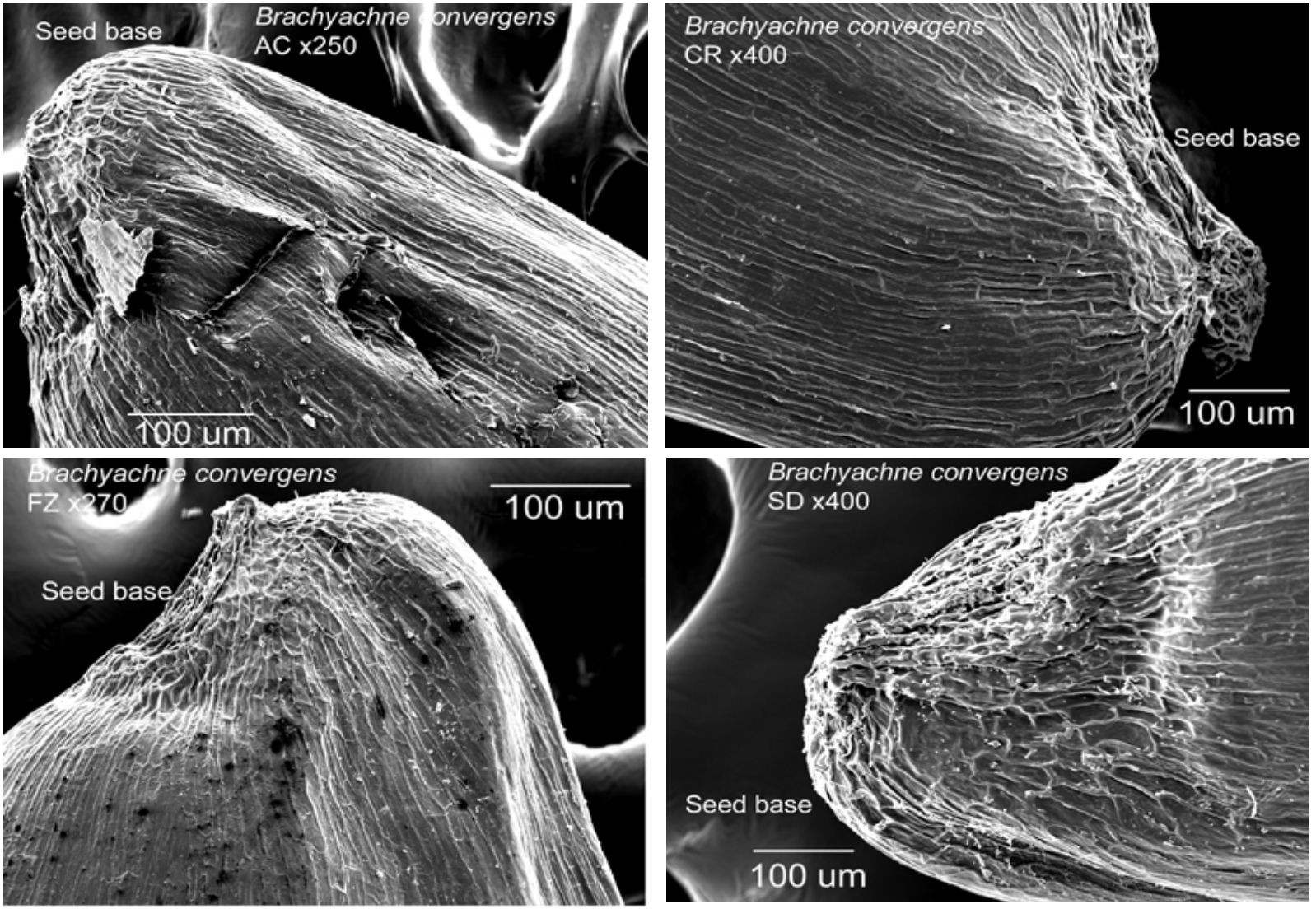


Figure 5.3 Micrographs of the bases of *Brachyachne convergens* seed.

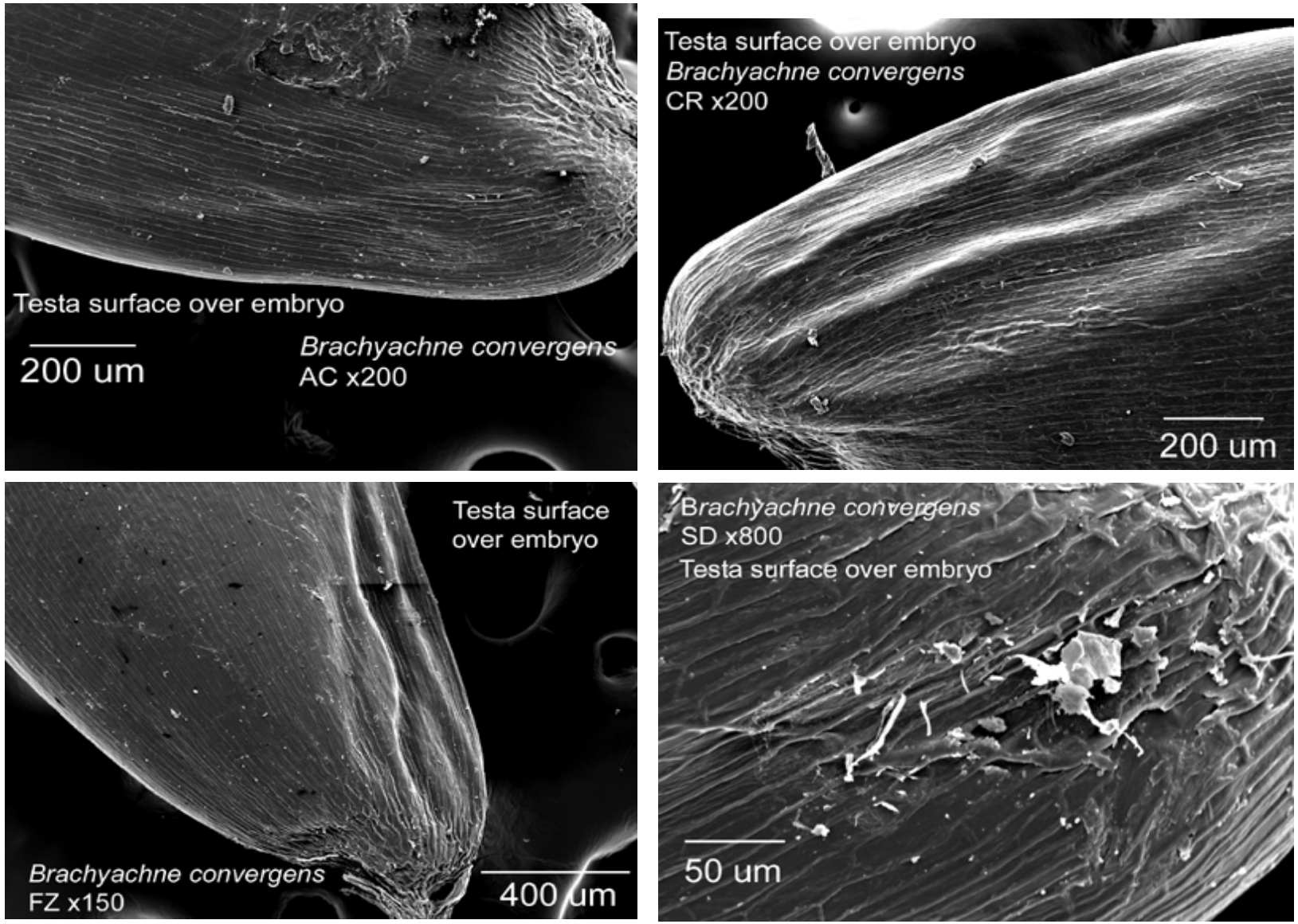


Figure 5.4 Micrographs of the surface cells over the embryos of *Brachyachne convergens* seed.

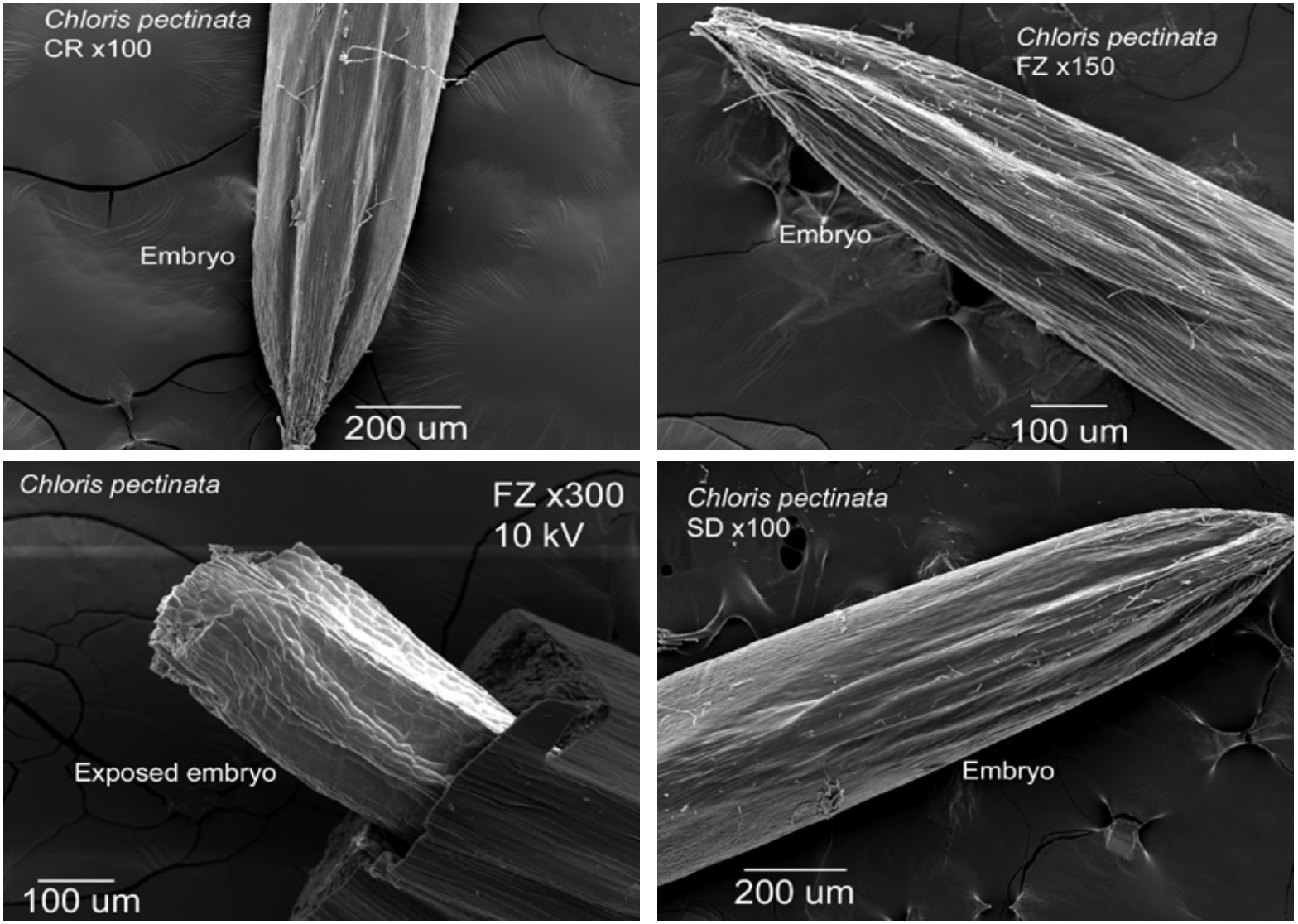


Figure 5.5 Micrographs of the embryo ends of *Chloris pectinata* seed and the exposed embryo of a freezer treatment seed.

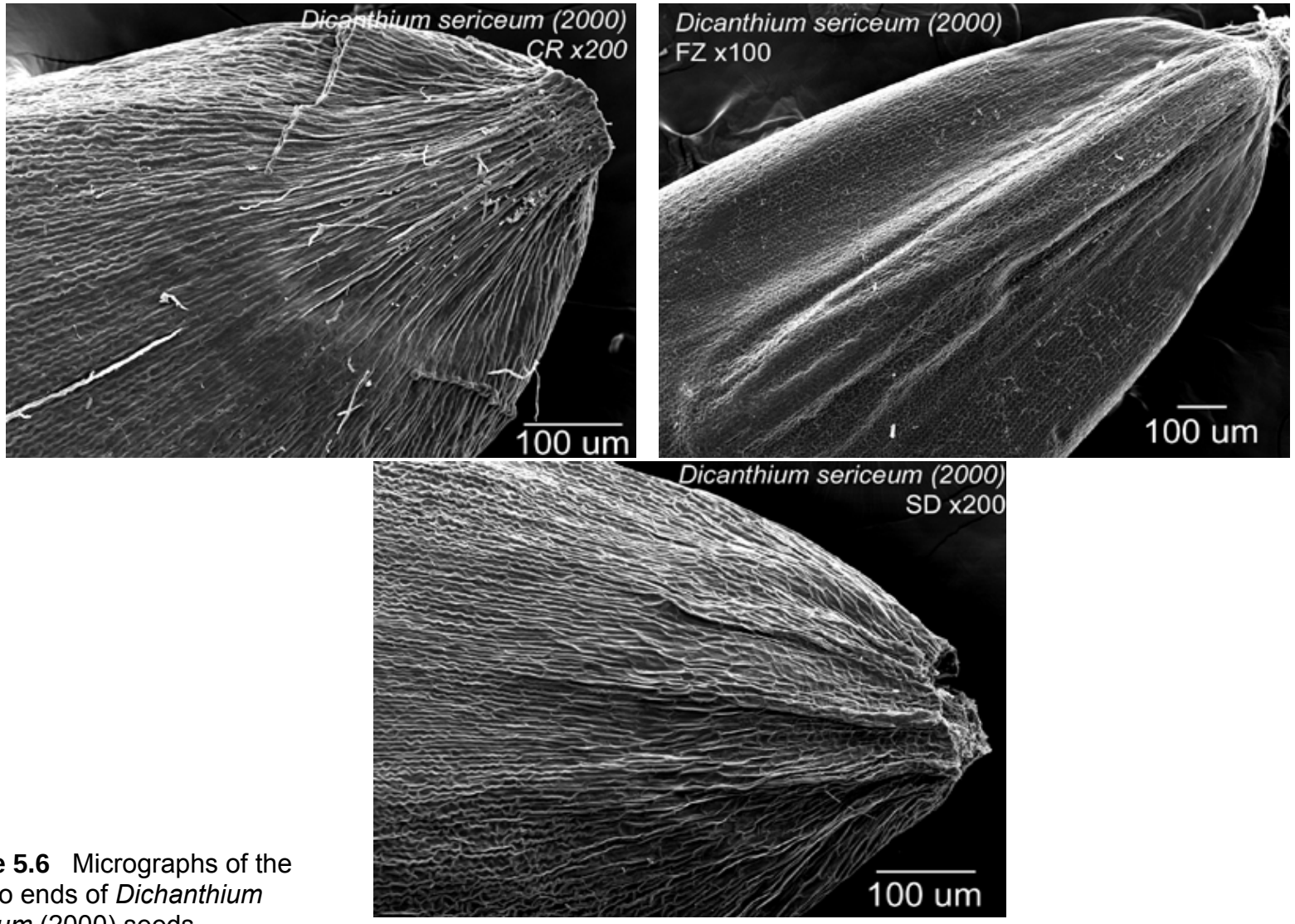


Figure 5.6 Micrographs of the embryo ends of *Dichanthium sericeum* (2000) seeds

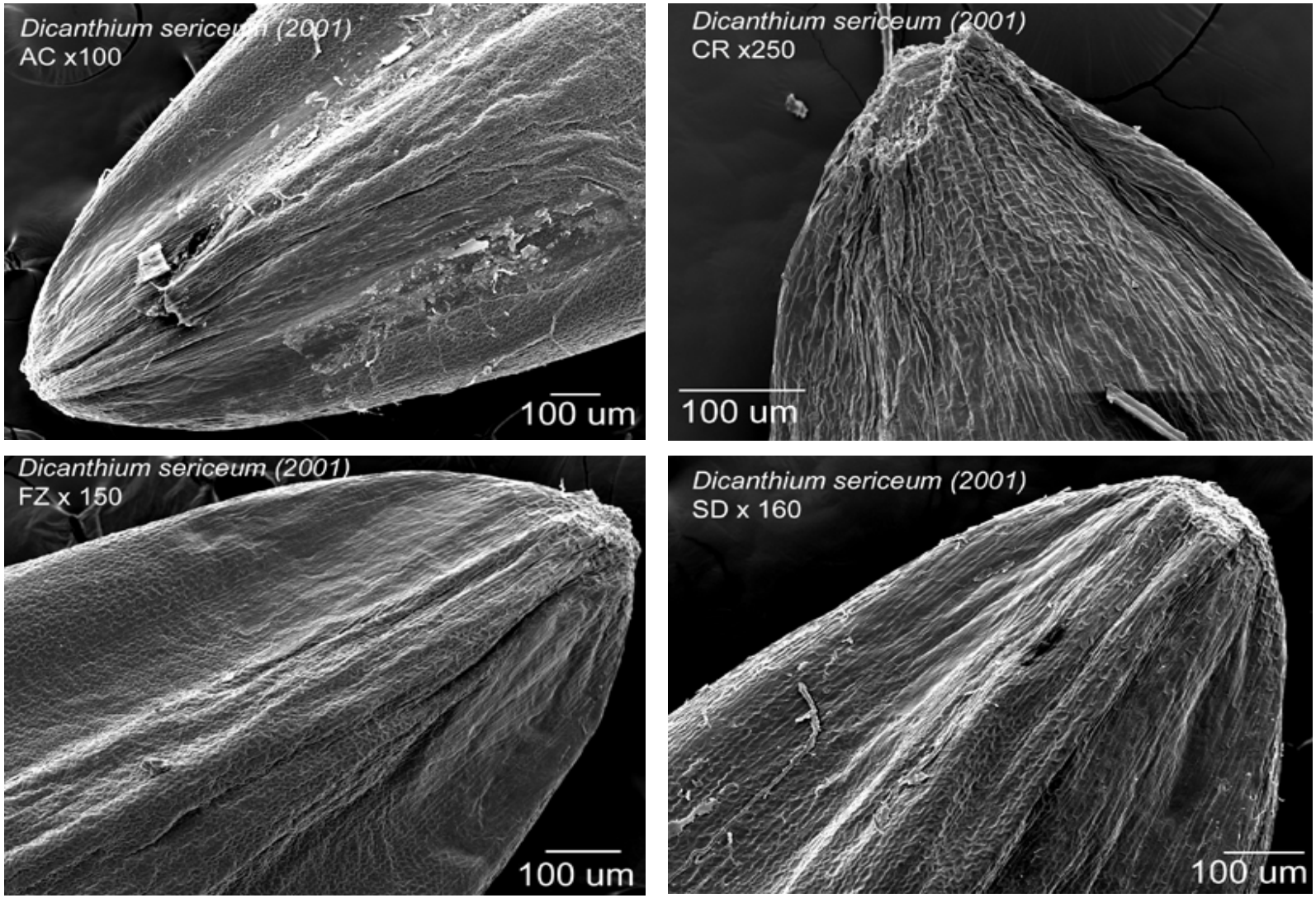


Figure 5.7 Micrographs of the embryo ends of *Dichanthium sericeum* (2001) seeds

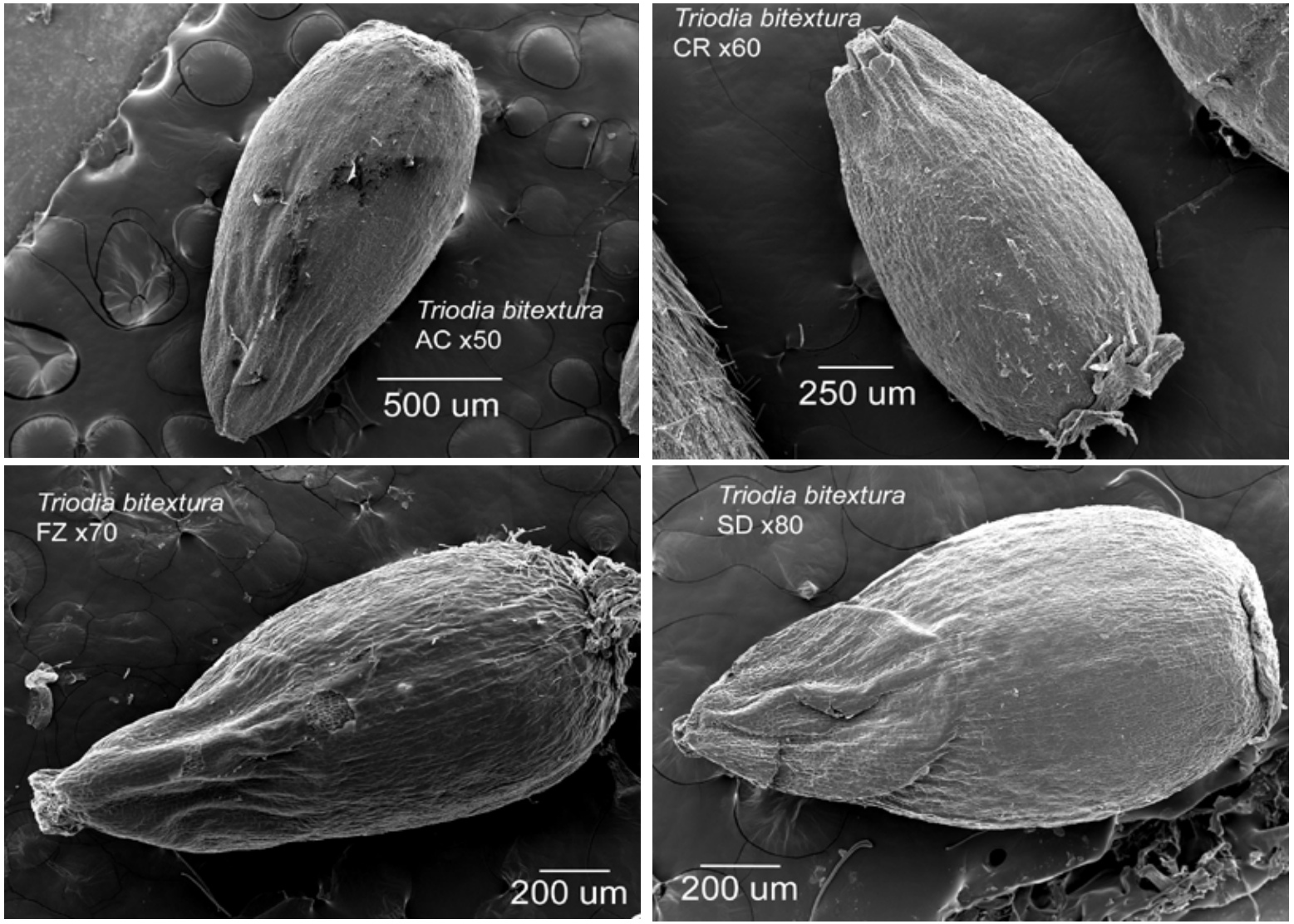


Figure 5.8 Micrographs of the entire caryopsis of *Triodia bitextura* seed

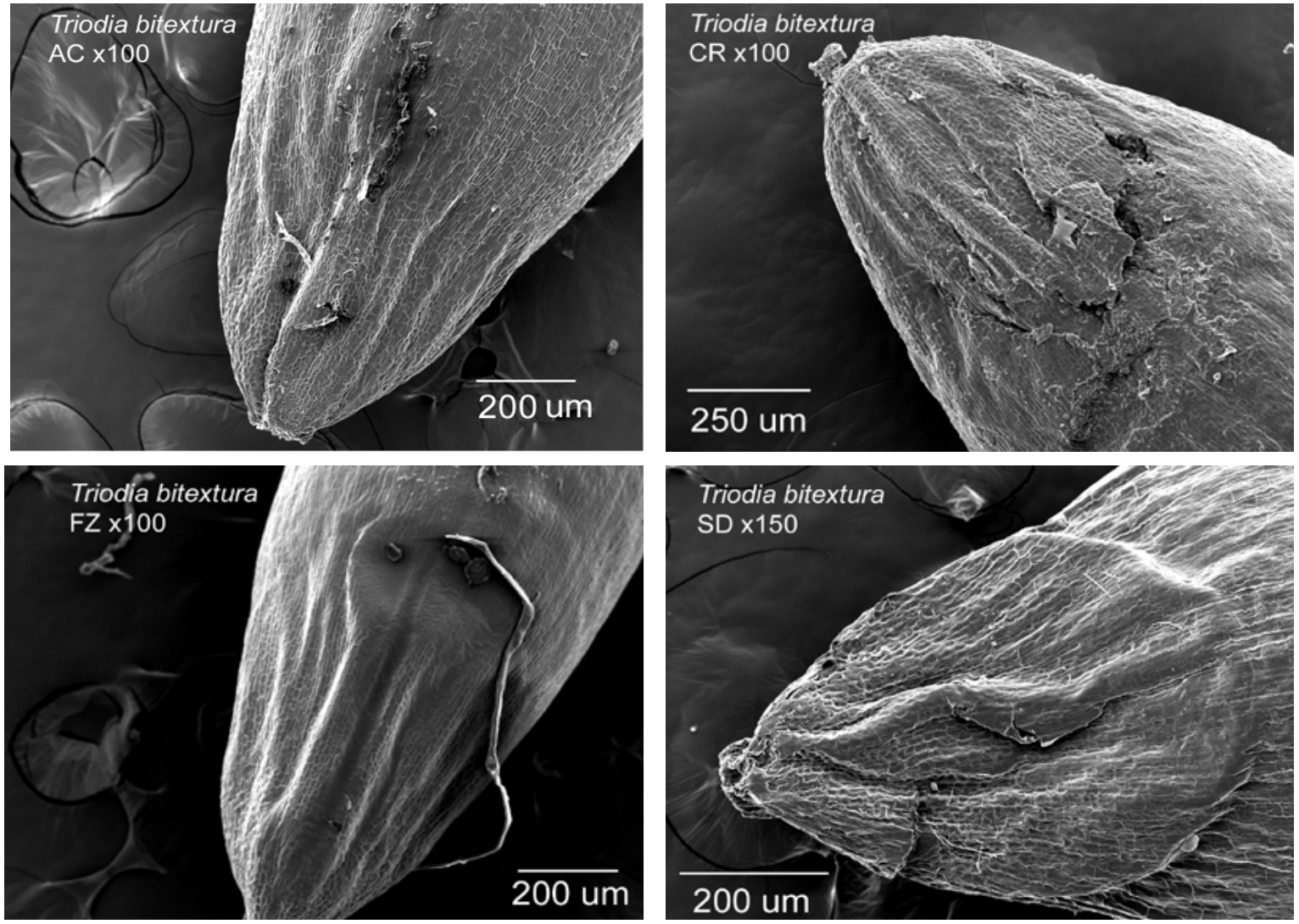


Figure 5.9 Micrographs of the embryo end of the caryopsis of *Triodia bitextura* seed

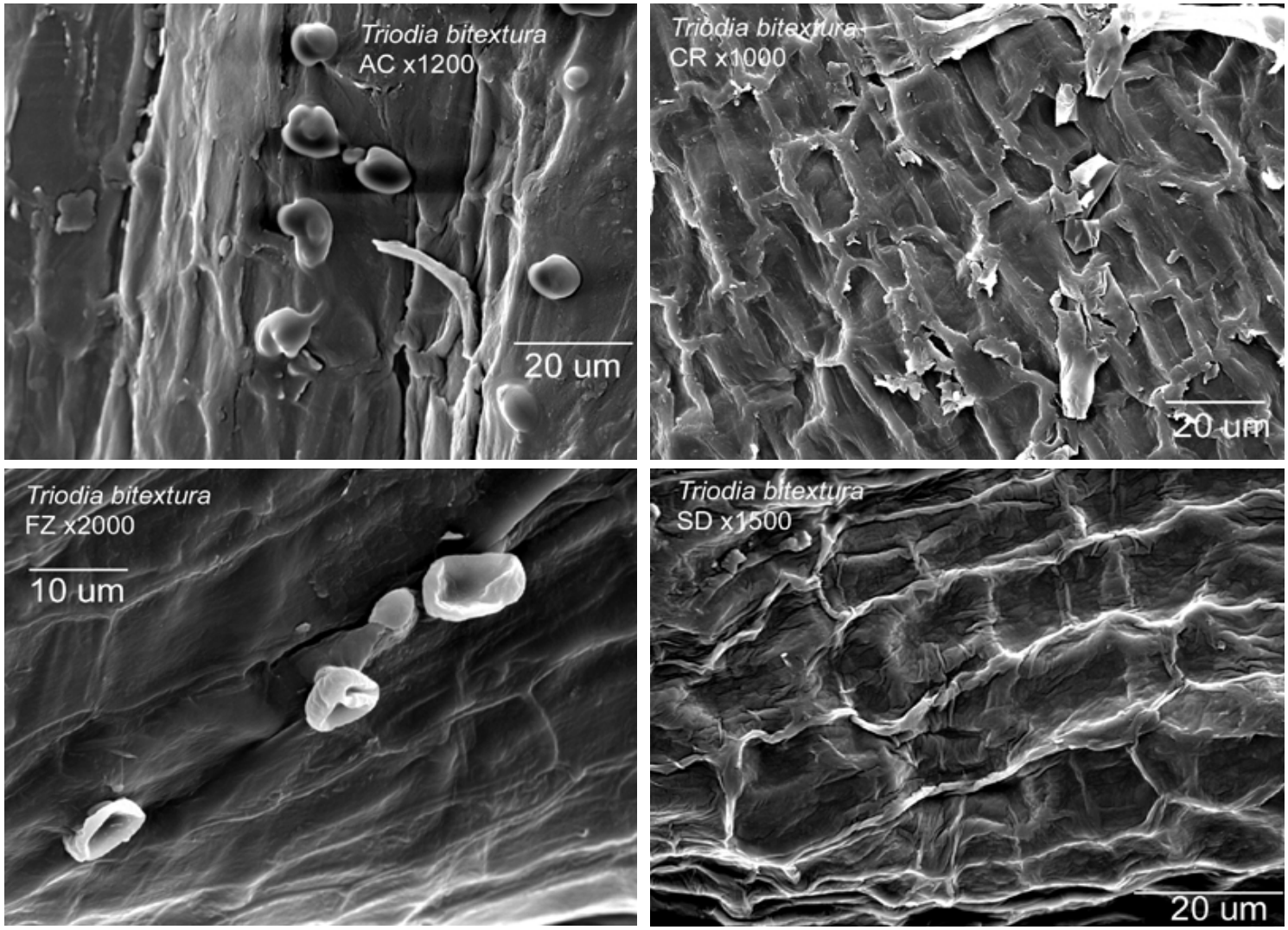


Figure 5.10 Micrographs of the surface cells over the embryo region of *Triodia bitextura* seed. AC and FZ images may be displaying sites of fungal infestation.

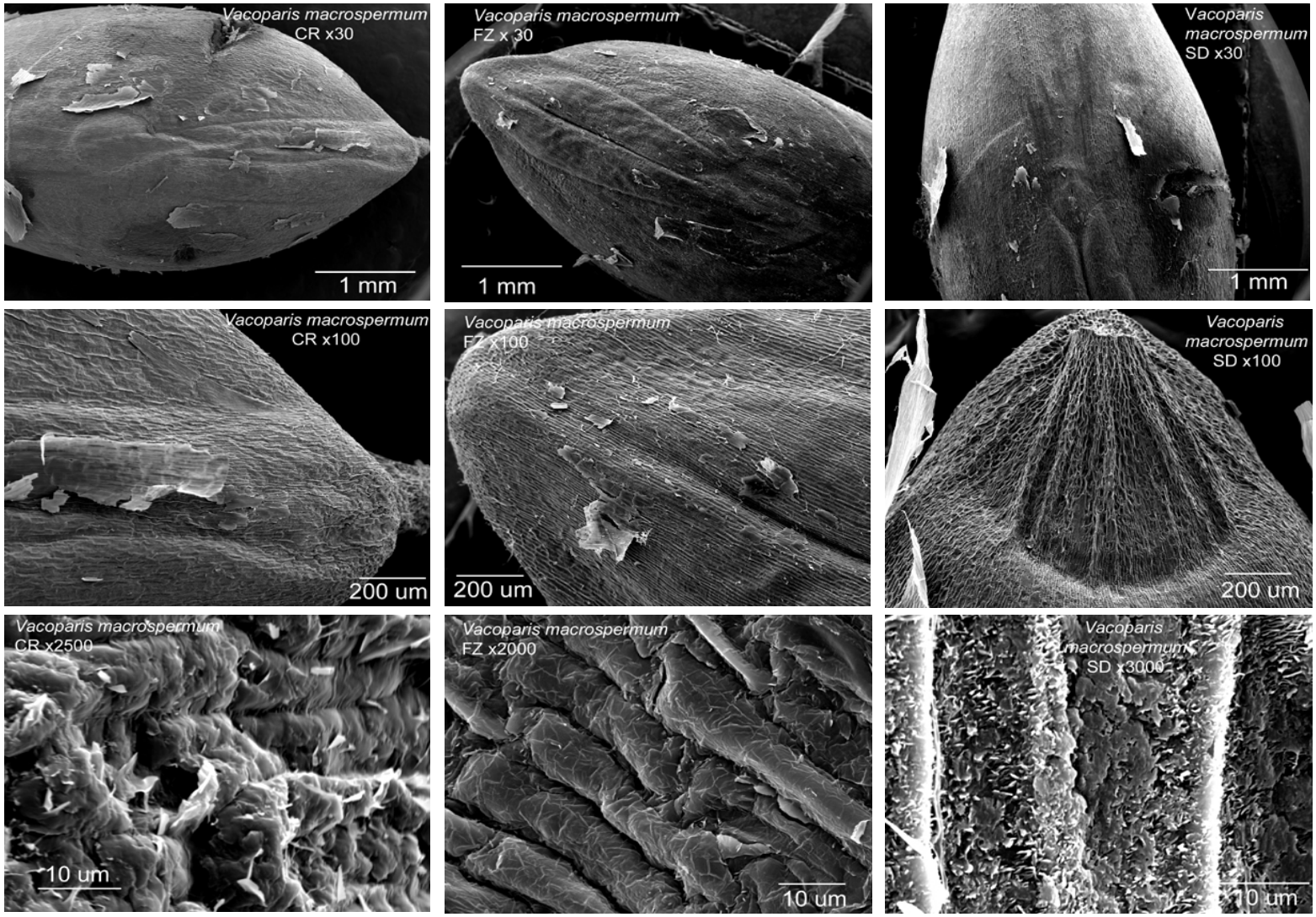


Figure 5.11 Micrographs of *Vacoparis macrospermum* showing whole caryopsis, enlargement of embryo end of seed and testa surface cells that lie over the embryo region of the caryopsis.

Accordingly, the integrity of the seed coat is of the utmost importance in maintaining the viability of the seed, and not allowing it to age as a result of oxidation. It is also crucial to keep the seed from fungal or bacterial and maintain protein and starch reserves within the seed.

Overall, the testa, or seed coats of the examined samples remained intact, unless small cracks were made in the surface of the seeds whilst being handled with forceps. Some treatments did show signs of fungal infestation, which could result in the fungus breaking down starch reserves and raising the ratio of fatty acids within cells, resulting in the non-synthesis of proteins and loss of seed viability (Maguire 1977). *Vacoparis macrospermum* (formerly *Sorghum macrospermum*) in past trials (Chapter 2) demonstrated a positive germination response to hot storage and it is still unknown as to whether or not this fungal presence is detrimental to this species, or whether it will prove to be a symbiotic relationship and stimulate seed germination.

Often the caryopsis is afforded protection by being enveloped in glumes, or tightly bound within the palea and lemma as in *Aristida* spp. (Figures 5.1 and 5.12). In this instance an additional mechanical and often chemical dormancy is imposed on the seed as the glume often contains germination-inhibiting chemicals. Or, as for S*porobolus* spp. and *Oryza sativa* (L.), a protein matrix that becomes mucilaginous upon contact with water restricting the diffusion of oxygen to the embryo and so halt development of the embryo (Desai 2004).

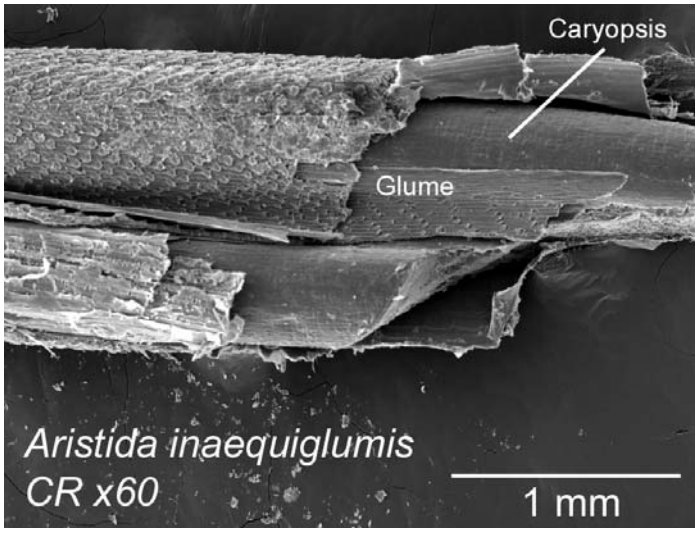


Figure 5.12 Broken seed illustrating the effectiveness of the glumes covering the caryopsis of *Aristida inaequiglumis*

The glumes, however, will have some physical constraint that may break down over time, or be affected by environmental conditions such as heat. In the case of *Dichanthium sericeum* (Figure 5.13) the glumes have wax plugged pores that may be susceptible to the heat from bushfires. Testing will be required to see if heat causes the wax to melt and expose the caryopsis to oxygen. Germination is further stimulated by the directing of water to the embryo region of the seed via the hollow hairs extruding from the surface of the glumes that siphon and channel water directly to the seed (Paterson et al. 2001). *Dichanthium sericeum* also has backward facing bristles at the base of the seed have a dual role as aerofoils, directing the vertical fall of the seed, and as anchor points for the lodgement of the seed in soil or leaf-litter microsites as the seed is being pushed by the unravelling of the seed hygroscopic awns (Peart 1981).

5.6 Conclusion

Examination of the surface areas of seeds in stored in different conditions provided a valuable database with respect to the taxonomy of these species, possible methods or mechanisms of germination and insight into the effects of various storage treatments on these species. This in itself is useful in that

there were no apparent external storage effects the surface features may be used in taxonomical studies as have the surface features of *Opuntia, Phyllanthus, Petunia* and *Lycopersicon esculentum* (Degano et al. 1997; Watanabe et al. 1999; Chakrabarti et al. 2003; Chaudhary and Khan 2003). Studies of which have focused on seed surface features as defined by SEM examination.

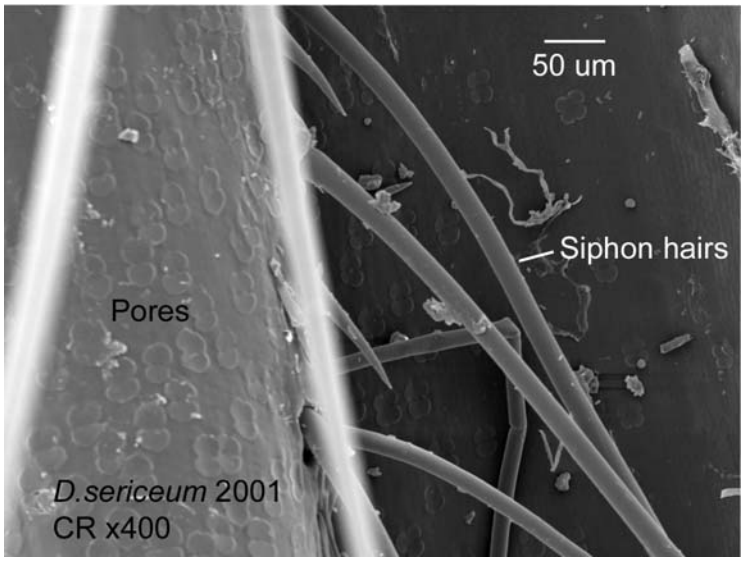
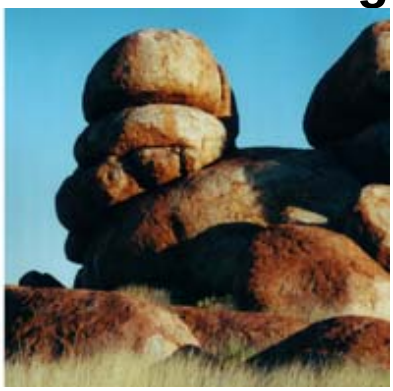


Figure 5.13 - Pores and siphon hairs on surface of *Dichanthium sericeum* seed

The lack of differences in features of the seed coat for different storage conditions suggests that any storage effects will be internal, either structural or biochemical, and so require other means of quantification, such as enzyme analysis, or as reported in Chapter 6, an analytical profiling of the proteins present in the seeds with respect to the storage treatments.

Chapter 6
Analysis of *Triodia bitextura* Seed Proteins via Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis as a Response to Various Storage Conditions



6.1 Abstract

Seeds of *Triodia bitextura* (Lazarides) were taken from four storage treatments and examined using 1D SDS-PAGE at the beginning and at 12 hourly intervals to 36 hours of imbibition. Results showed that Shed storage treatments had a detrimental effect on macro-proteins within the seed which in turn inhibited germination.

6.2 Introduction

Electrophoresis is the separation of macromolecules through a matrix by means of an electric field. Proteins are macromolecules composed primarily of a string (or strings) of amino acids also known as polypeptides.

In shape, proteins may be generally separated into globular and repeated folding structural groups. Globular proteins have convoluted and complex tertiary and quaternary structures folded around a hydrophobic axis and are capable of long-range interactions with other molecules. Repeating structures are composed of tandem blocks of 20 – 40 amino acids that form elongated

non-globular structures. They undergo regular interactions with other molecules, are able to be readily mutated by extension, shortening, deletion, insertion or duplication of blocks of the repeated amino acids (Main et al. 2005).

The molecular weight of a protein (measured in Daltons (D) or kilo Daltons (kD)) is as a result of the number and type (or sequence) of amino acids in its core polypeptide chain (Main et al. 2005).

Seeds contain a large variety of proteins in the form of storage proteins and proteolytic and hydrolytic enzymes (Mayer and Poljakoff-Mayber 1989). The storage proteins are located in the aleurone layer and in protein bodies in the starchy endosperm with lesser amounts in the scutellum and the embryonic axis with each specific protein essentially acting as a storage reserve of amino acids. They are broadly classified into four major groups, albumins, globulins, prolamins and glutelins, on the basis of their solubility, although these groups may contain otherwise unrelated proteins (Bewley and Black 1994; Müntz 1998).

One of the earliest events in the germination of seed is the breaking down of the storage proteins by enzyme reactions with the subsequent synthesis of new proteins and enzymes required for embryonic growth. The germination process is accompanied by a synthesis or evolution of different types of proteins present (Ketring 1977; Müntz 1998).

Both seed germination and vigour are directly affected by storage conditions in some native tropical grass species (Fesuk and Ashwath 2004), which accordingly will result in a change of metabolic activity and so a change in the ratio of the proteins that are present in the seed. This can include the

beneficial after-ripening of seeds, the rate of which is noted as increasing in proportion to an increase in the storage temperature. However, this current study was conducted with respect to long term storage of seeds. As seed quality decreases, there will also be an increase in the activity of hydrolytic enzymes with a subsequent decrease in proteins and a subsequent increase in fatty acids (Maguire 1977; Sharif-Zadeh and Murdoch 2001).

The process of electrophoresis is preceded by the lysis of the cells of the sample being tested to expose or free the native (natural) proteins that are in the cell membranes and storage bodies followed by their subsequent denaturising and conversion to a solute and by means of a sample buffer solution. The negatively-charged soluble proteins are then loaded into a well set into a polyacrylamide gel immersed in a buffer solution between two electrodes. A current is run for a time and intensity that will vary according to the type of electrophoresis that is being undertaken with a resultant migration of the proteins through the gel matrix (Caprette 1996).

Polyacrylamide gels provide a matrix through which the protein molecules can migrate. The rate at which the proteins move through the matrix is limited by the size of the pores in the gel and the molecular weight and magnitude of the charge of the molecule. The pore size of the gel is dictated by the percentage of acrylamide that is present in the gel, and so, the greater the acrylamide percentage, the smaller the pore size. A medium concentration gel (12%) will incorporate proteins of sequence length from 190 to 1900 amino acids or 20 to 2000 kDa and so allow the formation of banding causes by like sized proteins grouping as they progress at the same rate through the gel (Caprette 1996; EnCor Biotechnology 2005).

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) may be carried out within one dimension (1D wherein the protein samples move in a straight line along the gel), or in two-dimensions (2D whereby the proteins initially run along the gel, then are run at right angles to further differentiate the clusters of proteins along the 1D track) or with gels that increase percentage composition of acrylamide (and in so doing restrict the rate of movement of the smaller weighted proteins).

The current trials were conducted using 1D PAGE with protein gel markers (PGM) to allow for the approximate calculation of the molecular weight of the seed proteins. The presence or absence of proteins in some storage treatment sample that differ to other storage treatments in the same run indicates that the treatment has altered the nature and ratio of proteins present in the seeds during progressive stages of imbibition and so give an insight into the viability of the seed (Caprette 1996; Ahmad et al. 2005).

6.3 Materials and Methods

Awns were removed from seeds of *Triodia bitextura* that had been stored under four different temperature conditions (Cool room, air-condition room, freezer and garden shed), using approximately 40 seeds per replicate per treatment. Each sample was finely ground in an Eppendorf tube with a hand stone and 250 µL of commercially prepared sample buffer was added (from a five times dilution of a 100 mL sample composed of 1M Tris/HCl (31.25 mL pH:6.8), SDS powder (10 g), Glycerol (25 mL), Bromophenol Blue (2% in ethanol – 750 µL), 2- mercaptoethanol (5 µL) and distilled water to 100 mL). The tube was agitated and then rested at room temperature for 5 mins. Tubes

were then centrifuged and the supernatant drawn off and heated in a water bath set to 95 ^{0}C (Bio-Tek 2005; Rybicki and Purves 2005).

Seeds undergoing germination/imbibition, were incubated at 25 ^{0}C, as the seeds were so small humidification was bypassed). Up to 40 seeds per storage treatment were removed for assay at 12 hours, 24 hours and 36 hours from the beginning of incubation (Nkang and Ashwath 2004). The remainder of seeds (approximately 70-80 seeds per storage treatment) were left in incubation for anecdotal observation of the resultant germination of seeds from any of the storage treatments.

Sample lots of 10 µL of supernatant from each storage treatment were micro-pipetted into three wells of a commercially prepared fifteen well 12% polyacrylamide gel that was set into an MV20 Vertical Electrophoresis apparatus using a 70/125 V dual voltage power source (Model EVT300). The electrophoresis procedure was run for approximately 1 hour and 30 minutes, using a TGS running buffer in the tank (composed of 196 mM glycine, 0.1% SDS, 50 mM Tris-HCl (pH 8.3) made by diluting a 10x stock solution)(Rybicki and Purves 2005).

Gels from the electrophoresis apparatus were removed (with the gel being notched on the lower left corner to maintain aspect and placement of reps) and placed in a fixative bath (50% methanol, 10% glacial acetic acid and 40% distilled water) for 10 minutes. The gel was then transferred to a flat surface, and stained with Protein InstaStain® (impregnated with Coomassie Blue stain) for 30 minutes (Edvotek 2000). The gel was then placed into a destaining bath (using same fixative solution) for 12 -15 hours, before being digitally photographed.

Analysis of the gels was accomplished by marking the boundaries of each protein group and by measuring the distance travelled by the various bands and establishing the relative mobility or retention factor (Rf) for each band in each storage treatment (Caprette 1996). Each storage treatment had three samples per replicate and the travelled distance was marked as being in the centre of the outer boundaries of the protein groups. Hence Figure 6.1 displays the movement of the protein groups and shows a compilation of the boundaries for each treatment (Caprette 1996).

The molecular weights of the protein groups were calculated by measuring the differences in the molecular weight of the various proteins in the PGM and then dividing those differences by the Rf of the proteins to establish a movement standard for those particular molecular weights. The molecular weights of the unknown seed proteins was then extrapolated from these data for the two PGM between which they were positioned (EnCor Biotechnology 2005). Even though the movement of the protein groups was measured using Photoshop® software and the measurement was accurate to 0.01 mm, there will be error in the results from both the centering of the movement benchmark in the protein group and the placement of the outer boundary marks for the triplet of samples per replicate. This error was not translated onto Figure 6.2 (although error bars of 5% have been inserted) as it was the absence of large protein groups in the initial stages of imbibition that have clearly demonstrated the effect of the storage regime.

6.4 Results and Discussion

The mobility of protein groups is illustrated in Figure 6.2 with each assay showing the collective results of three samples (In the form of “I “outlining the

outer boundaries of the three sample groups) per storage treatments run on two separately prepared gels. The bold circles for the shed storage conditions indicate the largest protein molecule present in the seeds of that treatment. Note that they are smaller than the largest protein molecules from the other cooler storage treatments.

The molecular weight with respect to mobility is illustrated in Figure 6.3 for each of the assays. It was noted that of the seeds left in incubation, the Air condition, Cool room and Freezer storage treatments all had germinants (in the range of five to ten percent) while nil germinants were noted for the Shed storage seeds after 14 days.

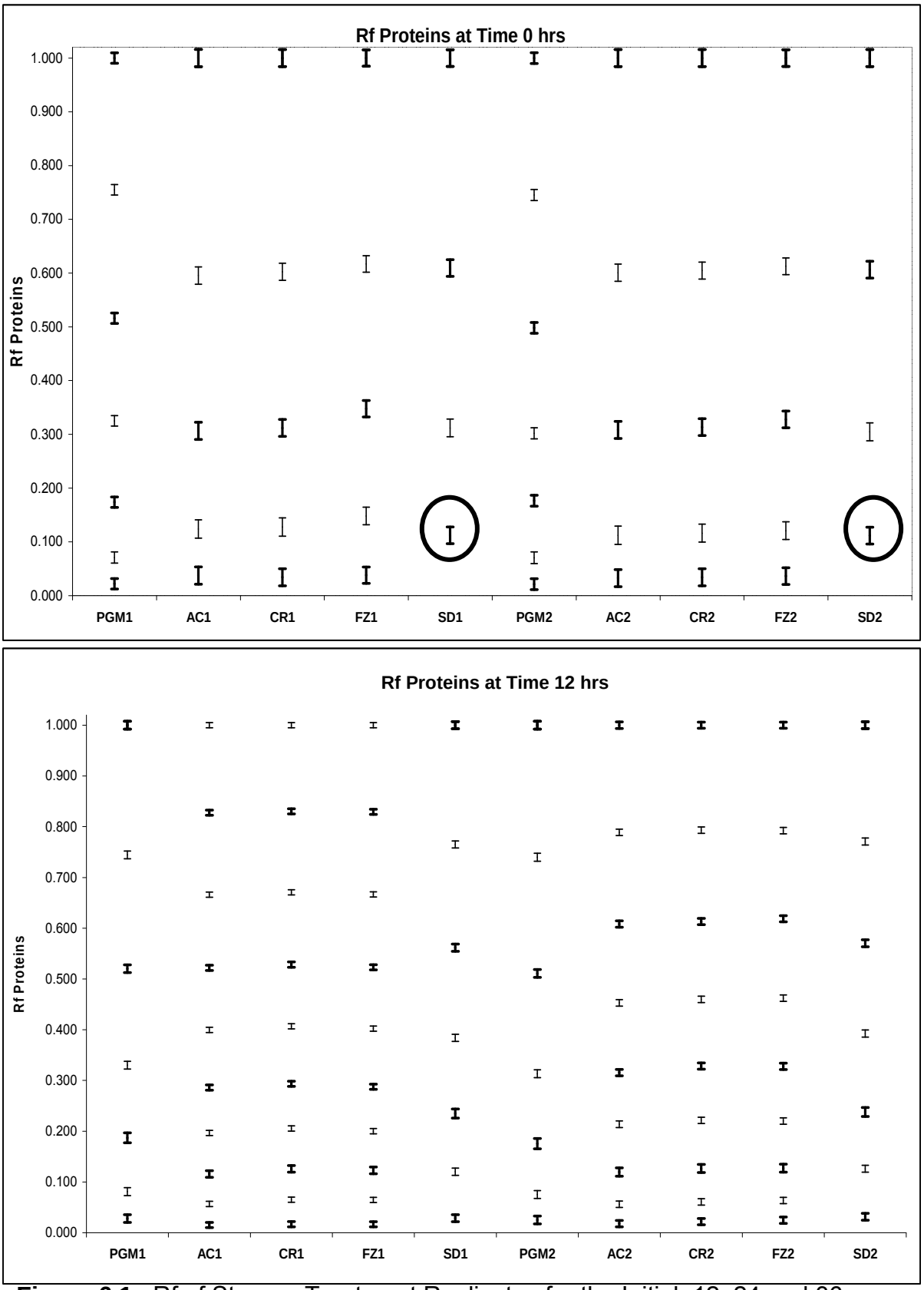
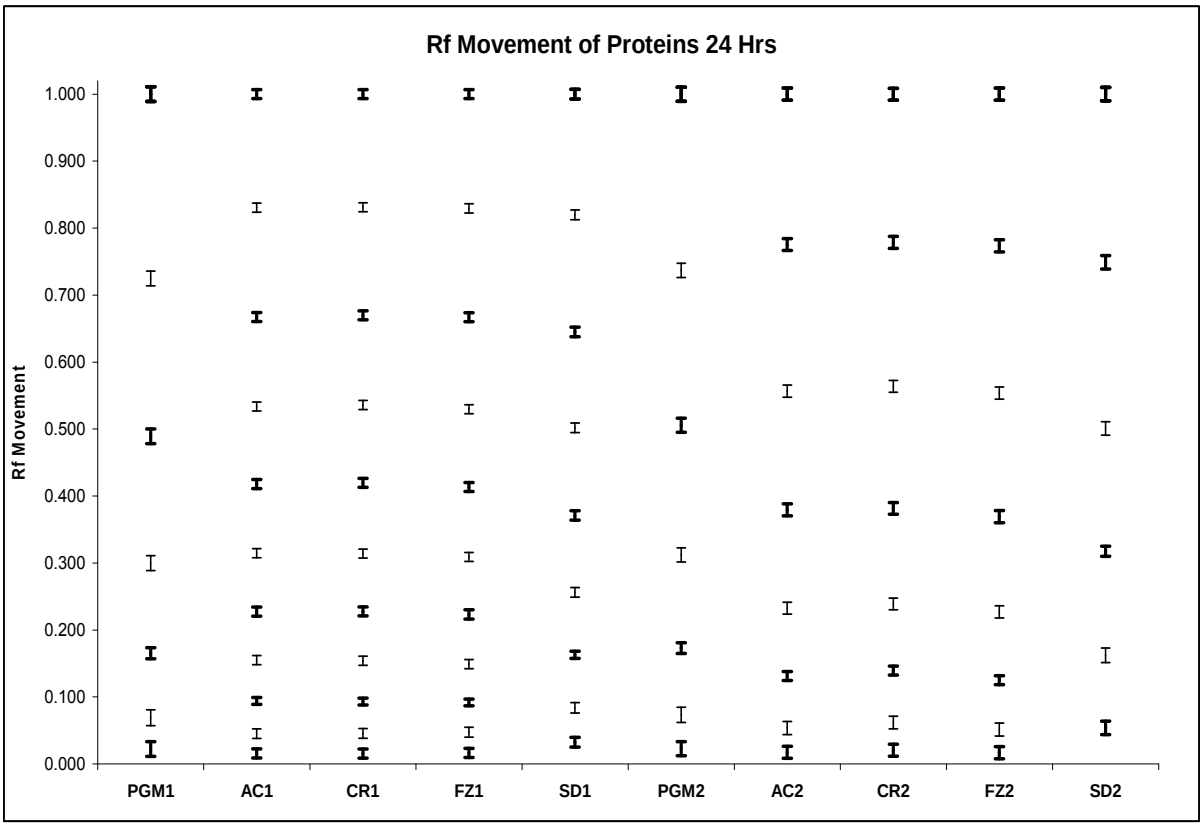


Figure 6.1 Rf of Storage Treatment Replicates for the Initial, 12, 24 and 36 hour Assays. The bold markings allow easier differentiation between groups as they progressed along the gel. The numbers 1 or 2 represent the replicates for each assay, PGM – Protein Gel Marker, AC – Air Conditioning, CR – Cool Room, FZ – Freezer and SD – Shed storage treatments



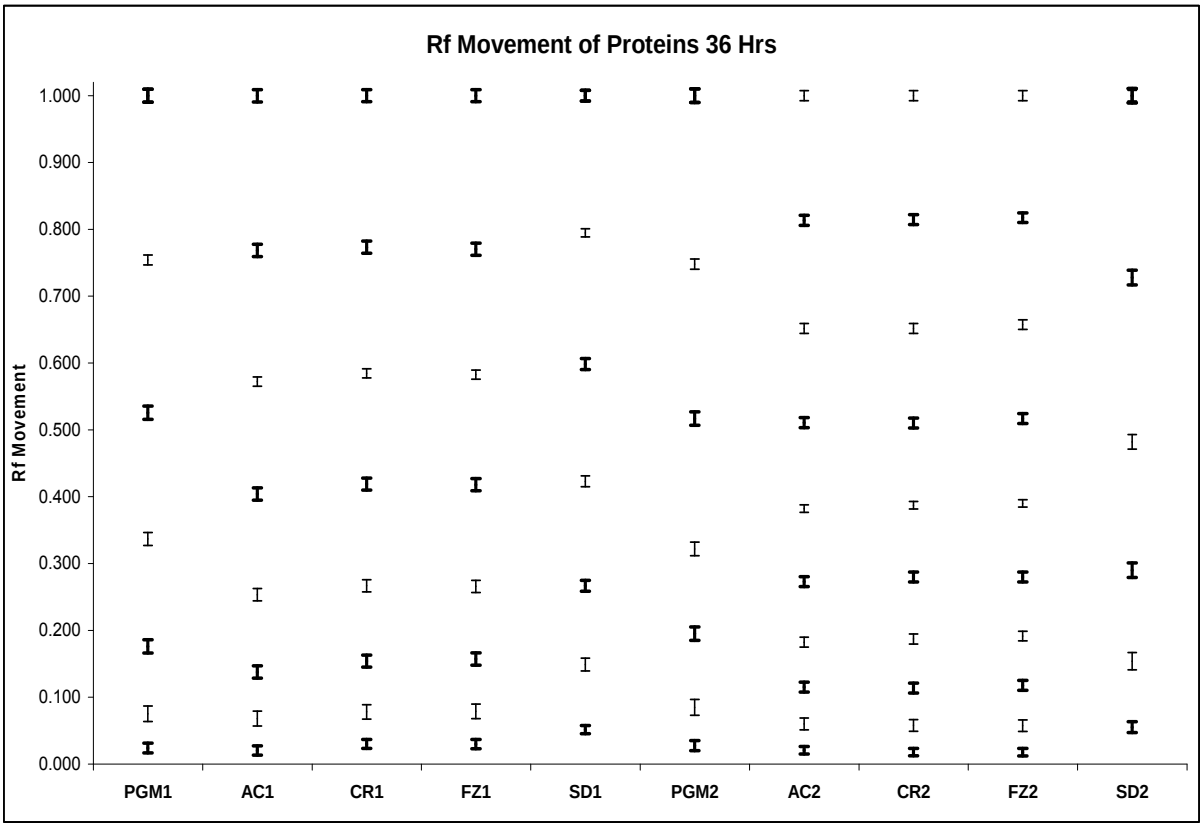


Figure 6.1 continued.

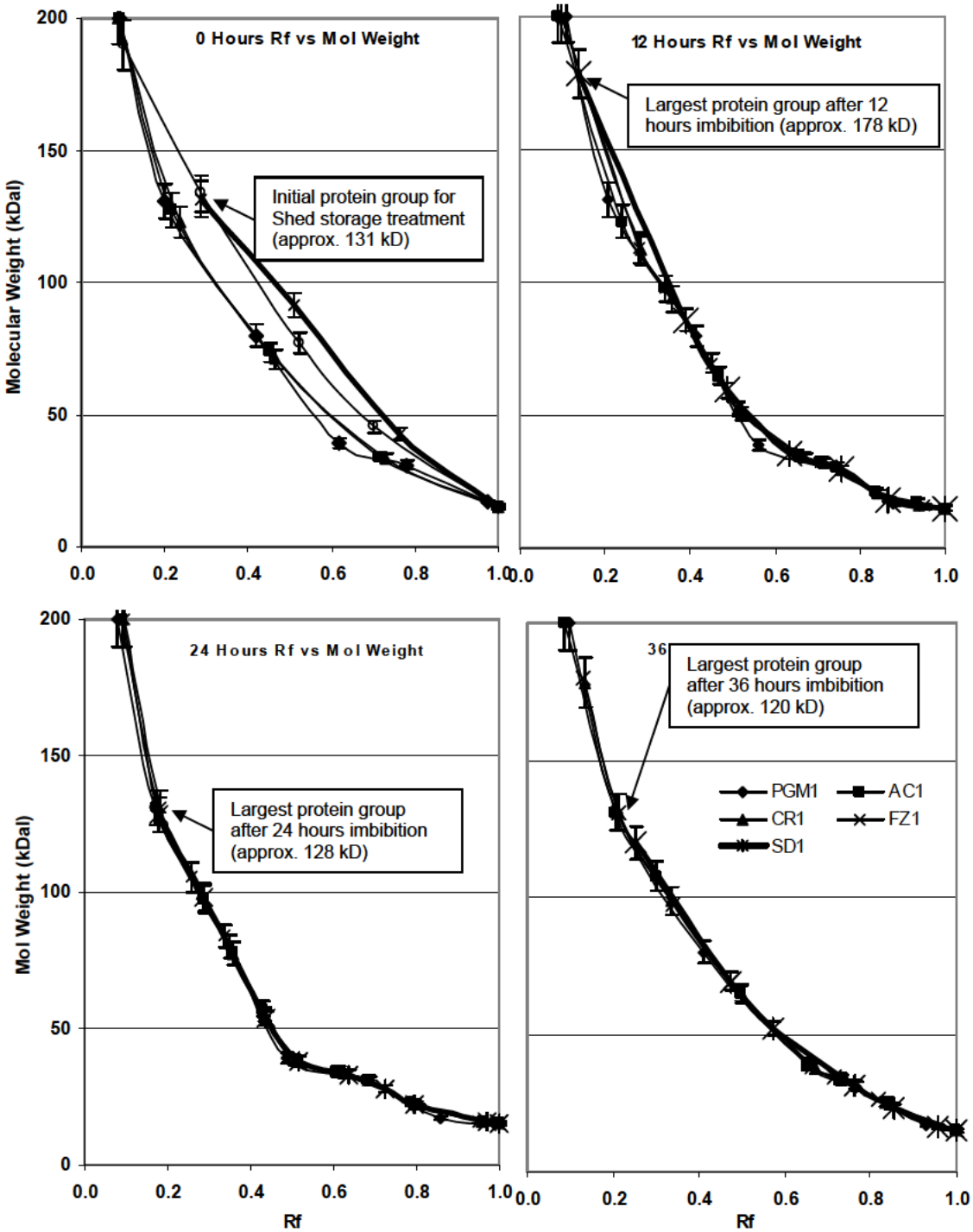


Figure 6.2 Rf vs Molecular weight of protein groups for each assay. PGM – Protein Gel Marker, AC – Air Conditioning, CR – Cool Room, FZ – Freezer and SD – Shed storage treatments.

Possibly one of the most difficult aspects of conducting an SDS-PAGE on native grass seed is that none of the proteins present have been identified by any laboratory analytical process, such as X-ray crystallography, 1D SDS-

PAGE coupled with LS/MS/MS or software programs such as the FINDER package (Shaw 1993; Ahmad et al. 2005).

However, the goal of this experiment was to establish whether or not the use of various storage regimes resulted in the presence or absence of groups of proteins within the stored seeds at 12-hourly intervals starting from the dry seed just prior to imbibition.

Figure 6.1 is a visual display of the breakdown and resynthesis of proteins during seed germination with each assay displaying new groupings of proteins over time. The Time 0 assay shows that the three cooler storage treatments have no significant change in the size or groupings of their proteins; however, the shed storage treatment clearly shows the absence of a large protein group (initial protein circled).

Figure 6.2 displays the molecular weight at 0 hours and shows that the molecular weight of the initial Shed treatment protein is in the vicinity of 131 kD, a sharp contrast to the 190 – 200 kD molecular weights of the cool storage groups. Table 6.1 (generated using *SPSS v13.0* software) displays the LSD of the relative movement (Rf) and the molecular weight (kD) of the largest protein molecule present in seed from each of the four storage treatments for the T = 0 hours gel run. Note the significant difference between the Rf and molecular size of the shed stored seed and the seed from the three other treatments.

It has long been known, and indeed a number of algorithms have been constructed to predict the relationship, that seed viability may be roughly doubled by a 5 ^{0}C reduction in storage temperature and that this relationship is linked to the moisture content of the seeds (Roberts 1972). These equations, however, are quite generalised and do not take into account adaptations of

seeds from species that have developed biochemical survival mechanisms while living in environments that have extreme temperature regimes.

Table 6.1 Comparisons of LSD's of the Rf (movement) and size (kD) of the largest protein molecule of *Triodia bitextura* seed sample between storage conditions at T = 0 Hours

ANOVA results of storage conditions of Rf movement and size (kD) of largest protein molecule at T=0 hours

Dependent Variable		(I) Storage	(J) Storage	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Rf	LSD	Aircond	Coolroom	-.005000	.005610	.423	-.02058	.01058
			Freezer	-.010000	.005610	.149	-.02558	.00558
			Shed	-.198597*	.005610	.000	-.21417	-.18302
		Coolroom	Aircond	.005000	.005610	.423	-.01058	.02058
			Freezer	-.005000	.005610	.423	-.02058	.01058
			Shed	-.193597*	.005610	.000	-.20917	-.17802
		Freezer	Aircond	.010000	.005610	.149	-.00558	.02558
			Coolroom	.005000	.005610	.423	-.01058	.02058
			Shed	-.188597*	.005610	.000	-.20417	-.17302
		Shed	Aircond	.198597*	.005610	.000	.18302	.21417
			Coolroom	.193597*	.005610	.000	.17802	.20917
			Freezer	.188597*	.005610	.000	.17302	.20417
kD	LSD	Aircond	Coolroom	1442.000	7123.982	.849	-18337.35	21221.35
			Freezer	883.000	7123.982	.907	-18896.35	20662.35
			Shed	71365.000*	7123.982	.001	51585.65	91144.35
		Coolroom	Aircond	-1442.000	7123.982	.849	-21221.35	18337.35
			Freezer	-559.000	7123.982	.941	-20338.35	19220.35
			Shed	69923.000*	7123.982	.001	50143.65	89702.35
		Freezer	Aircond	-883.000	7123.982	.907	-20662.35	18896.35
			Coolroom	559.000	7123.982	.941	-19220.35	20338.35
			Shed	70482.000*	7123.982	.001	50702.65	90261.35
		Shed	Aircond	-71365.000*	7123.982	.001	-91144.35	-51585.65
			Coolroom	-69923.000*	7123.982	.001	-89702.35	-50143.65
			Freezer	-70482.000*	7123.982	.001	-90261.35	-50702.65

*. The mean difference is significant at the .05 level.

As evidenced from Chapter 5, there was no discernable difference in the surface morphology, or evidence of any signs of stress between the storage treatments for this species. Therefore any difference is internal, either biochemical or structural.

The data in Table 6.1 and Figure 6.1 at Time = 0 hours indicate that there is a difference in the protein composition of the Shed storage treatment. During imbibition there was a synthesis of new proteins that would have been drawn from the amino acid pool provided by the (possibly) heat degraded macro-protein molecules of the endosperm or embryonic axis (Bewley and Black

1994). It is also known that some species of seeds have heat stress mechanisms in the form of extraction from cytoplasm and positioning to membrane-bound organelles specialised for the storage of proteins and chaperones (specialised proteins that may reactivate or refold proteins denatured by heat stress (Glover and Lindquist 1998; Müntz 1998)).

This is not to say that possible protein degradation is the only cause of the loss of viability (as previously noted by the anecdotal observations that the Shed stored seeds failed to produce any germinants); degradation of cellular membranes and the resultant leakage of electrolytes during imbibition will also contribute to a seeds non-viability, which is then followed by impairment of biosynthetic mechanisms (such as protein synthesis which includes the production of enzymes), reduced storage potential (which is also the degradation of storage proteins) resulting in seed death (Heydecker 1977). Trials listed by Heydecker (1977) used temperature stress conditions where seeds were exposed to temperatures of 40 – 45 ^{0}C. Compare this to native Australian seeds that are exposed to ground temperatures circa 65 – 70 ^{0}C, and in shed storage to temperatures circa 60 ^{0}C (Mott 1972; Fesuk and Ashwath 2004).

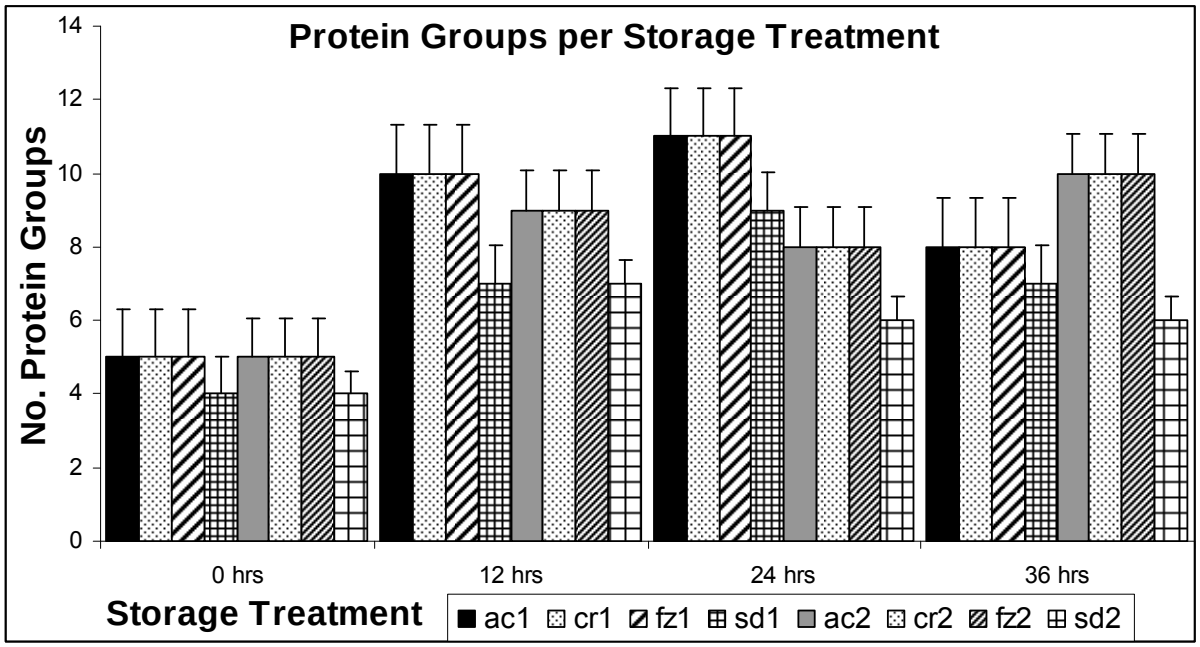


Figure 6.3 Protein groups per storage treatment. Error bars denote SE of protein groups per storage treatment replicate across time periods.

Figure 6.3 shows that there is no significant difference between the number of stained protein groups between the imbibition assays, however, the differences in values do suggest a difference between these groups and the protein groups from Time 0. This is an expected result due to past research in the *de novo* synthesis of protein during imbibition. There really is no significant statistical difference between storage treatments in the Time 0 assay although the Shed treatment does present a reduced amount of protein groups in comparison to the cooler treatments. This correlates well with experimentation that showed non-viable seeds has disorganised protein synthesising mechanisms that was in tandem with the seeds inability to synthesis RNA (Desai 2004).

6.5 Conclusion

In conclusion, sustained heat stress does result in protein degradation of *T. bitextura* seeds as evidenced by a marginally reduced size of protein

macromolecules present in the seeds before imbibition. Cold or cool storage is required, in this instance, for the inhibition of protein degradation and in turn to extend the viable life of *T. bitextura* seed. There is a general lack of knowledge with respect to proteins in Australian native grasses and to counter this, species-specific research is required to protein map target pastoral or rehabilitation species, such as *T. bitextura* to assist in the design of species-specific storage and germination treatment protocols.

Chapter 7
General Discussion

In closing, this chapter is presented to bring together the results of this research into a cohesive and inter-related unit of work.

At the beginning of this research little was known as to the germination requirements of seed of Australian native grasses. Indeed, there is still much to know, however, through the efforts of researchers around the country, and through the sharing of knowledge via traditional communication methods, the scientific community is learning more each day. This thesis contributes to this body of knowledge.

There is a market for the commercial production of native grass seed for the use in pastoral restoration, mining rehabilitation and road embankments and rail works. Yet, it is neither wise nor possible to begin such ventures without knowing what the germination or storage requirements of the seed are. Does this seed have an after-ripening time that must be taken into account? Will the seed germinate under the conditions in which it will be sown? Will the growing plant establish itself in an environment in which it has no history of adaptation?

What has happened to seed if it doesn't germinate? Is the batch of seed non-viable, or has it entered some dormant phase? Has the manner in which the seed been harvested or stored affected the biochemistry of the seed?

The current study has answered many of these questions. Many species do have an after-ripening period. Different geographic regions will have different environmental conditions, in terms of soil types, pH, mineral content which will affect some species of seed more so than other species.

Therefore, the provenance of seed is all important. The provenance should not only include the germinability of the seed, but its length and mode of storage, its seed quality in terms of seed plus seed trash, the location of harvest and associated soil types/conditions and a fact sheet for that species that outlines methods of sowing, sowing rates, suitability for various tasks (e.g. batter stabilisation, salt tolerance, erosion control, pasture fodder) and recommended times of sowing as per dormancy cycles. Not all these factors are known as yet, particularly the dormancy cycles, but this research has been completed to facilitate the discovery of these final details.

Continuance of the storage trials (Chapter 3) combined with research that examines not only germination rates, but the physiology of the seed at regular time periods during the seeds storage, will further expand our knowledge base of Australian native grass species. For example, the science of proteomics has given us a powerful diagnostic tool in the study of seed, yet it is to be fully utilised for our Australian species.

Let us now consider the objectives from Section 1.6.3

1. Observe the effectiveness of the planting (by GANT) of native grass species in a range of field sites across the Northern Territory, with respect

to edaphic, climatic and habitat alteration as a result of human activity (e.g. mining, dredging or grazing)

2. Test the effects of various storage methods and smoke germination treatment for each of the selected grass species used in the study.
3. Assess the establishment and stabilisation potential of three native grass species (*Brachyachne convergens, Chloris pectinata* and *Iseilema vaginiflorum*) selected by GANT for their potential both as fodder and batter stabilisers) and compare their performance to a commonly used grass (*Chloris gayana*) for slope stabilisation in locations such as railway embankments or landfill batters. Stabilisation of these structures will minimise their impact on the surrounding natural (or rehabilitated) environment.
4. Examine the effects of various storage treatments via sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) on the composition of proteins in the seeds of *Triodia bitextura* (selected for its response to storage treatments and its importance in desert rehabilitation).
5. Test, via SEM imagery, the effects of various storage treatments on the seedcoat (testa) of species of native grasses.

Chapters 3, 5 and 6 address the germination of seed, and in doing so address objectives 2, 4 and 5. They investigate the effects of storage on the germinability of seed utilising a variety of germination treatments, the storage effect on the seed external morphology by means of SEM and examine a facet of the internal biochemistry of the seed using SDS-PAGE technology.

7.1 Soil seedbank observations

Chapter 2 examined GANT field sites in Northern Territory that had been planted with selected native Australian grass species to assess their ability to rehabilitate land that had been altered by human activities. Some conditions were extreme (e.g. Mt Todd spoil heaps and Bing Bong sea floor dredge spoil), some, such as Limbunya, Hayfield and Benmara, were over-utilised, while Willaroo, through grazing and fire management unsuited to that area was infested with *Jatropha gossypiifolia.*

These trials were important; for too long exotic species of grasses have been introduced into the Australian environment to remedy problems caused by mining or erosion that had been a result of over-grazing. *Vetiver* is a grass species widely used in stabilisation and rehabilitation in saline environments and mining areas (Truong and Loch 2004), but now its close native Australian relative *Chrysopogon elongatus* has proven to be effective in establishing itself in the saline dredge spoils of Bing Bong and able to spread vegetatively by means of below-ground stolons. A difficult grass to germinate, *Chrysopogon elongatus* was able to be established very successfully by GANT using plugs, as is *Vetiver*.

Several scientists have trialled and reported on the effective use of fire in combination with native grasses, as a rangeland management tool for both pasture improvement and weed eradication (Anderson et al. 1988; Grice and Slatter 1996; Ash et al. 1997; McCarthy and Gill 1997; Orr and Paton 1997). This practise was trialled at the Willaroo site using tall Australian native grasses (*Sarga intrans*) as another independent study reported that although this species had a high fuel load early in the wet season, burning off would not

overly effect further growth of the grass (Lazarides et al. 1991). The use of this grass species provided hot fires when *Jatropha gossypiifolia* was at its most vulnerable in its new growth stage with the result that the weed was eradicated from the site.

Another valuable grass for revegetating in hot and exposed conditions and alkaline soils was *Brachyachne convergens*. This short annual has proven that it can germinate in a range of soil types, including soils with high pH. It has been found growing well in soils at Adria Downs (Birdsville, QLD) in soils of pH 9.5 (Shaw 2006b) and in fact grows over a wide portion of Australia (Figure 7.1). It should be noted that *Brachyachne convergens* did not occur at any site in the current trials/observations where the soil pH was below 7.

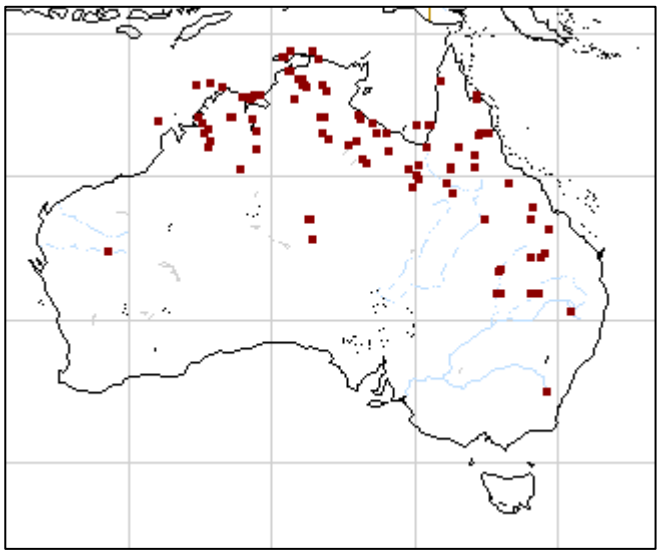


Figure 7.1 *Brachyachne convergens* CANB Collection Sites (ANBG 2003).

The greatest value of these observations is that they gave direction for the selection of native species, such as *Brachyachne,* for further testing.

7.2 Germination and storage trials

The storage treatments from Chapter 3 also produced very positive results, with some species considered to be tropical (e.g. *Astrebla squarrosa* which

has limited value as a pasture plant, but may be important in the preservation of the Gouldian Finch) having their longevity greatly increased via freezer storage (Figure 3.1). Other species, such as *Brachyachne convergens*, which is proving to be an important species for erosion control, colonisation, and batter stabilisation, has an after-ripening period of up to two years (dependent upon temperature) and responds negatively to cold storage having demonstrated the need for warm or ambient temperature storage.

After-ripening is the shedding of primary dormancy and the maturation of the seed after seed-drop or harvest. It is related to both humidity and temperature, with the fastest rates of ripening occurring in warmer temperatures. This means that the duration of after-ripening is both species-specific and environmentally-influenced (Murdoch and Ellis 2000; Desai 2004). Even within species there are the effects of the environment in which the maternal plant has developed to be considered, and variations in light, temperature and soil type can greatly influence the seeds being formed and the level of dormancy which the seeds achieve (Fenner and Thompson 2005).

This was manifestly demonstrated in the germination responses of the two seedlots of *Dichanthium sericeum*. Collected a year apart, from the same site, the two seedlots had different levels of germination that were not only influenced by their after-ripening, but possibly by their environmental conditions as well. The 2001 seedlot showed a very poor germination response in the initial trials, however, the seeds provided high germination percentages in response to storage in air conditioning and in response to smoke.

The 2000 seedlot, even though from the same site had a germination response less than half that of the 2001 seedlot, and showed the greatest response to freezer storage, not air conditioning (Figure 3.1). The 2000 seedlot spent its first 12 months in an aired room in Katherine NT, whereas the 2001 seedlot was transported to CQU Rockhampton very soon after harvest, and split into its storage regimes. This difference in storage conditions may be the reason for the difference in germination, or it could be that the seed was harvested at slightly different stages in its maturity, the weather conditions were somewhat different, or some other reason underpinned the difference. However, the need to quantify such effects can be appreciated for future harvesting and storage procedures.

After-ripening was also demonstrated by *Iseilema vaginiflorum* and *Brachyachne convergens*, both showing significantly increased germination responses three years after-harvest (Figure 3.1), although neither demonstrated any significant response to smoke treatment. Treatment of seeds with smoke, which is seen as a general germination stimulant, produced limited results for the majority of species, yet most importantly as well as increasing the germination percentage of *Dichanthium sericeum,* it also increased the germination rate of *Triodia bitextura*, a species that could prove quite important in mining rehabilitation, and for which the protein study results indicated that this species requires cold storage to maintain optimal viability.

Knowing the response of seed to long term storage is valuable; however, it is also necessary to have knowledge of how the stored seed may be utilised in terms of environmental rehabilitation and erosion control. Hence, the addressing of objectives 1 and 6 with results from field trials and environmental research. Questions may be raised here with respect to what

species of native grasses are best able to be used in rehabilitation of acidic soils, over-utilised pastures, or batter stabilisation? A grazier has 100 ha available for seed production – should native grasses be sown for seed, or is it more prudent in terms of bulk forage to plant exotic grasses such as buffel (*Cenchrus ciliaris*) or Rhodes grass (*Chloris gayana*)?

These questions have been addressed by the research of Chapters 3 and 4, which test and monitor the practical application of native grasses as rehabilitation tools, and their potential to be commercially grown products.

7.3 Stabilisation of batters field trial

This trait, in addition to its stoloniferous habit, made *Brachyachne convergens* an ideal choice for the batter stabilisation trials at the Rockhampton landfill site (Chapter 4). Although the other two annuals in this trial, *Chloris pectinata* and *Iseilema vaginiflorum* laid down protective mulch, *Chloris pectinata* had a weak spreading habit which did assist in slope stabilisation, and *Iseilema vaginiflorum* with a tufted habit, permitted the erosion of top soil around the base of the plant (Figure 4.10). Even its abundant seed drop could not counteract the open spaces which gave footholds to a range of weeds, the seeds of which would have been, of course, already in the batter soil.

Brachyachne convergens showed excellent surface-covering ability with a well-developed network of stolons. Its thatched mulch covering was several centimetres thick, affording prevention of germination of weeds in-between growing seasons (Table 4.2), and as Figure 7.2 shows it has the ability, once established, to slowly spread and stabilise neighbouring areas, yet be of no threat to other shrubs or trees that may have been sown in a mixed species

rehabilitation project. Due to this thick cover, the batter slope has been well protected for the past two years and nine months.

All stands at the landfill site of *Iseilema* are gone (however, there should be large repository of seed in the soil seedbank as layer of thatch still decompose on ground), in most places invaded by either *Chloris gayana* or Red Natal grass (*Melinis repens* (Willd.)Zizka)), or in heavy shade by broad-leaf forbs (Figure 7.3). *Chloris pectinata*, too, has been overgrown with *Melinis repens* leaving behind a stabilised batter, albeit not with the desired species (Figure 7.4).

Although the stands of *Chloris gayana* remain, the grass has become somewhat stunted and yellowed. This is an indication that the stands of *Chloris gayana* are suffering from nutrient stress and overcrowding, due mainly to their vigorous growth habit. It still presents a high fuel load, a trait that serves to make it undesirable in mixed seeding projects.

It would be interesting, although in this case impractical due to the presence of other landfill remediation trials close-by, to create a disturbance event such as fire, and record the subsequent germination response when the mulch has been burned and seeds in the soil seed bank are exposed to light. The effectiveness of the treatment would be influenced by intensity of fire, microtopography and amount of litter on the ground. Previous studies have shown that *Iseilema* is very responsive to low intensity burn-offs when light layers of litter are burnt. Some fires promote a germination response and more intense fires would have the result of killing the seed or hindering its germination process (Scanlan and O'Rourke 1982; Fenner and Thompson 2005).

Figure 7.2 *Brachyachne convergens* at the Rockhampton landfill site at 2 years 9 months after sowing. New germinations have appeared through the thick thatch shortly after winter rains. Note the absent of exotic grasses, the only weed species being a thistle that was able to penetrate the mulch.

Figure 7.3 May 2004 and July 2006. From the front is *Chloris pectinata, Iseilema vaginiflorum* and *Chloris gayana* in the background. In 2006 (34 months after sowing) only stunted growth of *Chloris gayana* remains.

Figure 7.4 *Chloris pectinata* (34 months after sowing) over-grown with exotic *Melinis repens*.

7.4 Comparison of storage treatments using 1D SDS-Page

The SDS-PAGE gels showed variation in the size of storage proteins within each of the seeds from the four storage treatments (Table 6.1). While the differences were not large, they were significant, certainly at the molecular level the differences could be large enough to disrupt the controlled break-down and subsequent recycling of the amino acids into the specific enzymes required for seed germination (for example endo-*β*-mannanase which is required for the weakening of cell walls to allow emergence of the radicle) (Müntz 1998; Welbaum et al. 1998).

The fact that the shed treatment at the initial phase of imbibition lacked protein molecules as large as the other three treatments indicates that to some degree protein denaturation has occurred. This finding is reinforced by the anecdotal observation of the germination of excess imbibed seed from the three cooler storage treatments; whereas there were no subsequent germinations from the excess shed-stored seeds.

Seed ageing is also caused by non-enzymatic reactions by reducing sugars or aldehydes and effect protein structure. Coupled with lipid peroxidation, in conjunction with Amadori and Maillard products, there is a complex chain of events that in turn is linked to the temperature of the storage treatment (Murthy and Sun 2000; Murthy et al. 2003).

It is that storage proteins are vulnerable to heat denaturating and chemical denaturing when in a dry state and so incapable of repairing themselves. Hence, the SDS-PAGE illustrates that there is a change at the molecular level in seeds as a result of storage temperatures. This is not a new finding as it has long been known that generally the higher the storage temperature and

humidity, the shorter the viability of the seed being stored (Roberts 1972). Similar trials using *Phaseolus vulgaris* (L.) seeds compared artificially-aged seeds to naturally-aged seeds that had been in collected over a 5 year period and stored at 15 ^{0}C before being submitted to SDS-PAGE. The gels showed changes in their protein patterns, and concurrent germination trials had a resultant decrease in germination that was greater in the naturally-aged seeds than the artificially-aged seeds. In the natural seeds the changes to the protein banding were slight, but the impact was pronounced (Desai 2004).

7.5 Comparison of storage treatments using SEM analysis

Many of the grass species collected had never before been subjected to this level of scrutiny; therefore in essence, much of this study was novel and has produced valuable knowledge of potentially commercial grass species and highlighted areas of research that are yet to be pursued. While the SEM examination of the seed coats did not show the existence of external effects to storage, the SEM images are on file and may be used to increase the taxonomic database for the species studied.

7.6 Conclusion

None of the chapters in this thesis should be considered stand alone works. If an Australian native grass species is to be examined fully, then it must not only be subjected to germination and storage trials, but it must also be tested for its versatility in adapting to other environments, and its traits should be carefully examined and utilised.

For example, *Brachyachne* had the stoloniferous habit that is good for slope stabilisation, erosion control and is recommended for mango growers in Northern Territory as its short length results in “the reduction of slashing costs

by 80%" (CCNT 2002). Another grass *Chrysopogon elongatus* has a tolerance for water with moderately high salt levels and it turns out, is better being planted as plugs rather than at seed (Table 3.1). For effective commercialisation there is after ripening, dormancy cycles, longevity and viability under various storage regimes to be considered. All these facets of a seed need to be tested and examined in order for the scientific community to gain insight into the particular species.

In order to effectively use Australian native grasses for restoration work, research that encompasses all the above facets must be done. However, it is expensive; who pays? This project was assisted by funding from the Victoria River District Conservation Association (VRDCA), Greening Australia Northern Territory (GANT), Central Queensland University and Plant Sciences Group and not to mention the hours of volunteer labour counting out seeds, and the odd cup of tea on a cattle station.

The impetus for this project began with agreement between GANT and the VRDCA that research must be carried out to give them direction in their rehabilitation efforts. Funding was made available to them from the Federal Government, but individual graziers contributed themselves as well.

So in answer to the question – "Who pays for all the research required to rebuild our land?" – all do, directly financially or with labour, indirectly with taxes.

"Who benefits from this research?" Again, we all do. A well-managed ecology gives strength to a country's economy in the form of a more efficient supply of produce and reduced costs in land maintenance and rehabilitation.

And, “Who can help?” We all can, each in our way. We can be involved directly in research ourselves, or in replanting projects, or even by educating ourselves and our children so that the mistakes of the past need never be repeated again.

The most valuable aspect of this study was the time factor, and being able to study seeds over long-term storage regimes. The study is open for continuation; the seed is still in secure storage awaiting the furtherance of funding and available research scientists.

Chapter 8
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Chapter 9
Appendices

Appendix 9.1 Permission for use of Maps and Information.

Hi Sam,
Apologies for the delayed reply. You may use the maps which are contained within the INRM Plan as these are already in the public domain. As you indicate the maps should be properly referenced and any changes indicated accordingly.

It would be appreciated if a copy of your report could be provided to NRETA as it may be extremely useful in the management of our natural resources in the NT.

Regards - IAN

Ian Lancaster
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From: "Samuel Fesuk" <s.fesuk@cqu.edu.au>@NTGEMAG
Sent: Friday, 6 January 2006 4:57 PM
To: Ian Lancaster
Subject: Permission to reproduce pdf maps in Masters thesis

Dear Ian,

As per our telephone conversation on the 6th January 2006, I am seeking permission to reproduce pdf maps from the appendices of the "Integrated Natural Resource Management Plan for the Northern Territory" in my VRDCA and GANT sponsored Masters thesis on the "Germination and Storage Studies of Selected Australian Tropical Native Grasses in Relation to their Ecology and Use in Land Rehabilitation".The maps would be fully referenced, of course, and any alterations would be restricted solely to the placement of markers indicating my research sites, which range from Mainoru in the north and Ti-Tree in the south, and from Limbunya to Benmara in the west and east respectively.

Thank you for your kind assistance in this matter, as at present I am writing the thesis for submission in March.

Yours sincerely

Sam Fesuk
Masters Student
Plant Sciences Group
Central Queensland University

Appendix 9.2 - Provenance of Seed Used

Table 9.1 - Provenance of Seed Used in Germination Trials – Chapter 3

Species	Date sent		Botanical province	Lat	Long	Date of harvest
Alloteropsis semialata	23/03/2001	1	Darwin & Gulf (25)	15 41 08	133 18 17	14/12/2000
Aristida inaequiglumis	23/03/2001	2	Darwin & Gulf (25)	Not noted		15/12/2000
Brachyachne convergens	23/03/2001	3	Barkly Tablelands (27)	18 42 12	134 30 25	11/02/2001
Chloris pectinata	23/03/2001	4	Central Region North (28)	18 52 07	134 30 20	10/02/2001
Chrysopogon elongatus	23/03/2001	5	Darwin & Gulf (25)	16 28 19	136 02 27	22/12/2000
Chrysopogon fallax	23/03/2001	6	Darwin & Gulf (25)	14 37 14	132 08 20	02/01/2001
Cymbopogon refractus	23/03/2001	7	Central Region North (28)	21 30 56	133 58 29	08/02/2001
Dactyloctenium radulans	23/03/2001	8	Barkly Tablelands (27)	21 34 52	133 47 27	09/02/2001
Dicanthium sericeum (2000)	23/03/2001	9	Darwin & Gulf (25)	16 28 19	136 02 27	28/04/2000
Eulalia aurea	23/03/2001	10	Darwin & Gulf (25)	15 49 39	133 23 20	23/12/2000
Iseilema vaginiflorum	23/03/2001	11	Barkly Tablelands (27)	18 42 12	134 30 25	11/02/2001
Oxychloris scariosa	23/03/2001	12	Central Region North (28)	21 34 52	133 47 27	09/02/2001
Paraneurachne muelleri	23/03/2001	13	Barkly Tablelands (27)	18 39 13	133 57 24	08/02/2001
Sorghum macrospermum	23/03/2001	14	Darwin & Gulf (25)	14 26 22	132 14 08	12/03/2001
Sporobolus actinocladus	23/03/2001	15	Central Region North (28)	21 34 52	133 47 27	08/02/2001
Sporobolus mitchellii	23/03/2001	16	Barkly Tablelands (27)	18 42 12	134 30 25	11/02/2001
Themeda triandra	23/03/2001	17	Darwin & Gulf (25)	14 37 14	132 08 20	05/01/2001
Triodia basedowii	23/03/2001	18	Central Region North (28)	21 30 56	133 58 29	08/02/2001
Triodia bitextura	23/03/2001	19	Barkly Tablelands (27)	18 45 24	134 01 00	18/01/2001
Triodia inutilis	23/03/2001	20	Barkly Tablelands (27)	16 33 23	134 44 23	12/12/2000
Triodia longiceps	23/03/2001	21	Barkly Tablelands (27)	Not noted		05/01/2001

Appendix 9.3 - Analysis of Variance and Post-hoc Test Results for Chapter 4

Table 9.2 - ANOVA and Post-hoc results (*p* – value = 0.05) for statistical analysis of the initial germination counts where 1= *Brachyachne convergens*, 2 = *Chloris gayana*, 3 = *Chloris pectinata* and 4 = *Iseilema vaginiflorum*.

ANOVA

Mean

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	45.137	3	15.046	6.375	.003
Within Groups	47.201	20	2.360		
Total	92.338	23			

Multiple Comparisons

Dependent Variable: Mean

	(I) Species	(J) Species	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1.00	2.00	.16667	.88695	.853	-1.6835	2.0168
		3.00	-1.39583	.88695	.131	-3.2460	.4543
		4.00	-3.25000*	.88695	.002	-5.1001	-1.3999
	2.00	1.00	-.16667	.88695	.853	-2.0168	1.6835
		3.00	-1.56250	.88695	.093	-3.4126	.2876
		4.00	-3.41667*	.88695	.001	-5.2668	-1.5665
	3.00	1.00	1.39583	.88695	.131	-.4543	3.2460
		2.00	1.56250	.88695	.093	-.2876	3.4126
		4.00	-1.85417*	.88695	.050	-3.7043	-.0040
	4.00	1.00	3.25000*	.88695	.002	1.3999	5.1001
		2.00	3.41667*	.88695	.001	1.5665	5.2668
		3.00	1.85417*	.88695	.050	.0040	3.7043
Dunnett T3	1.00	2.00	.16667	.55559	1.000	-1.7773	2.1106
		3.00	-1.39583	.48206	.102	-3.0370	.2453
		4.00	-3.25000	1.06572	.112	-7.2864	.7864
	2.00	1.00	-.16667	.55559	1.000	-2.1106	1.7773
		3.00	-1.56250	.66150	.193	-3.6918	.5668
		4.00	-3.41667	1.15800	.099	-7.4178	.5845
	3.00	1.00	1.39583	.48206	.102	-.2453	3.0370
		2.00	1.56250	.66150	.193	-.5668	3.6918
		4.00	-1.85417	1.12458	.526	-5.8445	2.1361
	4.00	1.00	3.25000	1.06572	.112	-.7864	7.2864
		2.00	3.41667	1.15800	.099	-.5845	7.4178
		3.00	1.85417	1.12458	.526	-2.1361	5.8445
Dunnett t (2-sided) [a]	1.00	4.00	-3.25000*	.88695	.004	-5.5032	-.9968
	2.00	4.00	-3.41667*	.88695	.003	-5.6698	-1.1635
	3.00	4.00	-1.85417	.88695	.121	-4.1073	.3990

*. The mean difference is significant at the .05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Table 9.3 - ANOVA and Post-hoc results (*p* – value = 0.05) for statistical analysis of the ground cover percentages where 1= *Brachyachne convergens*, 2 = *Chloris gayana*, 3 = *Chloris pectinata* and 4 = *Iseilema vaginiflorum*.

ANOVA

target cover

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	110083.025	3	36694.342	38.282	.000
Within Groups	111189.967	116	958.534		
Total	221272.992	119			

Multiple Comparisons

Dependent Variable: target cover

	(I) Species	(J) Species	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	1.00	2.00	-70.70000*	7.99389	.000	-91.5374	-49.8626
		3.00	.53333	7.99389	1.000	-20.3041	21.3707
		4.00	-3.00000	7.99389	.982	-23.8374	17.8374
	2.00	1.00	70.70000*	7.99389	.000	49.8626	91.5374
		3.00	71.23333*	7.99389	.000	50.3959	92.0707
		4.00	67.70000*	7.99389	.000	46.8626	88.5374
	3.00	1.00	-.53333	7.99389	1.000	-21.3707	20.3041
		2.00	-71.23333*	7.99389	.000	-92.0707	-50.3959
		4.00	-3.53333	7.99389	.971	-24.3707	17.3041
	4.00	1.00	3.00000	7.99389	.982	-17.8374	23.8374
		2.00	-67.70000*	7.99389	.000	-88.5374	-46.8626
		3.00	3.53333	7.99389	.971	-17.3041	24.3707
LSD	1.00	2.00	-70.70000*	7.99389	.000	-86.5329	-54.8671
		3.00	.53333	7.99389	.947	-15.2996	16.3662
		4.00	-3.00000	7.99389	.708	-18.8329	12.8329
	2.00	1.00	70.70000*	7.99389	.000	54.8671	86.5329
		3.00	71.23333*	7.99389	.000	55.4004	87.0662
		4.00	67.70000*	7.99389	.000	51.8671	83.5329
	3.00	1.00	-.53333	7.99389	.947	-16.3662	15.2996
		2.00	-71.23333*	7.99389	.000	-87.0662	-55.4004
		4.00	-3.53333	7.99389	.659	-19.3662	12.2996
	4.00	1.00	3.00000	7.99389	.708	-12.8329	18.8329
		2.00	-67.70000*	7.99389	.000	-83.5329	-51.8671
		3.00	3.53333	7.99389	.659	-12.2996	19.3662
Dunnett T3	1.00	2.00	-70.70000*	7.24389	.000	-91.0635	-50.3365
		3.00	.53333	9.33795	1.000	-24.8922	25.9589
		4.00	-3.00000	9.64786	1.000	-29.2475	23.2475
	2.00	1.00	70.70000*	7.24389	.000	50.3365	91.0635
		3.00	71.23333*	5.89266	.000	54.6683	87.7983
		4.00	67.70000*	6.37238	.000	49.7864	85.6136
	3.00	1.00	-.53333	9.33795	1.000	-25.9589	24.8922
		2.00	-71.23333*	5.89266	.000	-87.7983	-54.6683
		4.00	-3.53333	8.67932	.999	-27.1379	20.0713
	4.00	1.00	3.00000	9.64786	1.000	-23.2475	29.2475
		2.00	-67.70000*	6.37238	.000	-85.6136	-49.7864
		3.00	3.53333	8.67932	.999	-20.0713	27.1379

*. The mean difference is significant at the .05 level.