ASPECTS RELATED TO THE GERMINATION OF *THEMEDA TRIANDRA* SEED

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ABSTRACT

Themeda triandra is a grass species of economic importance in Southern and Eastern Africa, and Australia. The species is being lost from grasslands and savannas in these areas due to poor agricultural practice, rangeland degradation, opencast mining and increased afforestation. Based on the poor re-establishment of the species from seed in sub-climax grasslands, dogma holds that *T. triandra* can not be re-established from seed. Recent research has, however, highlighted the potential to establish this species from seed, but the use of seed of *T. triandra* in re-vegetation of disturbed areas is limited by poor understanding of the seed biology of the species and low seed availability. In this Thesis ways to maximise the use of available seed are reported. Areas investigated include optimisation of seed storage conditions, overcoming primary seed dormancy, promoting germination of available seed and pre-treatment of seed to improve germination. The Thesis closes with an investigation of the environmental limits of tolerance of seedlings from the *T. triandra* ecotypes studied, when grown under field conditions at reciprocal sites.

Two altitudinally and geographically distinct populations of *T. triandra* were studied; a high altitude grassland population at Cathedral Peak (Drakensberg: 1800 m) and a low altitude savanna population from the Umfolozi Game Reserve (Zululand: 90 m).

At seed shed *T. triandra* seed is dormant. The depth and duration of primary seed dormancy varies between populations, but appears to reflect severity of the winter period experienced. More than 95% of *T. triandra* seed from the Drakensberg population was dormant at seed shed, compared to 55% of seed from the Zululand population. In both populations dormancy is lost during dry after-ripening.

At seed shed *T. triandra* seed displays a high level of seed viability (>80%). Seed

viability decreases with increased storage time. Optimum seed storage, across the temperature range -15°C to 70°C, was achieved at 25°C (± 2°C), at which temperature seed was held for 40 months. During this period viability decreased from over 80% to 50% and dormancy was lost through dry after-ripening within four (Zululand) to eight (Drakensberg) months. Loss of dormancy can be accelerated at higher temperatures, but is accompanied by rapid loss of seed viability. In contrast, viability can be maintained in storage at sub zero temperatures, but loss of dormancy is retarded. Loss of dormancy coinsides with the onset of spring.

Dormant seed is capable of germination at a narrow range of constant temperatures (25°C to 40°C). With after-ripening, the range of temperatures at which germination takes place increases (15°C to 40°C) and the optimum temperature for germination decreases from 30°C in both populations to 25°C. After-ripened seed is capable of germination at lower water potentials than dormant seed. Similarly, seed from the low altitude population is capable of germination at lower water potentials (-1.0 MPa dormant: -1.5 MPa after-ripened) than seed from the high altitude population (-0.5 MPa dormant: -1.0 MPa after-ripened). Dormancy in seed from the high altitude population is overcome by prolonged stratification (30d). In contrast, seed from the low altitude population responds to short duration stratification (5d) with longer periods proving detrimental to seed germination. Germination of dormant and non-dormant seed of *T. triandra* does not differ significantly in the light or dark. Neither does photoperiod, or red / far-red light exposure significantly affect germination.

Seed response to light and temperature, as characterised under controlled conditions, was verified in a field seed burial experiment undertaken at the high altitude Drakensberg site during winter. Burial in soil does not affect the response of *T. triandra* seed to light or temperature. Loss of dormancy is accelerated in buried seed. After-ripened seed germinates over a wider range of temperatures

than dormant seed.

The mechanisms governing *T. triandra* seed dormancy and germination appear to be universal between ecotypes. Dormancy is enforced, in part, by the seed covering structures (glumes) which impose a mechanical restraint to radicle emergence. Approximately 85% of dormant seed, however, contains a dormant embryo. Embryo dormancy is enforced at seed shed by compounds inhibitory to seed germination. The germination process in *T. triandra* appears to be governed by endogenous gibberellins. Bioassay results reveal that endogenous gibberellin synthesis commences up to six hours sooner in after-ripened seed than in dormant seed and that the level, or concentration, of gibberellin-like compounds is substantially lower in after-ripened seed than in dormant seed. Similarly, the concentration of applied gibberellic acid required to achieve maximum germination of *T. triandra* seed decreased from 500 $mg.l^{-1}$ (8 week old seed) to 50 $mg.l^{-1}$ (78 week old seed) as dormancy is lost during after-ripening. Contrary to previous reports, boron does not promote *T. triandra* seed germination.

Plant-derived smoke significantly promotes *T. triandra* seed germination (5% to 43% for dormant seed from the Drakensberg population). The effectiveness of smoke in promoting germination increased with increasing seed imbibition suggesting smoke action at a metabolic level. This suggestion is reinforced by the ability of smoke to bring about the germination of seed which had failed to germinate in water. Moreover when smoke is applied in combination with gibberellic acid the final level of seed germination following combined treatment is significantly greater than the level of germination achieved in the presence of either smoke or gibberellic acid alone. A similar result is achieved with joint application of smoke and kinetin, although the results were not statistically significant. Furthermore, smoke treatment reversed ABA-induced inhibition of germination of non-dormant *T. triandra*, wheat, radish and sunflower seed to a level equal to or greater than that achieved using GA_3 or kinetin. The possibility that smoke

promotes seed germination by mimicking, or promoting the synthesis of endogenous gibberellins was investigated. Bioassay results revealed that smoke had no effect on increasing the level of endogenous gibberellin-like activity in *T. triandra* caryopses. The mechanism by which smoke acts to promote seed germination remains elusive, however results presented suggest that smoke may act to remove an ABA-induced block to seed germination. Consequently, it is suggested that smoke plays a permissive role in promotion of *T. triandra* seed germination by removing a block to the seed germination process thereby allowing endogenous gibberellins to act.

Treatments which significantly improved the level of *T. triandra* seed germination were evaluated as seed pre-treatments. Significant improvement in germination was obtained following smoke (aq) and gibberellic acid (100 $mg.l^{-1}$) pre-treatment of seed. The effects of pre-treatment were evident on germination of seed for up to 21 days after pre-treatment. Seed pre-treatment with smoke had no affect on subsequent seedling growth, but gibberellic acid pre-treated seedlings developed abnormally. In contrast, short duration exposure of dormant seed to high temperature (70°C for 7 days) increased germination, seedling height and tiller number. Priming of seed in polyethylene glycol (PEG 8000) for 7 days significantly improves the level of *T. triandra* seed germination. The use of seed pre-treatment to maximise germination of *T. triandra* seed is discussed.

Reciprocal transplanting of seedlings from both the Drakensberg and Zululand populations confirmed that the *T. triandra* populations under investigation are distinct ecotypes. Field transplant gardens were established in the Drakensberg, Zululand and at an intermediate altitude in Pietermaritzburg (800m). Less than 10% of planted seedlings died at any site. With increasing altitude of the field site, tiller number increased, but tiller allocation to reproduction decreased. Similarly, for both Zululand and Drakensberg seedling transplants the time taken to reach anthesis increased with increasing altitude and the proportion of transplants which

flowered decreased. These data are consistent with the climate of the field sites where the high altitude site experiences a short growing season and harsh winter while the Zululand site experiences a prolonged growing season and mild winter period. These data indicate that *T. triandra* ecotypes are tolerant of a wide range of environmental variables.

The application of the data presented in this Thesis, in maximising the use of available seed of *T. triandra* for use in re-vegetation, is discussed.

Declaration:

I hereby acknowledge that the work reported in this dissertation is the result of my own research, except where otherwise acknowledged.

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1 Introduction

1.1 *Themeda triandra*: the species and the problem

1.1.1 Introduction to the species

The tropical grass genus *Themeda* is a member of the tribe Andropogoneae of the sub-family Panicoideae (Clayton and Renvoize 1986). The genus comprises 18 species, many of which are considered important fodder grasses, at least while in the early stages of growth (Bor 1960). The genus predominates in Asia with the most widespread species, *T. triandra* Forsk., extending through Asia, central, eastern and southern Africa and Australasia (Clayton and Renvoize 1986; Lowe 1989). *Themeda triandra* (syn. *Themeda australis*) is frequently a dominant species of grassland and savanna regions in these areas. The species shows marked morphological variation. In southern Africa, particularly robust forms grow in the north western Transvaal and extend through Swaziland and Zululand to the Transkei coast. High altitude forms tend to be small and densely tufted. A rare, non-tufted, stoloniferous form also occurs on the southern KwaZulu Natal and Transkei coasts (Gibbs Russell and Spies 1988).

The basic chromosome number of *T. triandra* is 10, which conforms to that of the tribe Andropogoneae. Diploid to 11-ploid specimens have been reported (Spies and Gibbs Russell 1988). *Themeda triandra* is a facultative aposporic apomict (Spies and Gibbs Russell 1988) which accounts for the marked variation within the species. The agamic *T. triandra* complex has vast potential for variation, with sexual reproduction among facultative apomicts resulting in more complex hybrids and increasing variation through segregation of existing hybrids (Liebenberg, Lubbinge and Fossey 1993). Gluckmann (1951) described four varieties of

T. triandra, each variety with four different forms, but the characters used to separate these varieties are poorly correlated with habitat, distribution and chromosome number (Gibbs Russell, Watson, Koekemoer, Smook, Barker, Anderson and Dallwitz 1991).

Results obtained following growth of five widespread *T. triandra* populations under different temperature regimes led Groves (1975) to conclude that, as regards flowering of *T. triandra* in response to temperature, genetic differentiation of ecological importance has occurred within the taxon. Spatially separate *T. triandra* populations have also been reported to differ in growth response to temperature (Groves 1975; Downing and Groves 1985), recovery from defoliation (Rethman 1971; Oesterheld and McNaughton 1988), photoperiodic control of flowering (Evans and Knox 1969) and the occurrence of seed dormancy (Groves, Hagon and Ramakrishnan 1982). From an eco-physiological perspective it would thus be unwise to deal with the *T. triandra* complex as a single species. In this investigation the group has been considered at the level of individual ecotypes.

1.1.2 Distribution in southern Africa

Themeda triandra is widely distributed in southern Africa and is a dominant species of the grassland and savanna biomes (Tainton 1984; Gibbs Russell 1987). The species is also present in the fynbos biome of the south western Cape and to a lesser extent remnant patches of *T. triandra* grassland can be found in the semi-arid eastern portion of the Nama-Karoo (Gibbs Russell 1987).

The savanna biome of southern Africa can be separated into an arid (nutrient rich) and moist (nutrient poor) savanna (Huntley 1984). As a mesophytic grass of tropical origin (Tainton 1984) *T. triandra* is seldom found in arid savanna ecosystems. Within African savanna ecosystems plant species diversity and community structure are maintained by interaction between fire and the impact of

large herbivores (Trollope 1984a; McNaughton 1979; McNaughton and Sabuni 1988; Yeaton 1988). In this regard African savanna ecosystems differ markedly from Australian savannas which support few large native herbivores (Calaby 1980). Consequently, prior to the introduction of domestic stock fire was the major determinant of community structure in Australian savanna (Mott, Williams, Andrew and Gillison 1984).

Two main grassland types are also distinguished: (a) climatic climax grasslands in which climatic factors prevent plant succession to a woody community and (b) sub-climax grasslands in which succession would proceed from grassland to a wooded (shrubland, savanna or forest) community in the absence of biotic factors such as fire, grazing and soil type (Huntley 1984). *Themeda triandra* is a dominant species of both climatic and fire-climax grasslands in southern Africa (Tainton 1984). True climatic climax grasslands are only found at altitudes exceeding 2200 m on the highveld and Lesotho plateau. The moist grasslands covering much of the eastern part of southern Africa arose and are maintained by fire which prevents successional advance to either forest or savanna communities (Tainton 1984).

1.1.3 The importance of Themeda triandra as a grazing grass

> *"Themeda triandra is by far the most generally important of our grasses. It is the most palatable grass, and just as bread is the staff of life to us, so Themeda can be regarded as the staff of life to the grazing animal"*
>
> J.P.H. Acocks (in Zacharias 1990).

Themeda triandra has long been regarded as one of the most important native grasses of southern Africa (Tainton 1984), east Africa (Lowe 1989) and Australia (Mott 1978). The importance attached to *T. triandra* lies not solely in its palatability as suggested by Acocks (Zacharias 1990), but because the grass is

found in abundance over a wide range of habitats (Tainton, Bransby and Booysen 1979). Other native grasses may be more productive and nutritious than *T. triandra* (Weinmann 1955; Downing 1972) which becomes "sour" and unpalatable in high rainfall areas especially in the second growth season after being burnt (Tainton 1984). *Themeda triandra* veld, however, has the advantage of being stable under correct management (Tainton *et.al.* 1979). Consequently, *T. triandra* is widely used as a reference species by which to gauge the quality of native grassland (Tainton 1984). Management of grassland is generally geared towards maintenance of economically desirable species for grazing (Tainton 1984) and not for conservation of biotic diversity within grasslands (Davis and Heywood 1994).

1.1.4 Management practices and associated problems

In all veld types where *T. triandra* occurs, the grass is regarded as a "decreaser" species: that is a species which dominates in veld which is in good condition, but decreases in abundance under mismanagement (Trollope, Trollope and Bosch 1990). Mismanagement includes both over and under utilization. In both grassland and savanna areas *T. triandra* is selectively grazed because it is palatable and abundant. Repeated removal of the *T. triandra* stem apex, in simulated grazing experiments, reduced photosynthetic capacity and yield through a reduction in the number of leaves produced (Booysen, Tainton and Scott 1963), thereby limiting the competitive ability of the grass. Under heavy grazing pressure, *T. triandra* is consequently out-competed and replaced in the sward by less palatable, and hence undesirable, species (Tainton 1984). This point is best demonstrated by an example from Australian savanna ecosystems where, following European settlement and the introduction of cattle and sheep to Australian bunch spear grass (*Heteropogon contortus* (L.) Roem. & Schult.) savanna in the late 1800's, the species composition of the savanna understorey changed within forty years from a *T. triandra* dominated sward to one in which the less palatable *H. contortus* dominated (Shaw and Bisset 1955; Shaw 1957).

Natural fires have long played an important ecological role in shaping fire-dependant plant communities. The importance of fire as a factor in determining community structure increased once man harnessed fire as a tool. Archaeological evidence suggests that fire was first used by man in central (Clark 1969) and southern (Mason 1969) Africa between 180 000 to 150 000 BC (Maggs 1977), although Brain and Sillen (1988) present evidence which suggests that *Australopithecus robustus* made controlled use of fire in southern Africa 1.0 to 1.5 million years before present. Palynological evidence from Australia suggests an increased incidence of fire in the natural landscape with the arrival of Aboriginal man approximately 30 000 years before present (Kershaw 1984). Fire has progressed from being a common natural feature of the African grassland landscape to a tool regularly used by man to encourage new growth of grass for grazing, both by domestic stock (Tainton and Mentis 1984) and to attract wild animals which were then hunted (Marshall 1976). Post-fire regrowth is reported to have a higher nitrogen and mineral content than unburned grass in early spring (Tainton, Groves and Nash 1977).

The evolution of grassland community structure in Africa and Australia has thus been integrally associated with fire and it is widely held that dominant grass species, such as *Themeda triandra*, have evolved under a consistent fire regime. This belief is supported by evidence that *T. triandra*, and other decreaser species, decrease in abundance in the absence of fire (Granger 1976: Everson 1985). In contrast, the abundance of decreaser species is maintained under a regular burning regime, with annual winter and biennial spring burns favouring decreaser species (Edwards 1969: Everson 1985). Current management practice favours a biennial spring burning programme. The timing of the burn is critical, however, because summer burns, applied after the commencement of tiller initiation, rapidly bring about species change (Scotcher and Clarke 1980) attributed to fire-induced mortality of the tiller apex.

In bushveld and savanna areas fire is also used as a management tool to promote the maintenance of decreaser grass species. Fire is also widely used in savanna areas to control the encroachment of tree and shrub species (Trollope 1977). This practice is, however, only applied in areas of moist savanna and less frequently than in grassland areas. In the arid savanna areas in the west and north west of South Africa fire is used infrequently on account of the low grass fuel load (Tainton 1984).

The influence of fire on *T. triandra* extends beyond maintenance of individuals of the species in a sward. Burning promotes *T. triandra* seedling establishment. A post-burn flush of seedlings of *T. triandra* has been reported in eastern Cape savanna grasslands (Trollope 1984b), East African savanna grasslands (Lock and Milburn 1971; Ndawula-Senyimba 1972) and in montane grasslands in Natal (Everson 1994). In the absence of fire in montane grasslands, however, the reproductive allocation to seed production of *T. triandra* decreased (Everson 1994). Brown (1993a) cites unpublished results which indicate that germination of *T. triandra* seed is promoted by plant-derived smoke. Smoke is known to promote the germination of seed in a number of fynbos species (de Lange and Boucher 1990; Brown 1993a). Investigation of the influence of smoke on germination of *T. triandra* seed is reported in Chapter 7 of this Thesis.

1.1.5 Themeda triandra seedling establishment in the field.

Although a post-fire flush of *T. triandra* seedlings has been reported (Lock and Milburn 1971; Ndawula-Senyimba 1972; Trollope 1984b; Everson 1994) few investigations have followed subsequent seedling survival. Everson (1994) reported that although a high number of seedlings emerge following a fire, few seedlings actually survived the first winter season. This observation supports the contentions of Danckwerts (1984) and Everson (1985) that persistence of *T. triandra* in a sward, in both semi-arid and moist environments, is by vegetative reproduction. Data of this nature, in combination with the knowledge that the loss

of dominant palatable grass species in fire-climax and climax grasslands is irreversible (Tainton 1984), have led to the widespread belief that *Themeda triandra*, and associated palatable species, do not re-establish from seed. Recent research has challenged this dogma.

1.1.6 The potential to re-establish Themeda triandra from seed

Hagon, Groves and Chan (1975) reported that artificial re-establishment of *T. triandra, Bothriochloa macra, Danthonia* spp. and *Stipa bigeniculata* was possible and these authors provided recommended seeding rates and commented on the potential to propagate the species vegetatively. Similarly, Rommel, Stampa and Stampa (1988), McDougall (1989), Sindel and Groves (1990) and Sindel, Davidson, Kilby and Groves (1993) highlight the potential to successfully establish *T. triandra* from seed. Successful establishment of *T. triandra* from vegetative propagules has also been reported (McDougall 1989), although the limitations of this method are highlighted.

The establishment of *T. triandra* from seed is, however, limited by seed availability (Sindel *et.al.* 1993). Seed yield from wild populations is relatively low at ± 10 $kg.ha^{-1}$ in Australia (Hagon *et.al.* 1975) and between ± 12.5 and 20 $kg.ha^{-1}$ in South Africa (Everson 1994). The commercial production of *T. triandra* seed is further limited by physiological and morphological constraints. Furthermore, there is a lack of synchronisity in flowering (Woodland 1964), maturation and ripening of *T. triandra* seed (McDougall 1989). This can be attributed to the high phenological variation present in wild species. Temporal variation in the ripening of *T. triandra* seed makes the maximisation of seed recovery at harvest difficult. At best, approximately 50 per cent of *T. triandra* seed is ripe at any one time (McDougall 1989). In addition, *T. triandra* exhibits a low level of spikelet fertility (one fertile spikelet out of seven)(Vickery 1961; Gibbs Russell *et.al.* 1991) and seeds shatter readily (McDougall 1989). To these already substantial constraints

must be added the morphology of the seed which, with both awn and callus, is difficult to handle at harvest and at planting.

Themeda triandra seed is dormant at seed shed, but the depth and duration of dormancy differs between populations (Groves *et.al.* 1982). An obvious solution to enable use of this species for revegetation would be to select and harvest seed from populations having a low level of seed dormancy. A limitation, however, is that ecotypes are frequently morphologically distinct and genetic differentiation of ecological importance has taken place within geographically separate populations of *Themeda triandra* (Groves 1975). Ecotypic differentiation assumes importance when wild species are to be used commercially because it becomes necessary to determine to what extent distinct ecotypes are able to survive and reproduce in climates outside of their geographical range. At present these data are not available for *T. triandra*. If this species is to be used successfully in revegetation of disturbed sites, it is essential that germination of available *T. triandra* seed be maximised.

1.2 Seeds

1.2.1 Introduction

A seed represents a physiologically and genetically distinct reproductive unit of a plant. The success of angiosperms has been attributed to the evolution of seeds. Through seeds, plants gain the ability to be dispersed both temporally and spatially, thereby increasing the chance of seedling establishment and facilitating perpetuation of the species. Temporal distribution of seed germination has been achieved by the occurrence of seed dormancy. Within wild species variation in the level of seed dormancy is widespread, both within localised (Baskin and Baskin 1973) and geographically distinct populations of the same species (Groves *et.al.* 1982; Baxter, van Staden and Granger 1993). The importance of seed dormancy

should be viewed in an ecological context, where each species, or species guild, occupies a different niche and environmental resources are partitioned. Grubb (1977) suggested that regeneration within climax (equilibrium) plant communities follows disturbance, which disrupts the competitive dominance of the climax species creating a gap, termed the regeneration niche. Gaps may arise through mortality of individuals within the community, or through small scale disturbance. Any plant within the community can become established within a gap if propagules of that plant are present in the disturbed area. Soil stored seed, seed on the soil surface or seed which is dispersed into a disturbed site constitute such propagules. Pickett (1980) argues that co-existence of plant species within a community occurs, not because resources are effectively partitioned as in equilibrium communities, but because the attainment of competitive equilibrium within communities is prevented. Thus, plant communities should be viewed as being strongly conditioned by disturbance and the opportunistic response of species to disturbance. The creation of gaps or patches in an established plant community, and the regeneration of individuals in gaps, maintains non-equilibrium co-existence in the communities thereby maintaining regional diversity and preventing the formation of a closed community of climax species. Irrespective of the theory favoured, the ability of a plant to capitalise on the increased resources available following disturbance is, in part, determined by the ability of a species to produce viable seed (Grime and Hillier 1992), the efficiency of seed dispersal (Willson 1992), the capacity of a seed to remain dormant within the soil seed bank (Leck, Parker and Simpson 1989) and the ability of seed to germinate.

1.2.2 Definitions and concepts of seed germination and dormancy

Seed germination describes a process whereby a dry seed becomes hydrated, metabolically active and which culminates in the emergence of the radicle or hypocotyl from the seed covering structures (Bewley and Black 1984). Radicle emergence need not be preceded by cell division and may be accomplished by cell expansion alone (Simpson 1990). In this Thesis the definition of germination as

described by Bewley and Black (1984) is followed. Seed germination is defined as the process commencing with the uptake of water and culminating in the successful emergence of the radicle from the seed covering structures. According to this definition the germination process terminates with radicle emergence. Thus, the mobilisation of stored reserve products within a seed is not considered to be part of seed germination, but rather is part of subsequent seedling growth. Obviously a complex sequence of metabolic events takes place within a seed between the initiation of water uptake and radicle emergence. Should any of these events be disrupted, or blocked either physiologically or structurally, the germination process may be retarded or arrested. Similarly, should favourable environmental conditions which prevailed at the onset of germination cease to exist, the germination process may be arrested. The failure of a viable seed to germinate indicates that the seed is either dormant or quiescent. Dormancy refers to the state in which seed germination does not occur under favourable conditions because there is a physical and / or physiological block in the sequence of events leading to germination. In contrast, a quiescent seed is physiologically and structurally capable of germination, but does not germinate because conditions do not favour germination.

Two broad categories of seed dormancy are recognised, namely primary and secondary (or induced) dormancy (Crocker 1916: Roberts 1972). Primary dormancy is inherent in a seed at the time of seed shed and is established during development and maturation of the seed (Bewley and Black 1984). Secondary dormancy, however, may be induced in mature seeds after the seed has been shed and following seed exposure to conditions unfavourable for germination (Koller, Mayer, Poljakoff-Mayber and Klein 1962). Once a state of secondary dormancy has been induced, the seed will no longer germinate under normal environmental conditions previously favourable to germination (Bewley and Black 1984). In this Thesis ways of overcoming primary seed dormancy in *T. triandra* are considered.

Two mechanisms of primary seed dormancy, coat imposed and embryo dormancy, are widely recognised (Bewley and Black 1984; Simpson 1990). As the name implies, in coat imposed dormancy, the seed covering structures prevent the germination of a non-dormant embryo. In contrast, in embryo dormancy the embryo is physiologically incapable of germination. These two dormancy mechanisms may exist independently or in combination. The mechanisms of primary seed dormancy have been further sub-divided by Baskin and Baskin (1989) to yield five categories of seed dormancy used to describe the performance of seed in the soil seed bank (Table 1.1).

Table 1.1: Types of primary seed dormancy used to characterise the performance of seed in the soil seed bank. (Baskin and Baskin 1989)

Type	Causes of dormancy	Characteristics of embryo
Physiological	Physiological inhibiting mechanism of germination in the embryo	Fully developed; dormant
Physical	Seed coat impermeable to water	Fully developed; non-dormant
Combinational	Impermeable seed coat; physiological inhibiting mechanism of germination in the embryo	Fully developed; dormant
Morphological	Underdeveloped embryo	Underdeveloped; non-dormant
Morphophysiological	Underdeveloped embryo; physiological inhibiting mechanism of germination in the embryo	Underdeveloped; dormant

Effectively two additional categories of seed dormancy, morphological and morphophysiological, have been added to account for under-development of the embryo as a contributing factor to seed dormancy. The description of physical dormancy, as provided by Baskin and Baskin (1989), is limited by the failure of the authors to take into consideration effects of the seed covering structures, other

than permeability to water, on the underlying embryo. The role of the seed covering structures in the imposition of dormancy in *Themeda triandra* is discussed further in Chapter 4. Similarly, means of overcoming embryo dormancy in *T. triandra* seed are discussed in Chapter 7.

1.2.3 The effect of the maternal environment on seed dormancy

Seasonal variation in the level of seed production in response to changing environmental variables is well known in agricultural crops (Smith and Pryor 1962; Aspinall, Nicholls and May 1964), but has also been documented for wild species characteristic of grasslands (Sarukhan and Harper 1973), shrub (Davis 1976) and forest communities (Baron 1969; Beveridge 1973; Jensen 1982; Nilsson and Wastljung 1987). Similarly, the level of primary seed dormancy is influenced by conditions present in the maternal environment at the time of seed development and maturation (Wurzburger and Koller 1976; Gutterman 1978; Gutterman 1991; Fenner 1991) as reported for the environmental variables temperature (Juntila 1973), light quality (Gutterman and Porath 1975; Cresswell and Grime 1981) and day length (Cumming 1963; Gutterman 1973, 1982, 1985; Gutterman and Porath 1975). In addition to the influence of environmental variables experienced at the time of seed development and maturation, on seed germinability, the age of the mother plant (Gutterman 1978) and seed position in the inflorescence (Datta, Evenari and Gutterman·1970; Thomas, Biddington and O'Toole 1979; Gray and Thomas 1982) influence subsequent seed germinability. Furthermore, Datta, Evenari and Gutterman (1972) report that for *Aegilops ovata* the position in the inflorescence of the caryopsis from which the mother plant was derived affects the germination of sibling seed. Altitude also exerts an influence on subsequent seed germination. For example, the germination of *Chenopodium bonus-henricus* seeds from a high altitude maternal population (2600 meters) was significantly less than that from a site at lower altitude (600 meters). This change in seed germination was accompanied by an increase in polyphenol compounds present in the seed

coat which were shown to inhibit seed germination (Dorne 1981).

Maternal environment therefore has a marked effect on the germination of sibling seed but, when dealing with wild seed populations from a range of maternal sites, it is difficult, if not impossible, to assign changes in seed germinability to any one environmental variable. Groves *et.al.* (1982) found marked differences in the level of germination of *T. triandra* seed collected from a wide range of maternal sites across Australia and Tasmania, but found no clear correlation between germinability and maternal climate. A better correlation between maternal environment and the level of seed germination may have been achieved had sites of similar latitude been selected across an altitudinal gradient so as to minimise the effect of variation in day length experienced at maternal sites. The time taken for *T. triandra* seed to lose dormancy during dry storage also differed markedly between different seed populations (Groves *et.al.* 1982).

1.2.4 Dry after-ripening of seed

Vegis (1964) recognised that the range of temperatures at which a seed may germinate decreased as the level of dormancy increased. Conversely, as dormancy was lost so the window of temperatures at which germination was possible widened. These transitional states are known as states of conditional dormancy (Karssen 1980). This concept has been expanded (Baskin and Baskin 1985) to account for seasonal changes in the level of dormancy of soil-stored seed (Baskin and Baskin 1978, 1981a, 1981b, 1983; Baskin, Baskin and McCormick 1987).

As dormancy is lost during after-ripening, a process describing the progressive loss of primary seed dormancy during dry storage, seeds gain the ability to germinate over an ever increasing window of environmental conditions. Conversely, under conditions unfavourable to germination secondary dormancy may be induced. As seeds enter a state of secondary dormancy the window of conditions over which

germination can occur, decreases, until germination is not possible under normally favourable environmental conditions. Thus, secondary dormancy is of ecological importance in controlling field germination of soil-stored seed.

1.2.5 The occurrence of dormancy in grasses

Seed dormancy in grasses is widespread and has been extensively researched. Research emphasis has largely been directed at cereal seed and has focused on maximisation of seed storage and overcoming dormancy / promoting germination of grain and cereal seed. The literature has been comprehensively reviewed by Simpson (1990) and will not be elaborated further. Relevant literature will be discussed in the context of the individual Chapters which follow.

1.3 Research hypotheses and objectives

As has already been pointed out, *Themeda triandra* is acknowledged as a dominant species of African and Australian grassland and savanna communities (Mott 1978; Tainton 1984; Lowe 1989; Everson 1994). The species decreases in abundance with over or under utilisation (Tainton 1984: Everson 1985) and regeneration from seed, following disturbance, is poor (Everson 1994). *Themeda triandra* is palatable and highly favoured as a grazing grass. There is thus a desire to see restoration of *T. triandra* dominated grassland. Contrary to the dogma which advocates that *T. triandra* cannot be re-established artificially (ie: from sown seed), recent research (McDougall 1989; Sindel and Groves 1990; Sindel *et.al.* 1993) has highlighted the potential for artificial re-establishment of *T. triandra*. The availability of *T. triandra* seed is, however, limited and by nature of the morphology of the grass is likely to always be so (Sindel *et.al.* 1993). A need therefore exists for optimisation of aspects related to the germination of *T. triandra* seed. The research reported in this Thesis was designed to address this identified need.

The breadth of research covered in this Thesis reflects the diversity of processes

which influence seed germination and ultimately seedling establishment. The objectives of this research were thus also diverse, but can be summarised as a single primary objective; "To improve the level of understanding of *T. triandra* seed germination behaviour in order to maximise the germination of available seed". To achieve this objective the following areas of research were pursued:
[see also Figure 1.1 for a flow chart of research areas covered]

(a) Optimisation of *T. triandra* seed storage and investigation of ways of accelerating the seed after-ripening process [Chapter 3].

(b) Clarification of the role of the seed coat (glumes) in the imposition of primary seed dormancy [Chapter 4].

(c) Investigation of the germination response of *T. triandra* seed to environmental variables [Chapters 5].

(d) Assessment of the germination response to environmental variables of field-aged (over-wintered) *T. triandra* seed to verify conclusions drawn from data obtained in Chapters 3 and 5 [Chapter 6].

(e) Investigation of means of overcoming primary seed dormancy in *T. triandra* and promotion of *T. triandra* seed germination to obtain a better understanding of the mechanism governing seed dormancy and germination [Chapter 7].

(f) Determination of the role of plant-derived smoke in promoting *T. triandra* seed germination. This component of the research encompassed investigation of (i) the mechanism by which smoke promotes *T. triandra* seed germination, (ii) the importance of the nature of the plant material burned and (iii) practical aspects regarding smoke stimulated germination of *T. triandra* seed [Chapter 8].

(g) Investigation into the pre-treatment of *T. triandra* seed to maximise germination and assessment of the effects of seed pre-treatment on *T. triandra* seedling growth [Chapter 9].

(h) The final area researched involved confirmation that ecotypic differentiation

has occurred between altitudinally separate *T. triandra* populations in KwaZulu Natal and assessment of the ability of altitudinally and morphologically distinct *T. triandra* ecotypes to survive and reproduce in reciprocal climates [Chapter 10].

The research covered in Chapters 3 to 9 above, relates directly to the primary objective listed. The field trials reported in Chapter 10 related not to maximisation of *T. triandra* seed germination, but rather to testing the ecotypic limits of tolerance of altitudinally separate *T. triandra* populations. With the availability and supply of *T. triandra* seed for use in revegetation likely to be limited, it is probable that *T. triandra* seed harvested in one region will be sown in regions dominated by different *T. triandra* ecotypes. Although not a desirable practice, and one which urgently requires further research attention, the ability of *T. triandra* ecotypes to survive and reproduce under reciprocal climatic conditions required investigation and has direct bearing on the increased use of *T. triandra* in revegetation of disturbed areas.

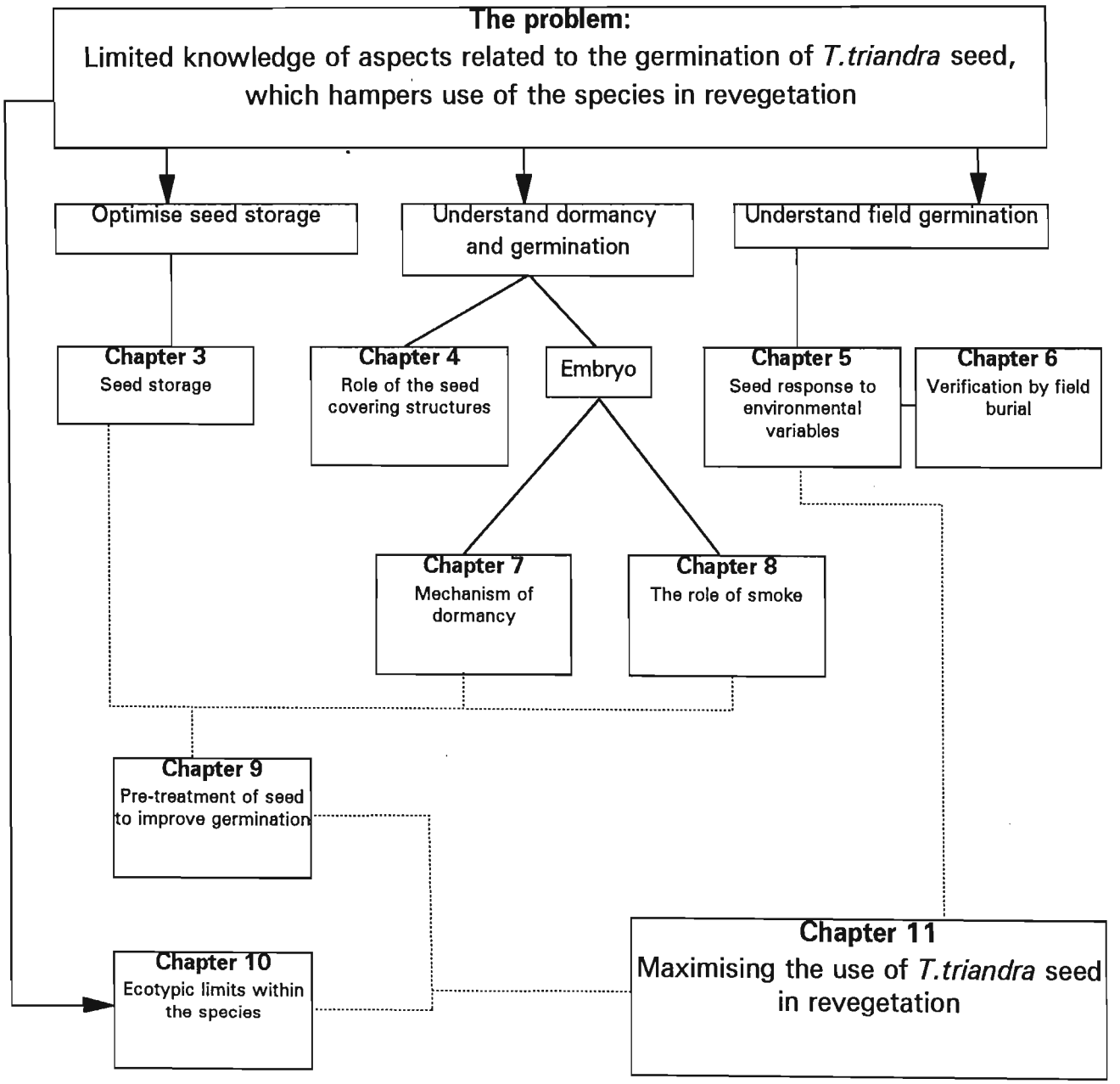


Figure 1.1: Flow chart detailing areas researched, chapters in which the research is reported and inter-relations between research components to achieve the primary objective of maximising use of *Themeda triandra* seed in revegetation.

CHAPTER 2: CHARACTERISATION OF THE PROBLEM AND THE SPECIES

2.1 Introduction

The mechanisms governing seed dormancy in *Themeda triandra* have, in part, been described. The role of the seed covering structures in imposing dormancy is attributed to a mechanical restraint (Martin 1975), while gibberellins (Groves *et.al* 1982), promoted endogenously by boron availability, govern release from seed dormancy (Cresswell and Nelson 1971; 1972; 1973). Furthermore, the response of non-dormant seed to light and temperature have been described (Hagon 1976; Mott 1978; Groves *et.al* 1982).

After evaluation of the published literature (Groves *et.al.* 1982; Hagon 1976) it was assumed that a deep level of dormancy would exist in seed produced at high altitudes (Drakensberg *T. triandra* populations) and that in low altitude, populations (Zululand) the level of seed dormancy in *T. triandra* would not be as pronounced. Two localities with *T. triandra*-dominated grassland or savanna populations were identified. These localities were at Cathedral Peak (Drakensberg) and in the Umfolozi Game Reserve (Zululand). Seed was harvested from these sites and germination assessed. Initial results of germination trials confirmed the assumption that the level of dormancy would be higher in the Drakensberg population than in the Zululand population, thereby justifying continuation of research involving seed from the identified localities. Furthermore, germination of intact seed was significantly lower than that of excised caryopses (Table 2.1), confirming that seed dormancy in the selected populations is, in part, imposed by the seed covering structures. Not all seed germinated following removal of the seed covering structures, indicating that in more than 50 percent of the Zululand and 75 percent of the Drakensberg seed population the embryo was dormant at seed shed.

Table 2.1: Comparison of the germination[1] of intact *Themeda triandra* seed with that of excised caryopses, from which the robust glumes had been removed. Within a column data followed by different letters are significantly different ($P \leq 0.05$)[2]

Seed state	Germination (%)			
	Drakensberg		Zululand	
Intact	5.2 (2.1)	a	34.0 (2.8)	a
Excised	24.2 (3.8)	b	48.7 (3.3)	b

1 Germination methods are described on pg. 31 (dormant seed populations.
2 One way ANOVA, followed by Tukeys Multiple Range test (Sokal and Rolf 1981)

2.2 Characterisation of the parental environment

2.2.1 Geographic location of parental seed sites

An east-west environmental gradient exists from the coast of Natal to the summit of the Drakensberg mountains, the highest peaks of which occur approximately 3000 m above sea level. The two selected sites were situated towards opposite extremes of this gradient, but at similar latitudes (Figure 2.1). The Zululand site was situated within the Umfolozi Game Reserve (28° 08′ S, 32° 17′ E) at the lower end of the gradient, at an altitude of 90 m above mean sea level. The selected site was situated in a shallow valley on a gentle south east facing slope. The savanna grassland was subject to regular controlled management burns and intermittent wild fires. The Drakensberg parent seed population was located at Cathedral Peak (28° 57′ S, 29° 14′ E) at an altitude of 1800 m. The site was located on a gentle east facing slope below the top of Mikes Pass in an area managed by the Natal Parks Board since 1986 and previously by the Directorate of Forestry since the mid 1930's, primarily for water conservation purposes. The grassland was undisturbed and subject to regular controlled management burns (biennial spring burns) and intermittent wild fires.

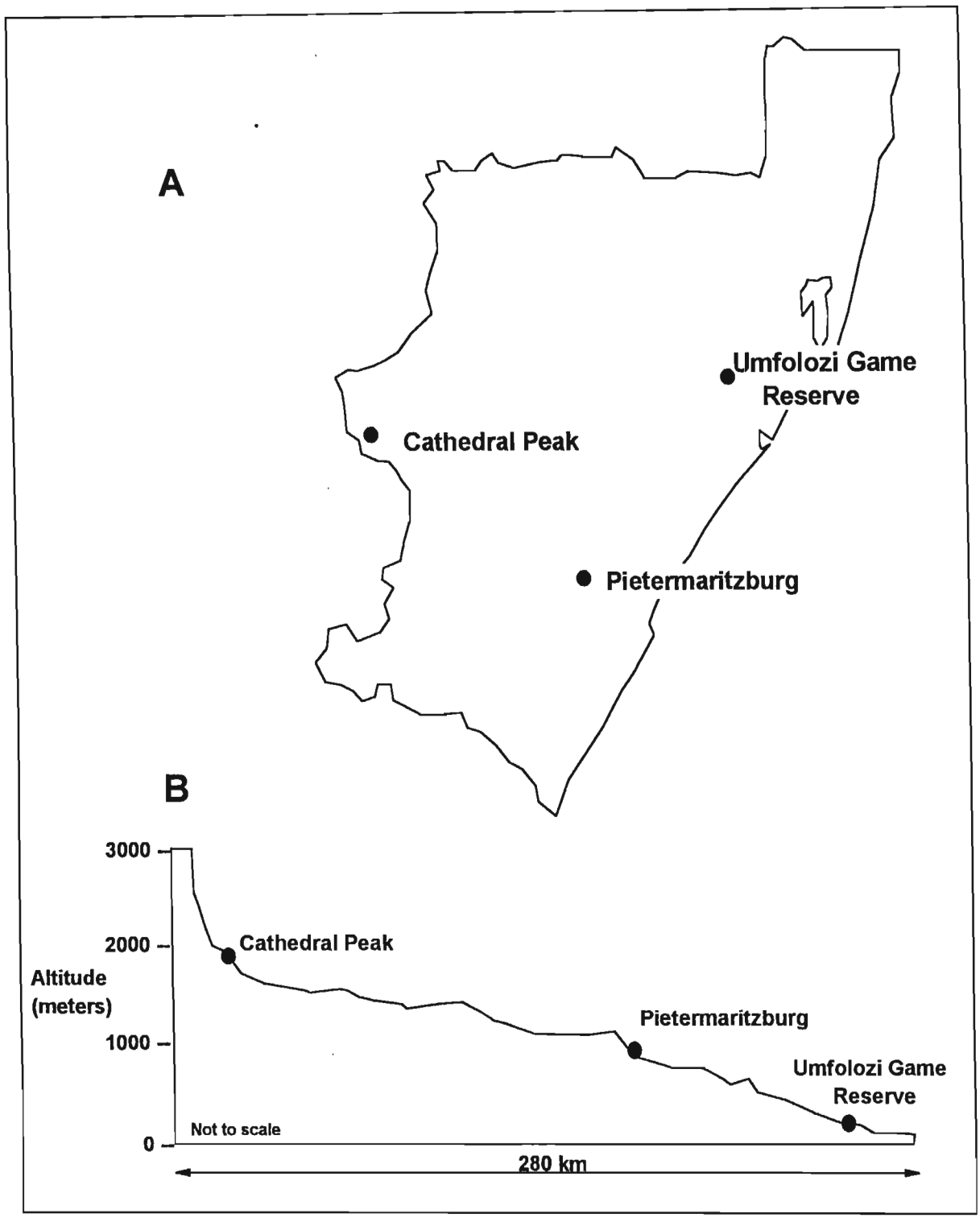


Figure 2.1: Locality of parental sites. A Geographic location of the seed collection sites at Cathedral Peak and the Umfolozi Game Reserve and location of the University on Natal (Pietermaritzburg) at which laboratory investigations were undertaken. B Diagrammatic representation of the altitudinal gradient along which the parental sites are situated.

2.2.2 Climate

Meterological data were obtained from the Computing Centre for Water Research (CCWR), University of Natal, Pietermaritzburg. Data from the nearest reliable meterological station were used. In Zululand, such data were obtained from the Hluhluwe station (altitude 45 m: 63 year record) and Drakensberg data from the meterological station at Cathedral Peak located within 5 kilometres of the parent site (altitude 1844 m: 45 year record). Climatic diagrams for each site are presented (Figure 2.2).

The mean annual rainfall of the Umfolozi Game Reserve is 1050 mm which is higher than that of the Hluhluwe station. The annual distribution of rainfall was however comparable, with 75 % of annual rainfall falling in summer between October and March (Figure 2.2). Mean annual rainfall at Cathedral Peak is 1355 mm, of which 82 % falls between October and March. Both sites experience a dry winter period lasting four months in Zululand (May to August) and two months at Cathedral Peak (June and July). Mean annual temperatures at Cathedral Peak are substantially lower than temperatures experienced in Zululand.

2.2.3 Vegetation

Vegetation within the Umfolozi Game Reserve was described by Acocks (1953) as eastern mixed bushveld at low elevations and by Phillips (1971) as interior lowland. This vegetation type constitutes a savanna and bushveld community in which the tree component is dominated by *Acacia nigrescens* and *Sclerocarpa caffra* with *A. karoo* forming dense thickets. In the savanna grasslands *Themeda triandra* dominates in open areas, but is replaced by *Panicum maximum* in shaded areas under trees. Over-utilisation results in a change in species composition. Less palatable species, including *Aristida congesta* sub.sp. *congesta*, *A. bipartita*, *Eragrostis* spp., *Schmidtia pappophoroides* and *Urochloa mosambicensis* increase in abundance at the expense of palatable dominants, including *T. triandra*.

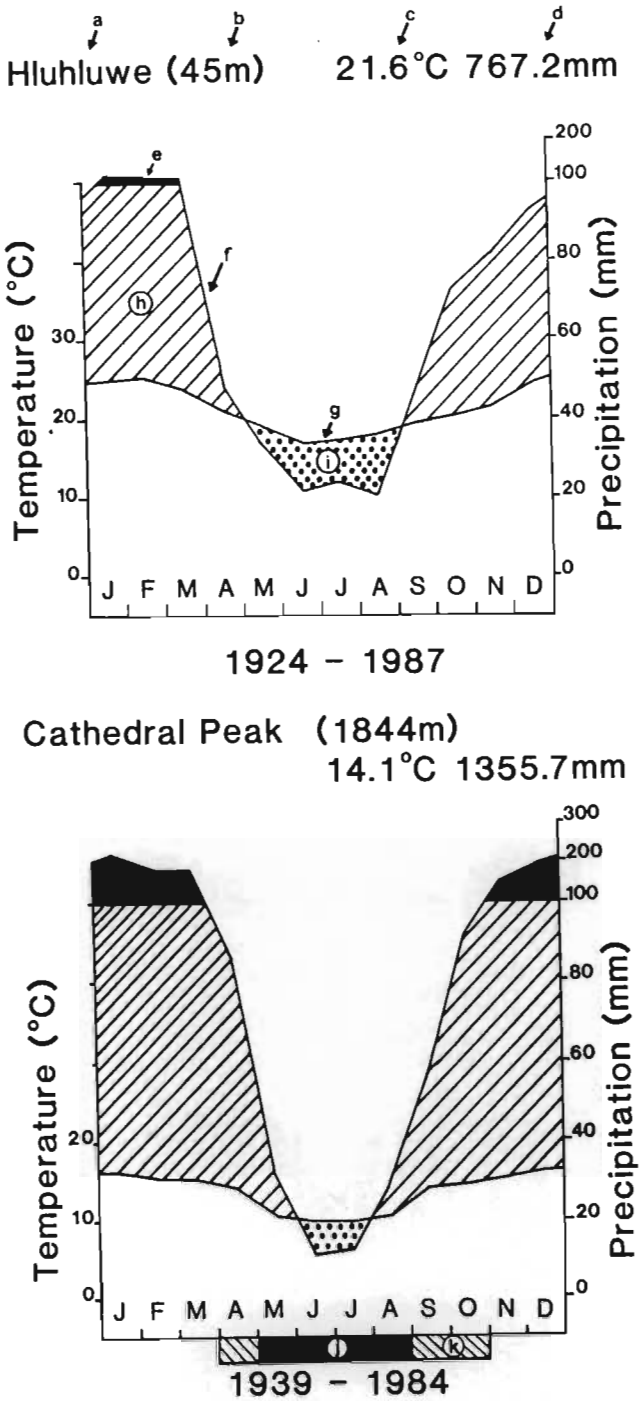


Figure 2.2 Climatic diagrams representative of climatic conditions at the low altitude Zululand and high altitude Drakensberg sites. Data used represents a period of 63 years and 45 years respectively. Data from meterological stations closest to the study sites were used. [a station; b height above sea level; c mean annual temperature; d mean annual precipitation; e mean monthly precipitation exceeding 100 mm with the scale reduced to 1/10 (black area); f curve showing mean monthly precipitation; g curve showing monthly mean temperatures; h humid period; i arid period; j minimum temperature 0°C; k frost]

The Drakensberg seed parental site was located in fire-climax grasslands (Acocks 1953), described by Phillips (1971) as highland to sub-montane grassland. The region is characterised by large expanses of open sub-climax grassland interspersed with localised densely wooded stream courses and forest patches on the cool moist south facing slopes. The grassland community is dominated by palatable tufted species including *Themeda triandra, Heteropogon contortus* and *Trachypogon spicatus*. This short-grass community is regarded as being highly stable (Tainton 1984). Degeneration is associated with a change in species composition rather that a reduction in basal area. Dominant species, including *T. triandra,* are replaced by species of lower palatability. Even if spared, the original species complement does not again attain dominance and pioneer forest species, including the woody shrubs *Buddleja salviifolia* and *Leucosidea sericea*, invade the grassland.

In the Zululand savanna environment *T. triandra* is particularly robust, growing to 1.5 m in height and having a small basal area. In contrast, in the fire-climax grasslands of the Natal Drakensberg *T. triandra* seldom exceeds 0.5 m in height and is densely tufted (Meredith 1955).

2.3 Seed Harvest

Mature seed bearing culms were hand-harvested in the field in late December in the Drakensberg and in mid-March in Zululand, when the majority of seed was mature. Seed maturity was visually assessed in the field. Cut, seed bearing, culms were transported to Pietermaritzburg on the same day of the harvest and spread out on plastic sheets in a greenhouse to air dry. When dry, the seeds were shaken loose from the reproductive culms. This procedure effectively screened the seed because only mature, black seeds were shed. The few immature, light coloured seeds which were shed were isolated and discarded at this time, or when seed was cleaned and counted for experimental use. It is therefore important to note that

all experiments, including the determination of seed viability, were conducted on ripe, mature dark brown *T. triandra* seeds. This seed fraction represented approximately 65 per cent of seed present in a stand at the time of harvest. McDougall (1989) reported ± 50 percent ripe *T. triandra* seed in Australian grasslands. After eight weeks of air drying seed was collected and stored at room temperature (25°C±3°C) in brown paper packets in a dark cupboard, or in glass bottles in a deep freeze (-15°C±2°C) dedicated for the storage of seed material. These storage conditions were selected to allow after-ripening of seed at room temperature and to prevent after-ripening through seed storage at sub-zero temperatures (Ellis, Hong and Roberts 1989). The later storage regime was employed to maintain seed in the dormant state for use in latter experiments. The optimisation of seed storage conditions for *T. triandra* is discussed further in Chapter 3.

2.4 Anatomy of the *Themeda triandra* seed dispersal unit.

The inflorescence of *T. triandra* is paniculate, bearing spikelets in clusters composed of 1-3 spikelet pairs surrounded by a whorl of four male or sterile sessile spikelets born on reduced axes. At maturity, each dispersal unit consists of a bearded callus, caryopsis and hydroscopic, geniculate awn (Figure 2.3 a). The caryopsis, consisting of embryo, scutellum and starchy endosperm, is enclosed by a membranous lower lemma (Figure 2.3 c). The upper lemma forms the geniculate awn. These structures are enclosed by a robust upper glume, which, as is characteristic of the Andropogoneae, is tightly overlapped by the tough lower glume (Figure 2.3 b, c) (Gibbs Russel *et.al.* 1991). The single-seeded dispersal unit (spikelet) is shed entire because the axis disarticulates below the glumes, affording good protection to the caryopsis (Simpson 1990).

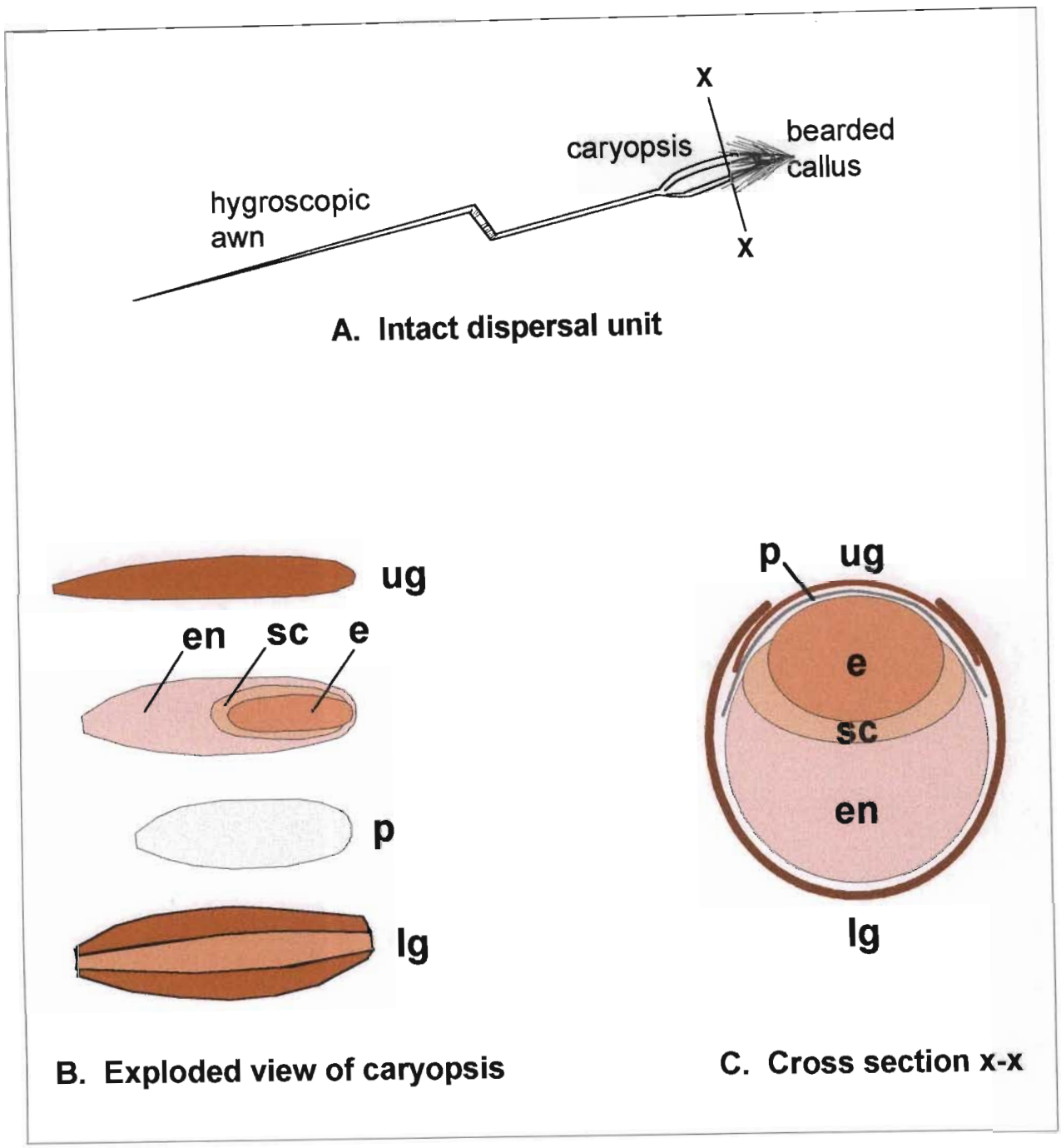


Figure 2.3: Structure of the *Themeda triandra* seed dispersal unit. A Components of the intact dispersal unit. B Exploded view of the caryopsis to indicate caryopsis parts. C Cross section through a caryopsis to indicated the orientation of caryopsis parts. [ug upper glume: lg lower glume: p palea: en endosperm: sc scutellum: e embryo]

For the purpose of this research the dispersal unit, consisting of upper and lower glumes, membranous lower lemma, the caryopsis and callus, but excluding the geniculate awn has been referred to as a **caryopsis**, or **seed** , and represents the basic "seed unit" manipulated experimentally. Where manipulation of the true caryopsis (embryo, scutellum and endosperm) has been undertaken this structure is referred to as an **excised caryopsis**. In all experiments the geniculate awn was removed to facilitate handling. Removal of the awn did not influence subsequent germination of the caryopsis.

2.5 Karyology

As described in Chapter 1, *Themeda triandra* is a facultative aposporic apomict (Spies and Gibbs Russel 1988) with a basic chromosome number of 10, but diploid to 11-ploid specimens have been recorded (Liebenberg 1986). *Themeda triandra* populations sampled by Liebenberg (1986) in the Natal Drakensberg were predominantly tetraploid, while in contrast, diploid, tetraploid and pentaploid populations were encountered in Zululand. Furthermore, within a population, individuals of different ploidy (Hayman 1960; Fossey and Liebenberg 1987) and individuals belonging to different apomictic clones (Fossey and Liebenberg 1992) occur. Cytological characterisation of the parent populations under investigation fell outside of the scope of this research.

CHAPTER 3: SEED VIABILITY, STORAGE AND AFTER-RIPENING.

3.1 Introduction

Seed viability is defined as the capacity of a seed to germinate under favourable conditions, in the absence of any blocks to germination. Viability is thus an indicator of potential germination and is distinct from seed vigour which, following the definitions of Perry (1978), McDonald (1980) and Basu (1994), is more wide ranging and defined as the capacity of a seed to germinate rapidly, uniformly and to achieve good field emergence over a wide range of environmental conditions. Loss of seed vigour precedes loss of viability (Abu-Shakara and Ching 1967; Bray and Dasgupta 1976). Seed viability is also distinct from seed longevity which reflects the period over which seed remains viable in storage.

Recovery of viable seed following periods of storage approaching, or exceeding, 100 years is not uncommon. A number of examples where seed longevity exceeds 75 years seeds are listed (Table 3.1). In all these instances seed was stored in the

Table 3.1: Documented examples where seed longevity exceeds 75 years.

Species	Longevity (years)	Germination	Storage	Reference
Albizzia julibrissin	147	-	Herbarium	*Anon 1942*
Anthryllis vulneraria	90	4 %	Laboratory	*Harrington 1972*
Avena spp.	123	21 %	Sealed glass tube[1]	*Aufhammer 1957*
Cassia multijuga	158	-	Museum	*Becquerel 1934*
Hordeum spp.	123	12 %	Sealed glass tube[1]	*Aufhammer 1957*
Leucaena leucocephala	99	3 out of 10	Museum	*Becquerel 1934*
Mimosa glomerata	81	5 out of 10	Museum	*Becquerel 1934*
Medicago sativa	78	22 %	Laboratory	*Harrington 1972*

dry state either in a laboratory, or as dry museum or herbarium material. Seeds of greater age have been recovered, most notably from Egyptian tombs dating back to 1350 and 4000 BC but, although intact, were not viable (Hallam 1973).

Two broad groups of seeds, orthodox and recalcitrant, are recognised on the basis of seed storage behaviour (Roberts 1973). Orthodox seeds, which include the majority of crop, cereal and grass species, are tolerant of drying to low moisture contents. Furthermore, the longevity of orthodox seed increases in a predictable and quantifiable manner as seed moisture content and temperature decreases (Roberts and Ellis 1982). Longevity of orthodox seed is improved by desiccation to seed water potentials as low as -350 MPa (Ellis, Hong and Roberts 1989). In contrast, recalcitrant seeds are intolerant of drying and do not survive desiccation below - 5.0 MPa (Roberts and Ellis 1989). Recently, a third intermediate category, in which dry seeds (-90 to -250 Mpa) suffer injury when exposed to low temperatures and further desiccation has been suggested (Ellis, Hong, Roberts and Tao 1990; Ellis, Hong and Roberts 1990). All examples covering the retention of seed viability during prolonged storage involve orthodox seed. *Themeda triandra* produces orthodox seed.

The objectives of seed storage are maintenance of seed viability and seed vigour. Although optimum storage conditions for crop and cereal seed are well documented (Roberts 1973; Ellis *et.al.* 1989; Ellis, Hong, Roberts and Tao 1990; Ellis, Hong and Roberts 1990) optimum storage conditions for seed of wild species have to be determined empirically. Certain rules of thumb do, however, apply for orthodox seeds (Harringtob 1973), namely;

(a) for each 1 % decrease in moisture content the storage life of the seed doubles. (Possibly more correct to say for 2 % decrease in moisture content).

(b) for each 10°F (5.6°C) decrease in temperature the seed storage life is

doubled. [seed moisture content calculated on a wet mass basis]

In addition, retention of seed viability during dry storage may be improved by storing the seed at reduced oxygen concentrations, achieved by storage under CO_2 (Bass 1973), N_2 (Roberts and Abdalla 1968) or under partial vacuum (Bass 1973). The beneficial effect of reduced oxygen concentration on the retention of seed viability decreases as both storage temperature and moisture content improve.

Seed deterioration during storage yields aged seeds with reduced viability and vigour and has been associated with a high degree of mitochondrial disruption (Hallam 1973; Hallam, Roberts and Osborne 1973; Berjak and Villiers 1972), reduced ATP content (Ching 1973; Anderson 1977), reduction in respiratory (Anderson 1970; Aspinall and Paleg 1971; Van Onckelen, Verbeek and Khan 1974) and hydrolytic (Throneberry and Smith 1955) enzyme activity and reduction in protein synthesis (Roberts and Osborne 1973) attributed to a decrease in the capacity to synthesize RNA (Anderson 1977) which results in a loss of metabolic vigour. Seed deterioration is also accompanied by increased solute leakage, attributed to the loss of membrane integrity (Abdul-Baki and Anderson 1970), but correlation between solute leakage and loss of viability is poor. Although metabolism can take place in air-dry seed, dependant on seed water content (Edwards 1976), mobilisation of stored reserves does not occur and is not associated with loss of seed viability during dry storage, unless through fungal hydrolysis of reserve compounds (Bewley and Black 1984).

Seed viability is widely assessed using the tetrazolium test which involves seed imbibition in solutions of 2,3,5 - triphenyltetrazolium chloride (or bromide). Enzymatic reduction (dehydrogenase enzymes) of the tetrazolium salt yields a red coloured compound called formazan. In seeds aged naturally, or stored under favourable conditions, this test yields a good correlation between reduction of the tetrazolium salt and enzymatic activity (MacKay 1972). It is, however, recognised

that viability may be over estimated following accelerated ageing where seed viability may be reduced without a similar reduction in dehydrogenase enzyme activity (Woodstock 1973). Donald (1994) reported that viability of after-ripened *Aegilops cylindrica* caryopses is underestimated using the tetrazolium assay.

In soil, seeds are also known to survive for long periods. The ability of a seed to remain viable in the soil seed bank confers a competitive advantage on that species by increasing the temporal distribution of propagules of the species. Loss of seed from the soil seed bank occurs primarily through germination (Taylorson 1972; Zorner 1981), although loss of viability and fatal germination, which increases with depth of burial, (Fenner 1985) can be significant. Pathogenic influences play a major role in determining the viability and longevity of soil-stored seed (Leck, Parker and Simpson 1989). Seed-borne bacteria do not play a major role in seed deterioration in dry storage and have not been shown to significantly decrease seed germination, unless bacterial infection has progressed to the point of seed decay (Bewley and Black 1984). Similarly, fungi exert little effect on seed stored at low moisture contents (relative humidity less than 68 %), but may accelerate the loss of seed viability at high moisture contents (Christensen 1972), as found in soil.

To enable wild seed to be used effectively in landscape restoration it is necessary that the level of seed viability in wild populations be known. In addition, the level of seed dormancy within wild populations and the duration of the after-ripening period required for dormancy to be lost should be quantified. Seed storage conditions required to facilitate after-ripening, but limit the loss of seed viability should also be optimised. These issues are addressed in this Chapter for *T. triandra*.

3.2 Methods

Seed moisture content was determined on a wet mass basis. Seed material was dried at 70°C for 24 hours (Anon 1985a), by which time constant mass had been attained. For each moisture content determination thirty replicates of three seeds, drawn randomly from the bulk seed store, were used. The moisture content of *T. triandra* seed in storage was determined at monthly intervals.

Seed viability was determined as recommended by the International Seed Testing Association (ISTA) (Anon 1985b). Naked *T. triandra* caryopses (glumes removed to expose the embryo and endosperm tissues) were pre-imbibed in distilled water for 24 hours prior to an eight hour incubation in one percent solution of 2,3,5 - Triphenyl tetrazolium chloride (TTC). Categories by which viability was determined for *T. triandra* were determined empirically and are indicated in Figure 3.1. Viability was determined at monthly intervals during seed storage. For each determination of viability three replicates of 50 caryopses were used. No seed was used for experimental purposes once viability fell below 60%. Hereafter in this thesis, all germination values are expressed as a percentage of viable seed, with viability determined for each population from the data presented in Figure 3.3.

To determine whether low moisture content, achieved by storing seed over silica gel, was detrimental to the germination and viability of seeds of *T. triandra*, seeds were placed into 5 ml pill vials situated inside 250 ml Schott bottles containing 40 g of silica gel. For each seed population, six replicates of 25 seeds were used. Germination and moisture content was determined after 0, 6, 12 and 24 hours and thereafter after 2, 4, 8, 16, 32 and 64 days. The experiment was conducted in the dark at 25°C ± 3°C. Following storage over silica gel, germination was assessed at 25°C ± 3°C. Hereafter, unless otherwise stated germination was assessed as follows; six replicates of 25 seeds were placed on two layers of

Whatmans No. 1 filter paper in 9-cm diameter disposable plastic Petri dishes and moistened with 4.5 ml of distilled water. Germination was assessed at either 30°C (dormant seed population) or at 25°C (non-dormant seed population) under constant light. A seed was considered germinated, and removed, when the emerged radicle exceeded 1 mm in length. Germination was assessed at two-day intervals and seldom occurred after the sixth day. All experiments were terminated on the tenth day.

Optimum storage conditions for *T. triandra* seed was determined by storing fresh (eight-week-old) *T. triandra* seed from both the Drakensberg and Zululand seed populations in brown paper packets at -15°C, 10°C, 25°C, 40°C, 70°C and 15/30°C (12h/12h). Changes in seed germination, moisture content and viability were monitored over a period of six months. At all storage temperatures, a temperature fluctuation of within ± 3°C occurred. After storage, germination was assessed at 30°C under constant light.

3.3 Results

Themeda triandra seed was considered viable when the radicle, plumule and scutellum were stained red (Figure 3.1). Seeds were classified non-viable if the radicle (Figure 3.1), plumule (Figure 3.1) or both radicle and plumule (Figure 3.1) tissues were unstained following incubation in TTC.

In both Drakensberg and Zululand populations of *T. triandra* seed, moisture content at the time of seed shed was between 15% and 16.5%, but decreased during post-harvest air drying to 10.1% (± 0.8%) and 7.0% (± 0.5%) respectively (Table 3.2). During storage at either room temperature (25°C ± 2°C), or when frozen (-15°C ± 2°C), seed moisture content for both seed populations remained within the range 7.0% - 8.1% and 7.2% - 8.1% respectively.

Storage temperature had a marked effect on the level of germination, viability and moisture content of seed of *T. triandra* seed (Figure 3.2). At -15°C little change in either germination, viability or moisture content was evident over a 6 month (24 week) storage period. A similar trend was evident at 10°C, although germination increased slightly after 8 weeks. In contrast, at 25°C and 15/35°C progressive after-ripening occurred and germination increased from 8% to 63% (25°C) and 51% (15/35°C) respectively. Over the same time period viability decreased from 81.3% to 72% (25°C) and 66% (15/35°C) respectively, but little change in seed moisture content occurred. At 40°C the after-ripening process was accelerated and 100% of viable seed germinated after only 16 weeks. Seed viability, however, decreased rapidly from 81% to 62% within 16 weeks and was only 8%

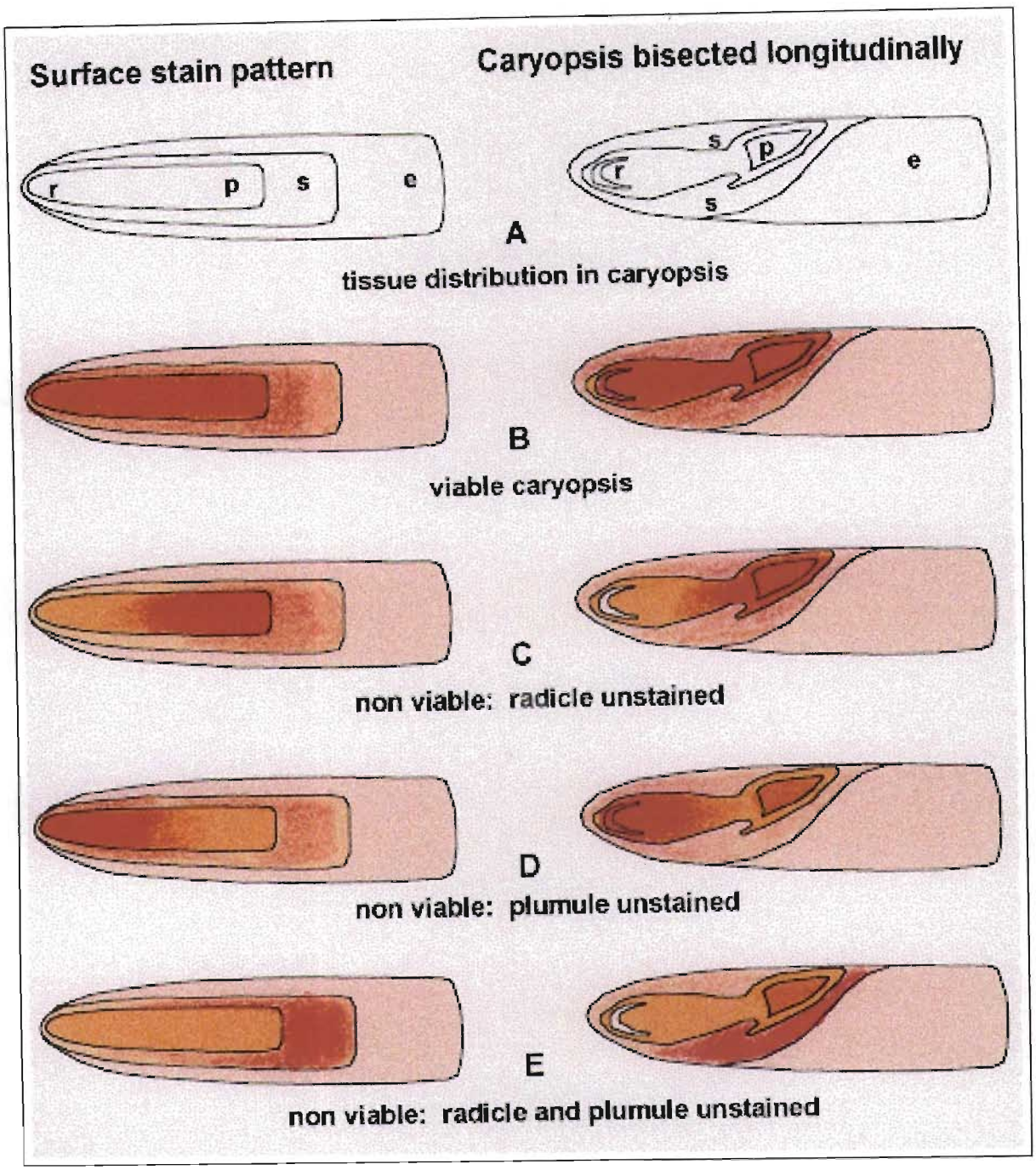


Figure 3.1: Evaluation of *Themeda triandra* seed viability based on the stain pattern present following incubation of caryopses in a 1 percent Tetrazolium solution. Red areas indicate viable tissue. The left hand figures represent the surface view of a typical caryopses, while the figures on the right hand side represent a longitudinal view through a typical caryopsis. **A** Diagram identifying caryopsis parts. **B** Viable caryopsis with radicle, plumule and scutellum stained. **C** Non-viable caryopsis, radicle unstained. **D** Non-viable caryopsis, plumule unstained. **E** Non-viable caryopsis, radicle and plumule unstained. [**r** radicle, **p** plumule, **e** endosperm, **s** scutellum].

on termination of the experiment after a total of 24 weeks. The loss of seed viability, and hence germinable seed, was accompanied by a decrease in seed

Table 3.2: Moisture content (% wet mass) of *Themeda triandra* seed at harvest, at the start of storage and during prolonged storage at 25°C±2°C or -15°C±2°C. Data represent mean±SE. Within square brackets, n = the number of moisture content determinations made during the period in storage.

Storage	Seed population	
	Drakensberg	Zululand
Seed Shed	15.5 (± 0.9)	16.4 (± 0.3)
2 Months air drying	10.1 (± 0.8)	7.0 (± 0.5)
After-ripened (25°C±2°C)	7.7 (± 0.4) [n=4: 34 months]	7.4 (± 0.4) [n=7: 40 months]
Frozen (-15°C±2°C)	8.0 (± 0.1) [n=6: 34 months]	7.4 (± 0.2) [n=5: 40 months]

moisture content. This trend was exaggerated at 70°C where short exposure of dry seed to high temperature promoted germination (see Chapter 7), but longer periods of seed exposure to high temperature caused a loss of seed viability. No viable seed was present after 4 weeks at 70°C.

At seed shed *T. triandra* seed from the Drakensberg is deeply dormant with less than 5% of seed germinating, although viability equals 82.5% (Figure 3.3 A). At room temperature (25°C±3°C) a period of after-ripening lasting 8 months was required for dormancy to be lost (> 80 % germination). Thereafter, germination of viable seed exceeded 80% for the duration of the experiment (36 months), but seed viability decreased from 82.5% to 55.3% after 36 months in storage at

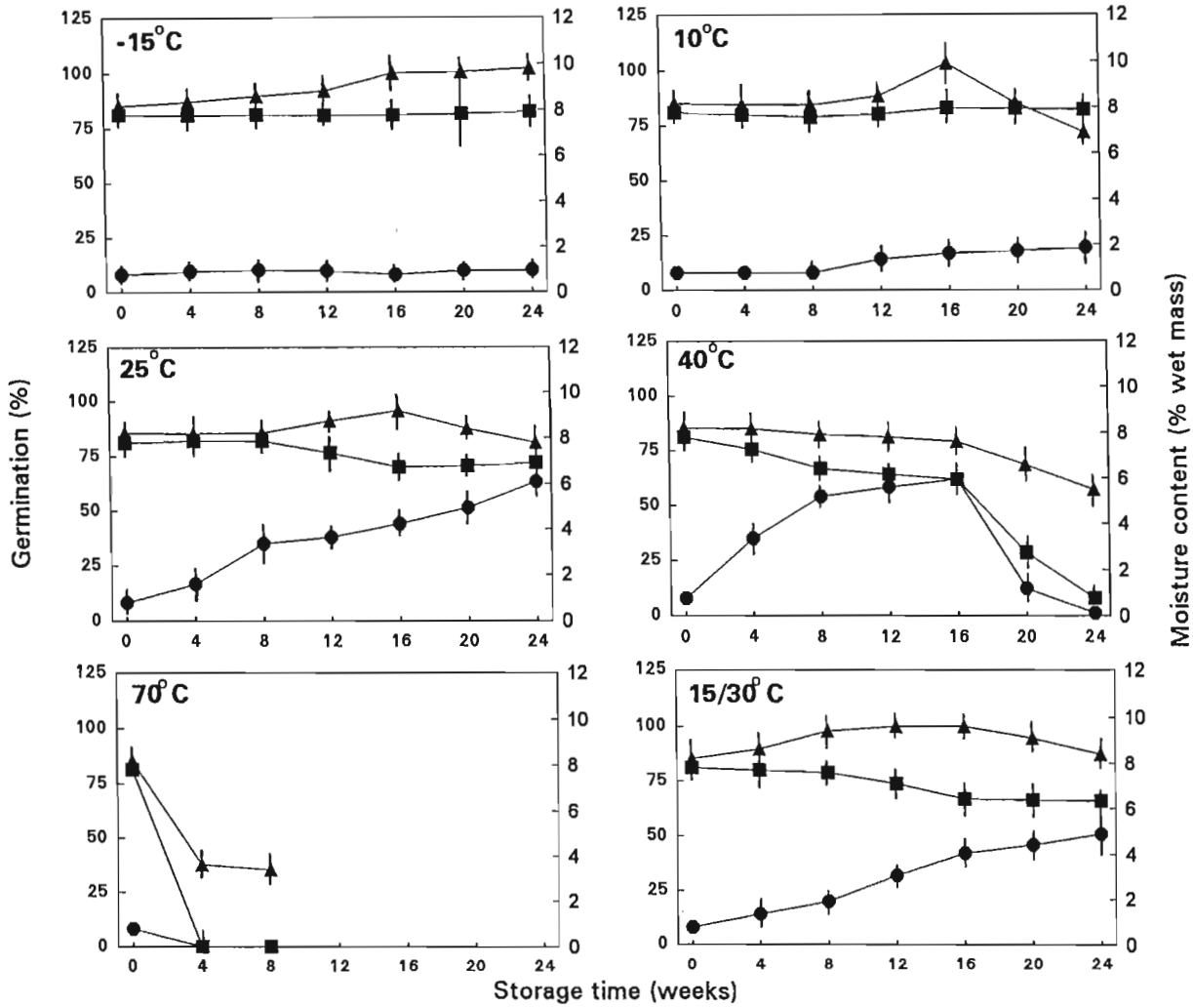


Figure 3.2: The effect of storage temperature on germination, viability and moisture content of *Themeda triandra* seed from the dormant Drakensberg population. Storage treatments were initiated on eight-week-old dormant seed. Bars represent ± SE. [▲ moisture content: ● germination: ■ viability]

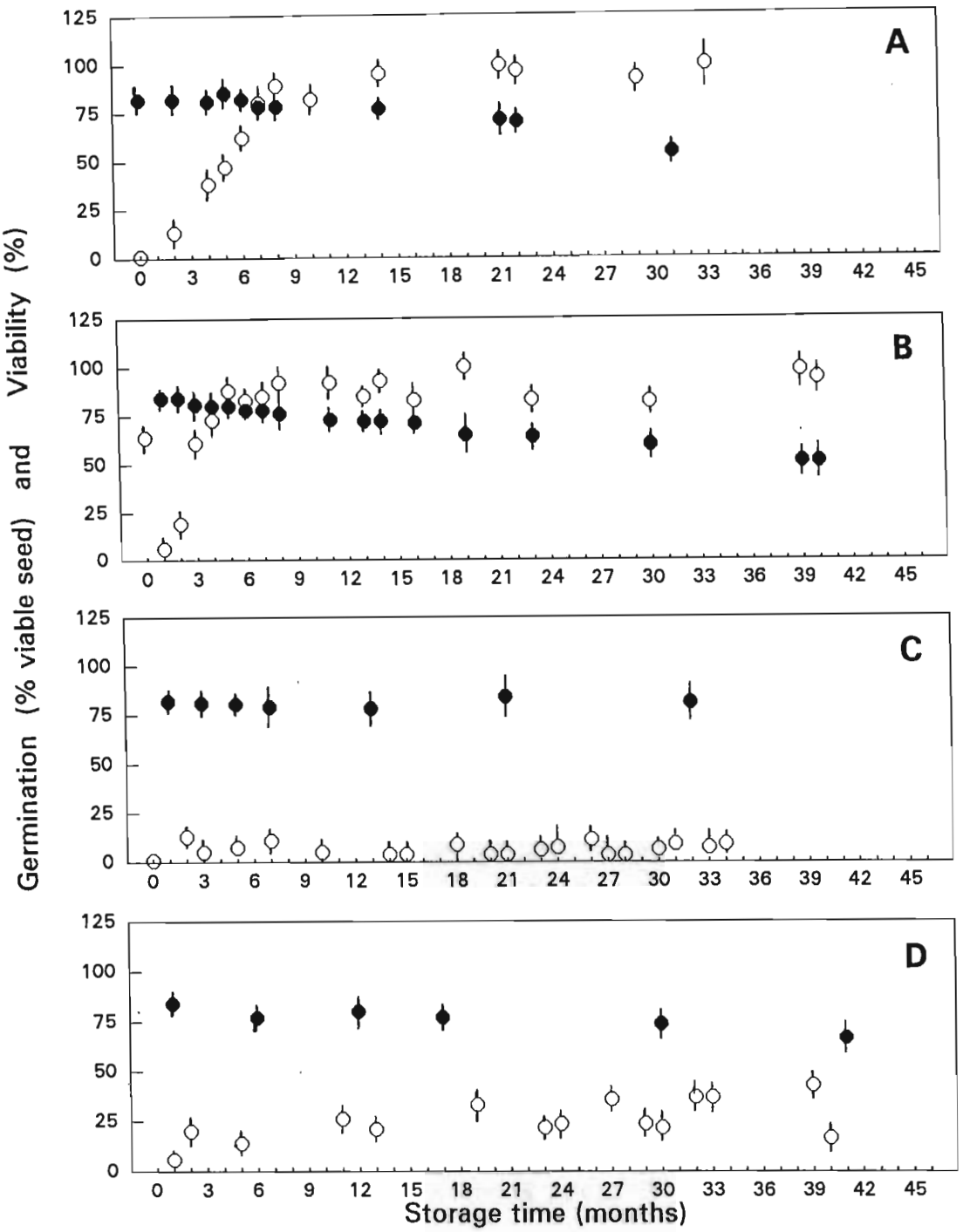


Figure 3.3: Change in germination and viability of *Themeda triandra* seed during dry storage at 25°C±3°C or -15°C±2°C. **A** Drakensberg and **B** Zululand seed stored at 25°C ± 3°C. **C** Drakensberg and **D** Zululand seed stored at -15°C±2°C. Drakensberg seed was harvested in December 1990 and Zululand seed in March 1990. Data represent mean ± SE. [● viability: ○ germination]

25°C ± 3°C. In contrast, Drakensberg *T. triandra* seed stored at -15°C ± 2°C underwent little change in either seed germination or viability. Seed viability decreased from 82.5% to 81.3% over the 3 year storage period (Figure 3.3 C).

Themeda triandra seed from Zululand, stored at room temperature, underwent a period of after-ripening during which time primary seed dormancy was lost. Furthermore, at 25°C seed viability decreased consistently from a level of 86% at seed shed to a level of 51% after 48 months in storage (Figure 3.3 B). Two points must be noted. Firstly, the period of after-ripening required for Zululand *T. triandra* seed to attain maximum germination was only 4 months in comparison to the 8 month period required for loss of dormancy in the Drakensberg *T. triandra* population. Secondly, at seed shed 60% of *T. triandra* seed from Zululand was non-dormant (compared to < 5% of Drakensberg seed), but within 3 weeks of seed shed more than 90% of Zululand seed became dormant and required a period of after-ripening to lose dormancy.

Storage of Zululand *T. triandra* seed at -15°C ± 2°C arrested both after-ripening and the loss of seed viability (Figure 3.3 D). In the case of the Zululand seed, however, a gradual increase in the level of germination and decrease in the level of viability did occur during storage at sub zero temperatures, but not to the extent evident in seed stored at room temperature.

Storage of non-dormant *T. triandra* seed over silica gel for a prolonged period of time (64 days) did not significantly affect germination of the Drakensberg or Zululand seed (Figure 3.4), although seed moisture content fell rapidly on exposure of seed of both ecotypes to silica gel.

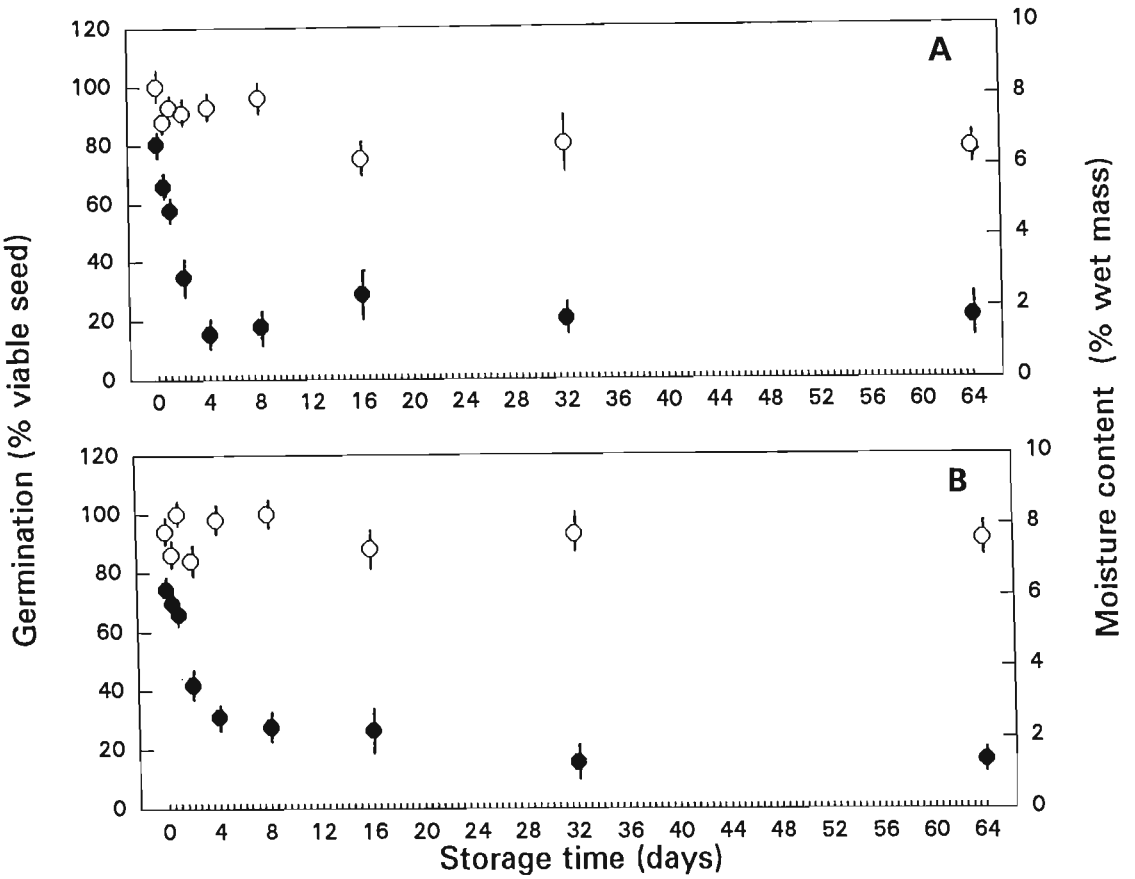


Figure 3.4: The effect of prolonged desiccation, caused by storage over silica gel, on germination and moisture content of non-dormant *Themeda triandra* seed from **A** Drakensberg and **B** Zululand populations. Data represent mean ± SE. [● moisture content: ○ germination]

3.4 Discussion

In wild species natural selection favours germination in response to specific environmental cues, thereby improving the probability of successful seedling establishment (Angevine and Chabot 1979). It is therefore not unexpected that intraspecific variation in seed germination response to environmental variables occurs between geographically and ecologically distinct populations (Thompson 1975). In *Dactylis glomerata* populations located across Europe a significant difference in seed germination response to temperature and light occurs.

Mediterranean populations possess a low level of seed dormancy while northern European populations have a high requirement for light and alternating temperature to promote germination (Probert, Smith and Birch 1985a). The requirement for increasingly specific germination cues appears to correspond to the increasing severity of the northern European winter period. Comparison of germination of seed of *D. glomerata* and *Silene vulgaris*, from geographically different sites within the British Isles (Probert, Smith and Birch 1985a) and Scandanavia (Thompson 1973) respectively, revealed that despite quantitative differences in seed germination response to environmental variables the magnitude of the response was consistent with the position of the parent ecological zone on a Mediterranean-to-northern European climatic gradient. Similarly, Groves, Hagon and Ramakrishnan (1982) report intraspecific variation in seed dormancy and germination characteristics in geographically and ecologically distinct Australian and Papua New Guinean *T. triandra* populations. The differences in depth of *T. triandra* seed dormancy and duration of the after-ripening period reported in this Chapter are discussed further in Chapter 5, in the context of changing seed germination responses to environmental variables associated with after-ripening. What is important to note, at this point, is that during dry storage of *T. triandra* seed at room temperature (25°C±3°C), for up to 48 months, the level of *T. triandra* seed viability remained above 50 percent. This result appears to contradict Everson's report (1994) that after fifteen months of dry storage at room temperature *T. triandra* seed viability had decreased from 90 percent, at seed shed, to 37 percent and had fallen to less than 20 percent after 40 months dry storage. Unfortunately, neither the storage temperature, nor the temperature range experienced during storage, was reported. Seed was, however, stored in paper packets in a field laboratory at Cathedral Peak where it was subject to diurnal temperature fluctuation and changing relative humidity (RH). The rapid loss of seed viability reported by Everson (1994) could therefore be due to fluctuating conditions of seed storage. However, there is little direct evidence to suggest that fluctuating storage conditions are harmful to seeds (Bewley and Black 1984) but,

it is well documented that the longevity of orthodox seed in dry storage is significantly affected by storage temperature and moisture content (Roberts 1973; Roberts and Ellis 1982). The difference between the longevity of *T. triandra* seed reported in this Chapter and that reported by Everson (1994), for seed collected from the same geographic location, highlights the importance of seed storage conditions in determining seed longevity. The high levels of seed viability at the time of seed shed reported in this Chapter (> 80 % for both populations) and 90 percent reported by Everson (1994) are substantially higher than the 41 percent viability reported for Australian *T. triandra* populations (M^{c}Dougall 1989). The viability values reported by M^{c}Dougall (1989) refer to all seed, both filled and unfilled, present on a reproductive culm at a point in time, whereas in this Thesis viability was determined on mature (brown/black) seed after seed sorting had taken place. The approach adopted in this Thesis was also used by Everson (1994) and provides a realistic measure of the level of viability in harvestable seed because immature seed , with white or green glumes, seldom falls from the inflorescence post-harvest and is also unlikely to be knocked from standing inflorescences during mechanical field harvesting of seed. For comparison, however, an 80 % level of viability as reported in this Chapter equates to a 52% level of viability of standing seed in the field.

The optimisation of storage conditions to maximise seed longevity may not always be the objective of dry seed storage. Storage of dry seed of a number of weedy grass species at 50°C for up to 14 days accelerated seed after-ripening. At 60°C a similar result was achieved in only 3 days, but further seed exposure to high temperature significantly reduced seed germination (Taylorson and Brown 1977). Similarly, storage of *Poa pratensis* seed at 38°C for 6 to 8 days accelerated after-ripening but, further treatment induced secondary dormancy (Phaneendranath and Funk 1981). Hagon (1976) reports that after-ripening of *T. australis* seed occurs naturally in 10 months (12/27°C), but dormancy is lost within one month at 24/62°C. The rapid loss of *T. triandra* seed dormancy at 40°C is attributed to

accelerated after-ripening. Retention of dry seed at elevated temperatures resulted in seed mortality. Temperature induced loss of seed viability was accelerated at 70°C. Accelerated after-ripening of *T. triandra* seed, as a dormancy breaking treatment, is discussed further in Chapter 7 and the effect of high temperature seed pre-treatments on subsequent seedling performance are discussed in Chapter 9.

At both 40°C and 70°C loss of viability was accompanied by a reduction in seed moisture content to below 6 percent. Storage of seed over silica gel for 9 weeks, however, during which time seed moisture content was consistently below 4 percent, had no significant effect on seed germination indicating that *T. triandra* seed mortality during accelerated after-ripening is temperature dependant.

The availability of *T. triandra* seed for revegetation purposes may always be limited because of the biological constraints on seed production (Sindel *et.al.* 1993). It is therefore essential that the use of available seed be maximised. As expected for an orthodox seed, after-ripening occurred at both 25°C and 15 / 35°C with no significant reduction in seed moisture content and a predictable decline in seed viability. In contrast, at both -15°C and 10°C little after-ripening occurred providing confirmation that, for the purposes of this research, seed should be stored at both -15°C and 25°C to ensure dormant and after-ripened seed populations respectively. Moreover, the rapid loss of viability in *T. triandra* seed stored at ambient temperature in the Drakensberg (Everson 1994) indicates that *T. triandra* seed longevity is adversely affected by fluctuation in temperature and moisture content. To maximise seed available for use in revegetation, seed should thus be stored at a uniform temperature of ± 25°C. Alternatively, once seed dormancy has been lost through after-ripening, cleaned seed should be stored at sub-zero temperatures (-15°C±2°C) until required. The best practice to adopt would be to ensure use of seed in the first season following harvest, to maximise germination by planting when seed viability is high and seeds are non-dormant.

CHAPTER 4: THE ROLE OF THE SEED COVERING STRUCTURES IN THE MAINTENANCE OF *Themeda triandra* SEED DORMANCY.

4.1 Introduction

In many species germination of a non-dormant embryo is prevented by structures covering the embryo and germination only follows modification or removal of these structures (Frank and Larson 1970; Wurzburger, Leshem and Koller 1974; Mott 1978; Young and Evans 1984; Auld 1986). The mechanisms governing the imposition of dormancy by a seed coat, or seed covering structure, are poorly understood. Bewley and Black (1984) suggest five ways that the seed covering structures may impose dormancy, namely by impeding the passage of water to the embryo, interfering with the passage of gasses to, or from, the embryo or modifying the light quality received by the embryo. Furthermore, the covering structures may contain, or prevent the release of, inhibitory compounds or impart a mechanical constraint to embryo germination.

Coat interference of water uptake is most commonly associated with hardseededness (Rolston 1978), as typified by the Fabaceae. Seed coat impermeability is widely attributed to impregnation of cells of the palisade layer with hydrophobic substances such as lignin, suberin and cutin and not to the presence of cuticular waxes on the outer surfaces of hard-coated seeds (Ballard 1973; Werker, Dafini and Negbi 1973; McKee, Pfeiffer and Mohsenin 1977; Rolston 1978). In hardcoated seeds, coat permeability to water in aged seeds is associated with the preferential passage of water through specific regions in the seed coat rather than a breakdown of the impermeable palisade layer within the entire seed coat. The site of water entry is in the chalazal region in members of the Malvaceae (Christiansen and Moore 1959), adjacent to the micropyle in the Convolvulaceae (Koller and Cohen 1959) and is associated with the hilum in

papilionoid legumes (Hamley 1932) and the strophiole in mimosoid legumes (Dell 1980; Hanna 1984).

Unlike the situation in hardcoated seeds, the seed covering structures in grasses (palea, lemma and glumes) do not completely envelop the caryopsis, but may still restrict germination of the embryo because the palea and lemma press tightly around the proximal (embryo) end of the caryopsis (Simpson 1990). Interference with the passage of water to the embryo is regarded by Simpson (1990) to be the most common factor responsible for coat imposed dormancy in grasses. Few experiments, however, adequately separate the effect of the seed covering structures in restricting the passage of water or gasses to the embryo. The literature abounds with examples suggesting that interference of the covering structures of grass seed with the passage of water (Atwood 1914; Probert, Smith and Birch 1985b), or oxygen (Mott 1974; Major and Wright 1974; Renard and Capelle 1976; Whiteman and Mendera 1982) plays a major role in the imposition of dormancy. Detailed anatomical investigation of the grass palaea, lemma and glumes, in relation to permeability to water and gasses is, however, limited. The presence of a lipid layer covering the surface of the inner epidermis of the lemma of *Aristida contorta* has been reported (Mott and Tynan 1974). Lipid distribution is continuous and dense in the region overlying the embryo, but is indistinct and discontinuous in other areas of the inner epidermis. This layer, which is intact in dormant caryopses and fractured in non-dormant after-ripened caryopses (Mott and Tynan 1974), does not prevent seed imbibition. This led Mott (1974) to conclude that restriction in gaseous exchange to the embryo is the cause of hull imposed dormancy in *A. contorta*. Another mechanism by which grass seed covering structures could interfere with the passage of gasses to the embryo has been suggested for rice (*Oryza sativa*). Hulls at maturity contain residual peroxidase activity which, on soaking of the seed, competes with the embryo for available oxygen thereby decreasing the rate of flow of oxygen to the embryo (Kuo and Chu 1982). Similarly, oxidation of phenolic compounds in the testa of apple seed

decrease the concentration of oxygen reaching the embryo (Côme and Tissaoui 1973).

Imposition of dormancy due to the presence of germination inhibiting compounds in seed covering structures is less common, but has been reported in *Elaeagnus angustifolia* (Hamilton and Carpenter 1976), *Elaeagnus umbellata* (Hamilton and Carpenter (1975) and *Chenopodium bonus-henricus* (Dorne 1981). In contrast, Wareing and Foda (1957) reported that the coat of *Xanthium pennsylvanicum* retards the movement of inhibitory compounds away from the embryo. For grasses similar evidence is limited and, at times, circumstantial. A germination inhibitor, possibly coumarin, has been reported in the glumes, palea and lemma of *Bouteloua curtipendula* (Major and Wright 1974) while the hulls of *Oryza sativa* contain an inhibitor, possibly ABA (Hayashi and Himeno 1973). An inhibitory monoepoxylignanolide is present in the hulls of *Aegilops ovata* (Lavie, Levy, Cohen, Evenari and Gutterman 1974). Leaching promotes the germination of *Panicum maximum* seed (Smith 1971) while compounds which inhibit lettuce seed germination, but not the germination of *Dactylis glomerata* seed, are present in the hulls of *D. glomerata* (Fendall and Canode 1971). Although a factor in hard-coated legume seed dormancy, the imposition of dormancy, as a mechanical constraint exerted by the covering structures surrounding grass caryopses, is not considered by Simpson (1990) to be significant. Similarly, although the seed coat has been shown to alter the quality of light reaching the embryo (Karssen 1970; Widell and Vogelmann 1988; Leite and Takaki 1994) evidence for grasses is lacking.

Previous research which involved manipulation, or removal, of the glumes of *T. triandra* is summarised in Table 4.1. West (1951) reported increased germination of *T. triandra* seed following physical or acid scarification of the glumes. Germination of *T. triandra* caryopses also increased significantly when the glumes are removed (Martin 1975; Mott 1978). In all instances glume removal was not followed by germination of all the exposed caryopses, indicating that a

level of embryo dormancy may also be present in the caryopses of *T. triandra*. This aspect has been discussed in Chapter 2. Martin (1975), after performing a variety of surgical incisions on the glumes of *T. triandra* seeds concluded that the glumes do not interfere with the passage of water or gasses to the embryo, nor do they contain, or prevent the loss of, an inhibitory compound, but that the glumes rather exerted a mechanical constraint on the underlying caryopsis preventing germination.

Table 4.1: Summary of previous research investigating the effect on seed germination of removal of, or damage to, the glumes of *Themeda triandra*.

Treatment	Germination (%)			Reference
	Intact seed	Glumes damaged	Naked caryopses	
Removal of callus & attachment between glumes.	1.0	5.0		*West (1951)*
Severe physical scarification of glumes.	1.0	11.0		
Acid scarification.	10.2	27.7		
Upper glume nicked above coleoptile	4.4	5.4		*Martin (1975)*
Lower glume slit longitudinally	4.4	33.8		
Glumes removed	4.4		41.3	
Glumes removed (light)	7.4		32.5	*Mott (1978)*
Glumes removed (dark)	13.7		29.8	

This component of the current investigation was initiated to clarify the role of the glumes of *T. triandra* in the imposition, or maintenance, of seed dormancy. The effect of the glumes on water uptake and passage of gasses to the caryopses was investigated. Furthermore, the possible presence of inhibitors of *T. triandra* seed germination in the glumes was investigated and the mechanical integrity of the

glumes tested.

4.2 Methods

To assess the role played by the glumes in the imposition of primary seed dormancy, the germination of intact seed of *T. triandra* was compared with that of caryopses from which the glumes had been removed. A significant seed coat effect was detected. To identify the causal nature of this effect a number of experiments were initiated. In all experiments dormant seed from the Drakensberg seed population was used.

To assess the influence of the glumes of *T. triandra* on water uptake, intact seeds and excised caryopses were placed on moistened filter paper at 30 °C. Petri dishes were prepared as for a standard germination test. Thirty replicates of three seed units were used. Replicate groups of seeds or caryopses were weighed in the dry state prior to commencement of imbibition, and at regular time intervals thereafter. Mass gain during imbibition was determined until germination took place.

Similarly, to assess whether the glumes of *T. triandra* restrict the passage of oxygen to the embryo, intact seeds were germinated in an atmosphere enriched with oxygen. Germination was assessed in gas tight vaccine bottles (120 ml) flushed with oxygen or air at regular intervals. Bottles were incubated at 30 °C under constant light.

Excised *T. triandra* caryopses were germinated in the presence of an aqueous extract prepared from the ground glumes of 200 seeds to determine whether the glumes of *T. triandra* contain a compound/s inhibitory to *T. triandra* seed germination. The extract was prepared by agitating the powdered glumes of 200 seeds in 30 ml of distilled water for 24 hours prior to filtration. The filtered extract was used in a standard germination assessment. Distilled water constituted the

control. A second control involving intact, dormant, seed was included to test the hypothesis that if an inhibitor was present in the glumes then germination of excised caryopses, in the presence of a glume extract, would equal germination of intact seed.

The glumes of *T. triandra* exert a mechanical restraint on radicle emergence (Martin 1975). To test this hypothesis portions of the glumes of intact seeds were surgically removed. Surgical manipulations involved (A) removal of a portion of the lower glume underlying the position of the embryo, (B) removal of a portion of the upper glume distal to the embryo, (C) severing the point of attachment between the upper and lower glumes, and (D) removal of a portion of the upper glume, overlying the position of the plumule in the embryo, followed by sealing of the exposed embryo surface with either silicon grease or petroleum jelly. Treatment D was always conducted under a stream of nitrogen to minimise diffusion of oxygen to the embryo. Germination of surgically manipulated seeds was assessed as per standard.

Hereafter in this thesis, unless otherwise stated, statistical analysis of data was as follows; prior to statistical analyses all percentage data were subjected to arcsine square root transformation of percentages to degrees (Sokal and Rolf 1981). Treatment differences were detected using Tukey's Multiple Range test.

4.3 Results

Removal of both glumes from *T. triandra* caryopses significantly increased seed germination, but did not result in germination of all viable caryopses (Table 4.2), indicating that both coat-and embryo-imposed dormancy mechanisms are present. The glumes did not, however, limit the passage of water (Figure 4.1) or oxygen (Table 4.3) to the embryo. Furthermore, the glumes did not contain compounds

which inhibit the germination of *T. triandra* caryopses (Table 4.4).

Table 4.2: Comparison of the germination of intact *Themeda triandra* seeds and excised caryopses (glumes removed). Within a column, data followed by a different letter is significantly different ($P \leq 0.05$).

Treatment	Germination (%)
seed	5.0 (2.8) a
caryopses	21.2 (4.3) b

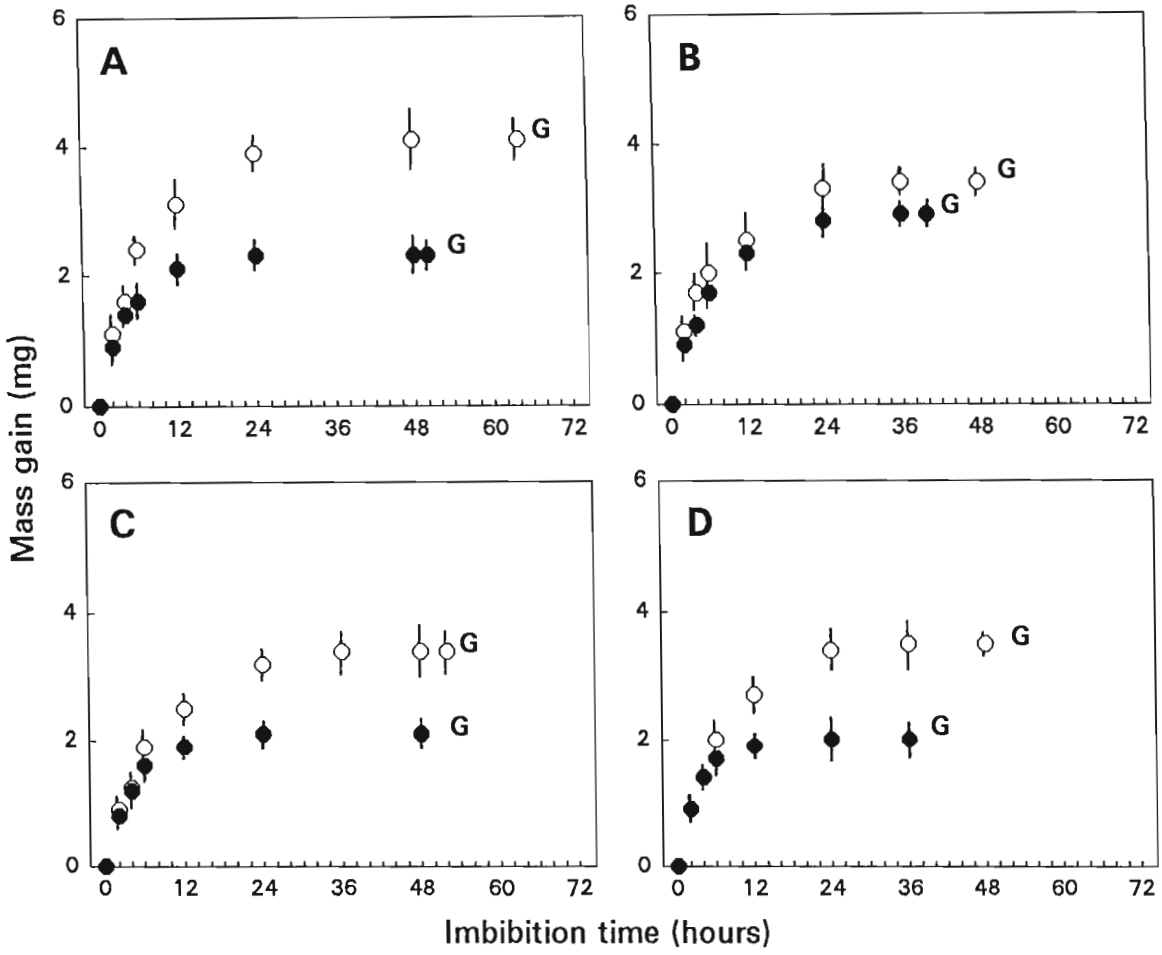


Figure 4.1: Comparison of water uptake by intact *T. triandra* seeds (open circles) and excised caryopses (closed circles). Data are presented as the increase in caryopsis mass in milligrams. G indicates the point at which seeds germinated. A Dormant seed and B non-dormant seed from the Drakensberg population. C dormant and D non-dormant seed from the Zululand population. Bars represent the standard error of the mean (n = 30 x 3 caryopses or seeds).

Table 4.3: Germination of dormant *Themeda triandra* seed in an atmosphere enriched with oxygen. Within a column data followed by a different letter is significantly different ($P \leq 0.05$).

Treatment	Germination (%)
control	17.0 (4.8) a
oxygen	13.5 (1.9) a

Table 4.4: The effect of an aqueous extract, derived from the glumes of *Themeda triandra* seeds, on germination of dormant *T. triandra* caryopses. Within a column, data followed by a different letter is significantly different ($P \leq 0.05$).

Treatment	Germination (%)
intact seed	5.0 (3.5) a
caryopses (water)	20.0 (4.8) b
caryopses (extract)	15.0 (2.0) b

Surgical manipulation of the upper and lower glumes confirmed that the glumes of *T. triandra* do not act as a barrier to the passage of water or oxygen to the embryo. Removal of parts of the glumes, although facilitating the passage of water and / or oxygen to the embryo, did not increase germination (Figure 4.2). Neither of the surgical manipulations applied disrupted the mechanical integrity of the glumes. When the point of attachment between the glumes was severed, germination increased to the level of excised caryopses. Similarly, when a weak point in the upper glume was created overlying the embryo, but sealed to prevent diffusion of oxygen or water to the embryo, the level of germination also increased to that of excised caryopses.

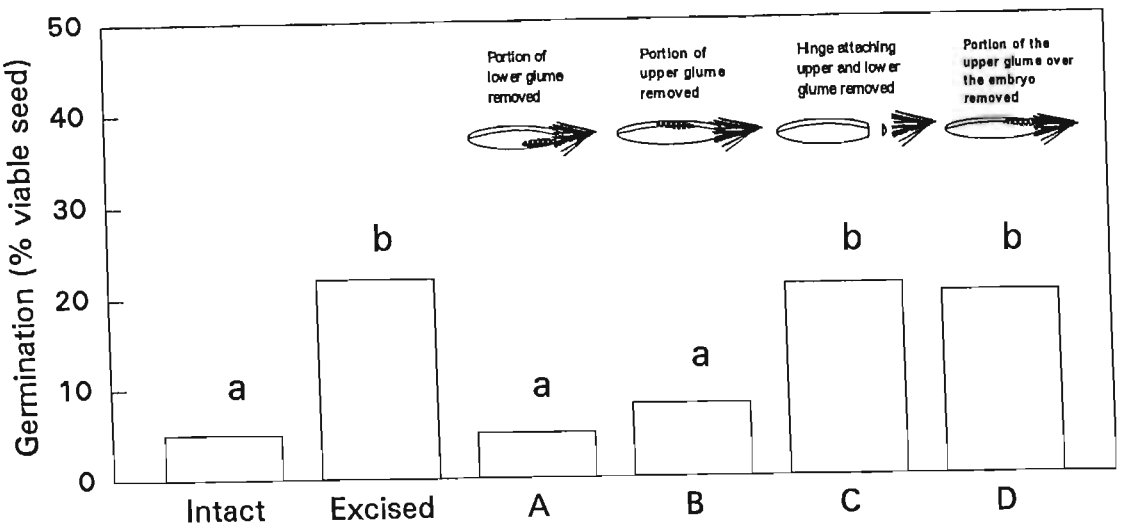


Figure 4.2: The effect of surgical removal of parts of the glumes of *Themeda triandra* seeds on germination. Surgical manipulations undertaken involved **A** removal of a portion of the lower glume underlying the embryo, **B** removal of a portion of the upper glume distal to the embryo, **C** severing the point of attachment (hinge) of the upper glume to the lower glume and **D** removal of a portion of the upper glume overlying the embryo, with the exposed embryo surface sealed to exclude water and gasses. Bars bearing different letters are significantly different ($P \leq 0.05$).

4.4 Discussion

The glumes of *Themeda triandra* are robust and tightly pressed around the proximal end of the caryopsis (see Figure 2.4). Simpson (1990) suggested that this arrangement of seed covering structures around the caryopsis, which is common in grasses, restricts the passage of gasses and / or water to the embryo. In this investigation the glumes of *T. triandra* neither prevented, nor significantly restricted, water passage to the underlying embryo (Figure 4.1). Similarly, surgical disruption of the glumes, and incubation of intact caryopses in an oxygen rich atmosphere, had no marked effect on germination. The treatments employed have

previously been successfully used to demonstrate that the covering structures of grass seeds impose dormancy by restriction of gas and / or water passage to the embryo (Roberts 1962; Frank and Larson 1970; Mott 1974; Wurzburger, Leshem and Koller 1974). Germination of excised caryopses of *Sorghum stipoideum, Chrysopogon latifolius* and *C. fallax* was markedly higher in the presence of light than in the dark (Mott 1978). In contrast, glume removal caused no difference in the germination response of excised caryopses of *Themeda triandra, Sorghum plumosum* (Mott 1978) and *Oryza sativa* (Roberts 1961) when germinated in light or dark. Germination response of *T. triandra* caryopses to different light treatments are reported in Chapter 5. The embryo of *T. triandra* is not light sensitive.

Coat imposed dormancy in *T. triandra* is therefore attributed to the robust glumes which exert a mechanical constraint on radicle emergence, as reported by Martin (1975). This conclusion is well demonstrated in the surgical treatments (Treatment D) which involved removal of a portion of the upper glume overlying the plumule, followed by sealing of the exposed embryo surface to exclude gas and water movement to the embryo. In each instance, the radicle emerged through the surgically created opening in the upper glume and not from the normal anterior position close to the proximal tip of the intact caryopsis.

Chapter 5: ENVIRONMENTAL FACTORS WHICH AFFECT *Themeda triandra* SEED DORMANCY

This Chapter incorporates the publication:
Baxter, B.J.M, van Staden, J. and Granger, J.E. (1993) Seed germination response to temperature, in two altitudinally separate populations of the perennial grass *Themeda triandra* Forsk. ***South African Journal of Science*** **89: 141-144**

5.1 Introduction

Themeda triandra is a widespread grass, dominant in grassland and savannah ecosystems in southern and east Africa and Australia. The two populations from which seed was collected for this study were located at the extremes of an environmental gradient spanning the province of KwaZulu Natal, South Africa. These two populations, from a high altitude site at Cathedral Peak in the Drakensberg and a low altitude site in the Umfolozi Game Reserve in Zululand, are regarded as separate ecotypes which are adapted to different regional climates. The seed parental environment of each ecotype has been characterised in Chapter 2.

At seed shed *T. triandra* caryopses are dormant and require a period of dry after-ripening, lasting 4 to 15 months, during which dormancy is lost (Groves *et.al.* 1982). The requirement for a period of dry after-ripening to overcome seed dormancy is common in grass and cereal species (Simpson 1990) and, in *Avena fatua* L. seed, is reported to facilitate germination over a wider range of temperatures, and at a faster rate, than occurs in dormant seed (Roberts and Smith 1977). Non-dormant *T. triandra* seed germinates over a wide range of temperatures (Groves *et.al.* 1982), but the response of dormant seed, at or soon after seed shed, to temperature has not been investigated. Increasingly it is being shown that the response of seed to environmental variables changes as seed dormancy is lost during after-ripening or ageing of seed (Baskin and Baskin 1980).

In addition, intraspecific variation in seed dormancy cycles, in response to regional climate, has been reported for the tree *Tsuga canadensis* (Stearns and Olson 1958), forbs *Silene dioica* (Thompson 1975) and *Hyacinthoides non-scripta* (Thompson and Cox 1978) and the grass *Dactylis glomerata* (Probert, Smith and Birch 1985a).

The effect of light on *T. triandra* seed germination has been investigated by a number of researchers but, no light requirement for germination has been reported (Lock and Milburn 1971: Hagon 1976: Mott 1978). Lock and Milburn (1971) did, however, report that a light requirement for *T. triandra* seed germination was induced during seed burial in soil. The effects of burial of seed in soil on germination of *T. triandra* seed is reported in Chapter 6.

Germination of non-dormant *T. triandra* seed from a variety of localities in Australia under conditions of moisture stress suggests adaptation to regional seed parental environments, as indicated by higher germination occurring at lower water potentials for seed from *T. triandra* populations from arid sites (Groves *et.al.* 1982). The germination response of freshly shed *T. triandra* seed under conditions of water stress has not been investigated.

In this component of the research the response of seed from two ecotypically distinct study populations to the environmental variables of temperature, light and moisture stress was assessed to ascertain whether ecotypic differences in seed germination response to environmental variables exist. Secondly, the response of *T. triandra* seed after seed shed, and once after-ripening had taken place, was compared to determine whether seed response to environmental variables changes as *T. triandra* seed ages and dormancy is lost. These points are of particular importance in furthering understanding of the dynamics of the seed biology of *T. triandra* to maximise germination of available seed during commercial utilisation of this important grass.

5.2 Methods

Dormant and non-dormant seeds from both the Drakensberg and Zululand populations were tested for germination response to light. In all experiments conducted, six replicates of 25 seeds were used. Germination was assessed at 30°C under constant light (12.4 μmol.m^{-2}.s^{-1}) or in light-tight boxes for dark treatments. Germination was monitored at two day intervals for 14 days. Dark incubated seeds were inspected under green safe light. The influence of photoperiod on seed germination was investigated at light / dark intervals of 12/12, 10/14 and 8/16 hours, with a 24 hour light and dark control. The effect of red light on *T. triandra* seed germination was also investigated. Dormant seed from both populations was exposed to 5, 20 or 60 minutes of red light (maximum wave length 660 nm; 1.4 μmol.m^{-2}.s^{-1}), or exposed to red light followed by exposure to far-red light (maximum wave length 730 nm; 1.7 μmol.m^{-2}.s^{-1}) in the ratio red / far red 5 / 5 and 20 / 20 minutes. A 24 hour light and a 24 hour dark control was included.

As light had no significant effect on germination of dormant or non-dormant seed all constant and alternating temperature germination trials were conducted in the dark. Seeds were, however, exposed to cool white light (12.4 μmol m^{-2} s^{-1}) provided by fluorescent tubes when germination was assessed at two day intervals. Germination was determined at six constant temperatures, 5, 15, 25, 30, 35 and 40°C. Germination of dormant Zululand seed was only assessed at 25, 30, 35 and 40°C. Seeds were also incubated at alternating temperatures of 5/20, 10/25, 15/25 and 20/30°C. Dormant Zululand seed was incubated at 15/30 and 20/35°C. Each temperature was maintained for 12 hours.

Dormant seed from both populations was also subjected to a period of stratification, which involved incubation of imbibed seeds at low temperatures.

Seeds were prepared as for a germination test, but were incubated on moistened filter paper in the dark at 5°C for 5, 10, 20 or 30 days before germination was assessed at 30°C under constant light. Dry-stored, unchilled seeds constituted the control. Germination rate was calculated at certain temperatures as the inverse of time to reach half maximum germination.

The germination of eight-week-old and after-ripened *T. triandra* seed, from both the Drakensberg and Zululand study populations, was assessed at a range of water potentials. The water potential gradient 0, -0.5, -1.0, -1.5 and -2.0 MPa was created utilizing Polyethylene glycol (PEG 8000) solutions of increasing concentration (Owen and Pill 1994). Seeds and Petri dishes were prepared as for a standard germination test, with the exception that PEG solutions were used in place of distilled water.

5.3 Results

No significant difference in germination of dormant and non-dormant *Themeda triandra* seed, from Drakensberg and Zululand populations, was recorded ($P \leq 0.05$) in the light or dark (Table 5.1). Similarly, neither photoperiod (Table 5.2), nor red / far red light (Table 5.3), significantly affected germination of *T. triandra* seed ($P \leq 0.05$).

Table 5.1: The effect of light on the germination of fresh and after-ripened *T. triandra* seed from Drakensberg and Zululand seed populations. Data represent mean germination ± SE. Within a column data followed by a different letter is significantly different ($P \leq 0.05$).

	Drakensberg		Zululand	
	Dormant	After-ripened	Dormant	After-ripened
Light	7.4 (2.6) a	94.5 (9.2) a	6.3 (2.4) a	89.7 (9.7) a
Dark	7.4 (1.4) a	96.4 (9.6) a	3.8 (1.3) a	83.3 (5.6) a

Table 5.2: The influence of photoperiod on the germination of fresh and after-ripened *T. triandra* seed from Drakensberg and Zululand seed populations. Data represent mean germination ± SE. Within a column data followed by a different letter is significantly different ($P \leq 0.05$).

Photoperiod	Drakensberg		Zululand	
(L/D)	Dormant	After-ripened	Dormant	After-ripened
12/12	7.4 (2.5) a	92.3 (9.2) a	6.2 (3.1) a	83.6 (8.2) a
10/14	7.4 (3.2) a	90.9 (9.0) a	6.2 (2.3) a	87.2 (6.8) a
8/16	9.9 (2.0) a	81.8 (8.0) a	4.9 (2.0) a	75.4 (8.1) a
24/0	7.4 (3.2) a	94.5 (9.2) a	6.3 (2.4) a	89.7 (9.7) a
0/24	7.4 (1.4) a	96.4 (9.6) a	3.8 (1.4) a	83.3 (5.6) a

Table 5.3: The effect of exposure to red or far-red light on germination of fresh and after-ripened *T. triandra* seed from Drakensberg and Zululand seed populations. Data represent mean germination ± SE. Within a column data followed by a different letter is significantly different ($P \leq 0.05$).

Light treatment	Drakensberg	Zululand
Control - dark	7.0 (1.9) a	11.0 (1.0) a
Control - light	4.0 (1.6) a	18.0 (4.8) a
R 5 min	8.0 (1.6) a	10.0 (3.5) a
R 20 min	9.0 (3.0) a	16.0 (2.8) a
R 60 min	7.0 (3.0) a	14.0 (4.0) a
R 5 min/FR 5 min	6.0 (3.5) a	13.0 (3.0) a
R 20 min/FR 20 min	4.0 (2.8) a	5.0 (1.9) a

Seed from all populations failed to germinate at 5°C (Figure 5.1). Germination of dormant Zululand seed was significantly greater at 30, 35 and 40°C than at 25°C (Figure 5.1). In contrast to the very specific temperature requirements of dormant seed, more than 75 % of non-dormant Zululand seed germinated at temperatures between 15 and 35°C. Maximum germination of non-dormant seed occurred at 30°C where germination equalled 90 %. At temperatures greater than 30°C

germination decreased sharply. Seventy five per cent of viable seed germinated at 35°C while significantly less seed (33 %) germinated at 40°C. Dormant seed from the Drakensberg population only germinated at temperatures between 25 and 35°C (Figure 5.1). Maximum germination (4%) was recorded at 30°C. Dormant seeds failed to germinate at 15 and 40°C. In contrast, germination of non-dormant seed occurred over a wide range of temperatures from 15°C to 40°C. Maximum germination was recorded at 25°C (90 %). At temperatures above 25°C germination decreased.

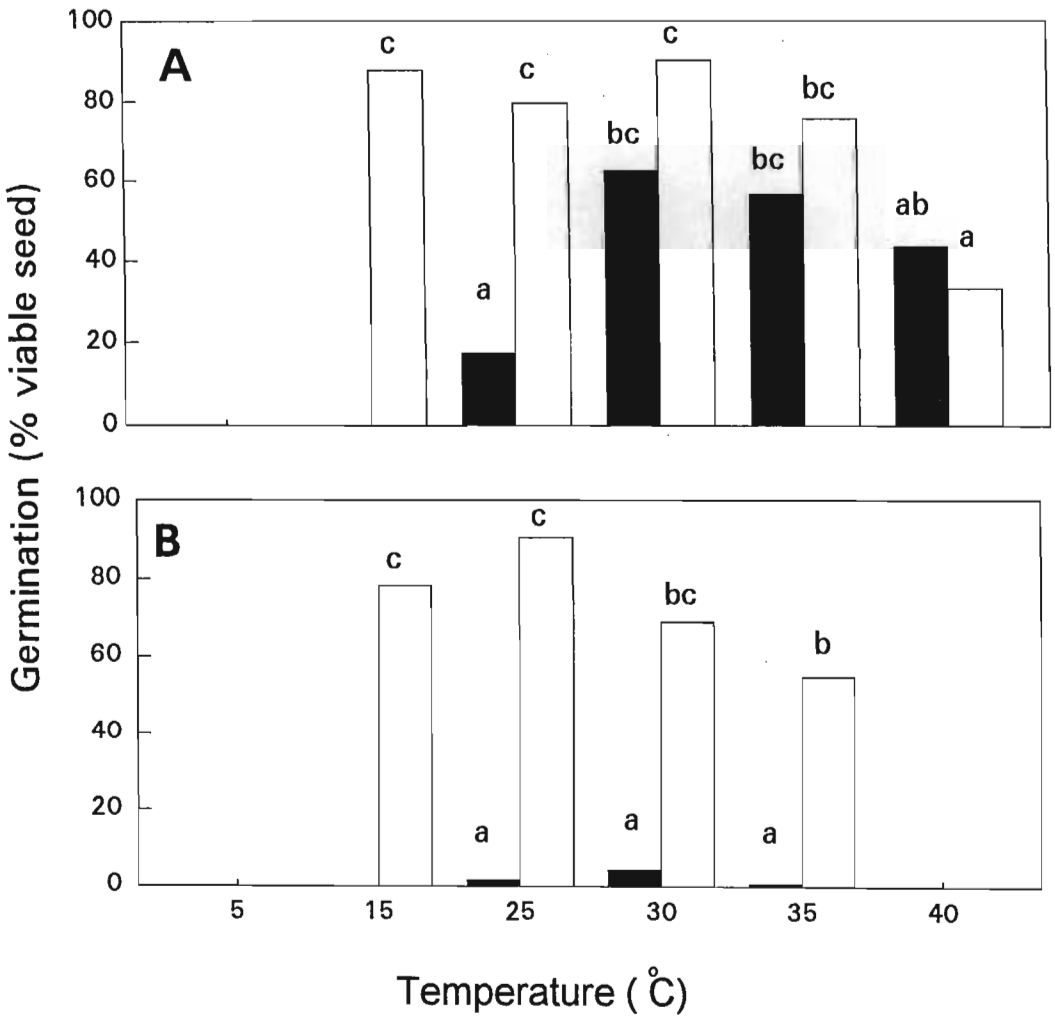


Figure 5.1: Germination of eight-week-old (solid bars) and after-ripened (open bars) *Themeda triandra* seed at a range of constant temperatures. A Zululand and B Drakensberg. Bars bearing the same letter are not significantly different ($P \leq 0.05$).

Thirty six percent of dormant Zululand seed germinated at 15/30°C and 15 % at 20/35°C (Figure 5.2). More than 85% of non-dormant Umfolozi seed germinated at all alternating temperatures tested. Maximum germination occurred at 20/30°C (99%). Between eight and nine per cent of dormant Drakensberg seed germinated at 15/25°C and 20/30°C (Figure 5.2). Less than three percent of viable seed germinated at lower temperatures. In contrast, more than 75 % of non-dormant seed germinated at all alternating temperatures tested. At 20/30°C 99% of viable seed germinated, while at 5/20°C germination equalled 76 per cent.

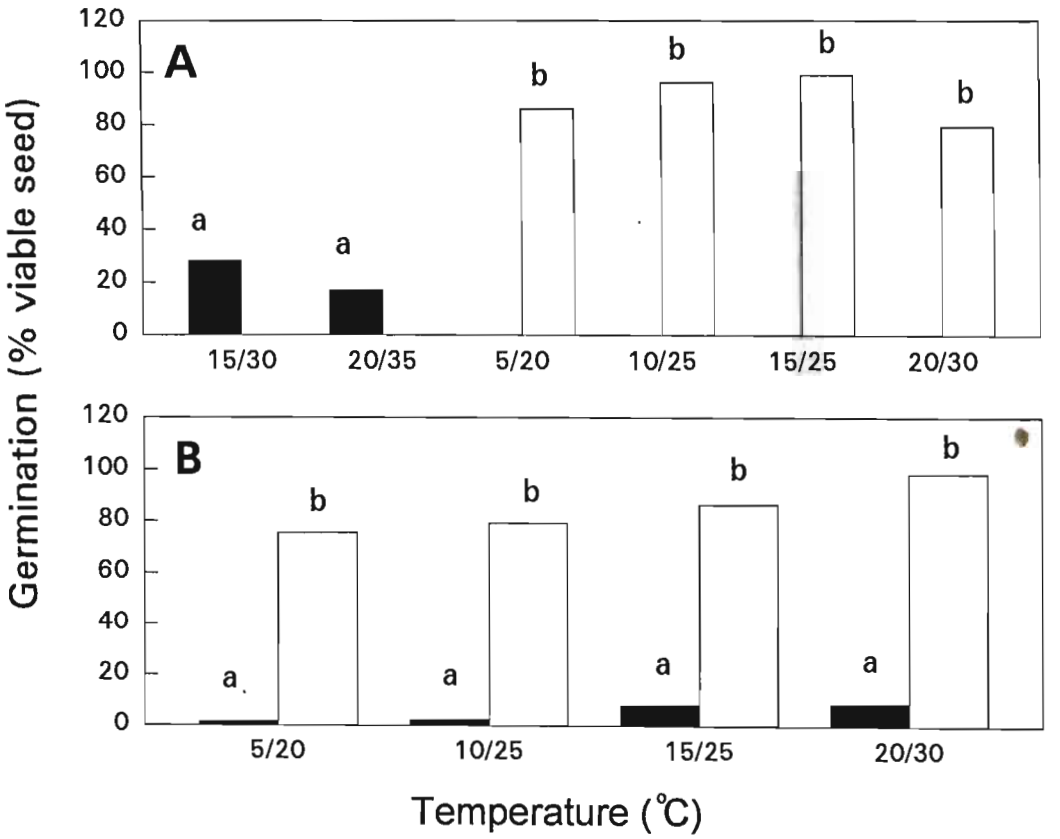


Figure 5.2: Germination of eight-week-old (solid bars) and after-ripened (open bars) *Themeda triandra* seed at a range of alternating temperatures. A Zululand and B Drakensberg. Columns bearing the same letter are not significantly different ($P \leq 0.05$).

The rate of germination, at both constant and alternating temperatures, increased with after-ripening (Table 5.4). In addition,the rate of germination at high temperatures was greater than that at low temperatures. The time taken for germination to commence increased as temperature decreased. These trends were evident at all temperatures tested and in both Drakensberg and Zululand seed populations.

Table 5.4: The effect of temperature on the germination rate of fresh (less than eight-weeks-old) and dry after-ripened *T. triandra* seed from Cathedral Peak. (Germination rate calculated as the reciprocal of time to reach half maximum germination).

Germination temperature (°C)	Germination rate	
	Fresh seed	After-ripened seed
30	0.18	0.33
15	-	0.13
20/30	0.21	0.33
5/20	0.10	0.17

Germination of *T. triandra* seed, from both Drakensberg and Zululand populations, increased following stratification (Figure 5.3). Maximum germination of Zululand seed (60.7 %) occurred after five days chilling, yet the magnitude of the germination response decreased with increased exposure to low temperature. In contrast, the longer the Drakensberg seed was exposed to low temperature the greater the increase in germination (Figure 5.3). After 30 days of stratification 68 % of viable Drakensberg seed germinated. Less than five per cent of non-stratified Drakensberg seed germinated. The rate of germination of stratified seed exceeded that of unstratified seed.

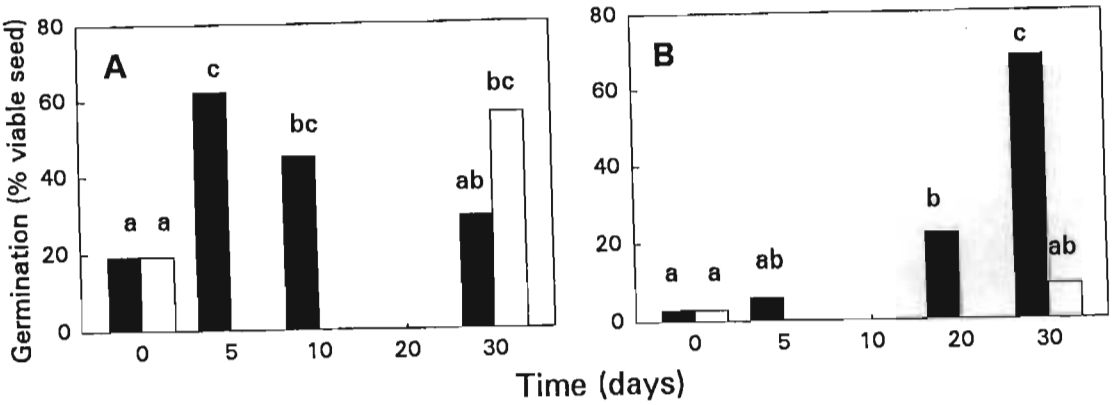


Figure 5.3: The effect of stratification on germination of eight-week-old *Themeda triandra* seed from A Zululand and B Drakensberg populations. Solid bars represent stratified seed, open bars represent dry after-ripened controls. Columns bearing different letters are significantly different ($P \leq 0.05$).

Zululand seed, both eight-weeks-old and after-ripened, germinated at lower water potentials than seed from the Drakensberg population (Figure 5.4). Furthermore, within each population after-ripening enabled seed germination at lower water potentials.

5.4 Discussion

Opportunistic, weedy or annual species, commonly exhibit light promoted seed germination which evolved to facilitate rapid colonisation of favourable sites created by disturbance. The lack of a light requirement for germination of seed of *T. triandra* is, however, not surprising because the grass is a sub-climax species dominant in stable grassland and savannah communities. It is not an invader of disturbed sites, or gaps (Everson 1994). Lock and Milburn (1971) did, however, report that burial of *T. triandra* seed in soil induced a light response. This possibility was investigated further and is reported in Chapter 6.

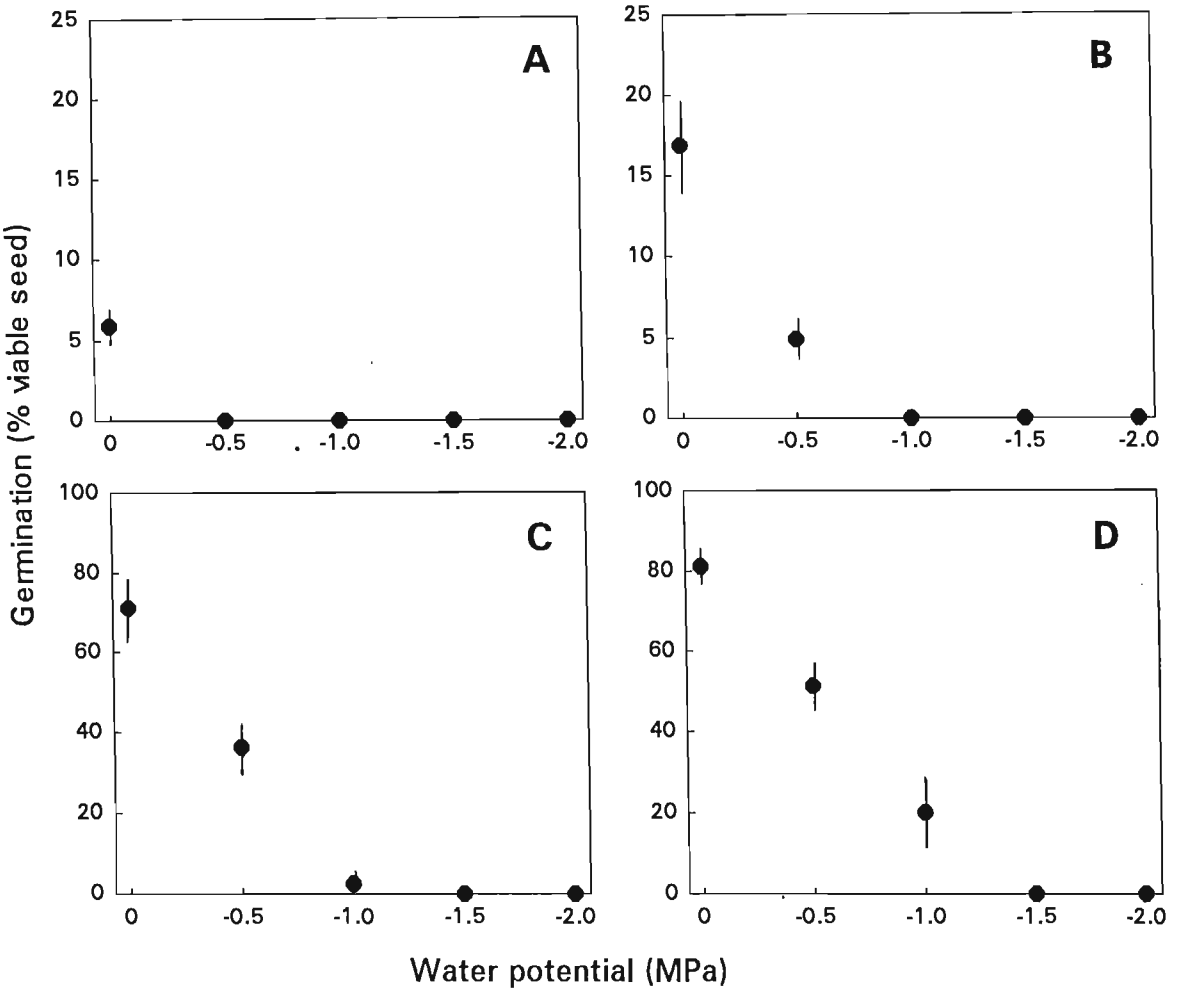


Figure 5.4: Germination of eight-week-old and non-dormant, after-ripened, *Themeda triandra* seed from Drakensberg and Zululand populations under conditions of increasing water stress achieved using PEG 8000. A. eight-week-old Drakensberg seed, B. eight-week-old Zululand seed, C. After-ripened Drakensberg seed, D. After-ripened Zululand seed. Note that the y-axis for figure A and B only extends to 25 % [Bars represent the standard error].

Variations in the level of seed dormancy within a population allows a spread in the timing of seedling emergence (Cresswell and Nelson 1972; Hagon 1976; Mott 1978 and Simpson 1990) increasing the chance of seedling survival in harsh and unpredictable environments. Similarly, changing patterns of seed dormancy may render seed germinable when seasonal environmental conditions are most

favourable for germination (Karssen 1982). The concept of changing dormancy levels in seeds was proposed by Vegis (1964), who suggested that as seeds become more dormant so the temperature range at which germination is possible narrows. The reduction in the range of temperatures suitable for seed germination may occur through a change in the upper or lower temperature limit for germination, or both. Seasonal changes in the response of seed to environmental variables, as dormancy is lost, has been reported and recently reviewed (Baskin and Baskin 1985).

Geographically distinct populations of *T. triandra*, in Australia and Papua New Guinea, differ in their seed dormancy and germination characteristics (Groves *et.al.* 1982). Similarly, in this study, it was found that the depth and duration of seed dormancy differed between populations located at different altitudes and in climatically different regions (Chapter 3). Inter-populational differences in the germination response of seed to temperature were also recorded. These differences are best considered in relation to climate at the collection sites. In both *T. triandra* populations studied, a proportion of viable seed possessed the ability to germinate immediately after seed shed, but only at high temperatures. The remainder of the seed possessed an after-ripening requirement which could be partially overcome by stratification.

Roberts and Ellis (1982) reported that after-ripened barley seed germinated over a wider range of temperatures than freshly harvested seed. The rate of barley seed germination increased with after-ripening. After-ripening also widened the temperature range for germination in Bluebunch wheatgrass (Roberts and Ellis 1982), and alleviated seed dependence on temperature in *Themeda tremula* (Pemadasa and Amarasinghe 1982). In this study the temperature range for germination and the germination rate of *T. triandra* seed, from high and low altitude populations, increased with after-ripening. The only comparable studies are those of Evans and Knox (1969), Hagon (1976), Mott (1978), Groves *et.al.* (1982) and

Gibbs-Russel and Spies (1988) in which temperature requirements for germination of non-dormant seed were investigated. Evans and Knox (1969) and Mott (1978) reported germination of non-dormant seed at temperatures ranging from approximately 14 to 42°C. Optimum germination (>80%) occurred between 24 and 35°C. Similarly, Groves *et al.* (1982) reported that of eight *T. triandra* populations, from sites which experienced a wide range of climates, seed from five populations had optimal values for germination between 20 and 30°C. In two populations optimum germination occurred between 20 and 40°C. In all 8 populations, however, germination was recorded at all temperatures between 15 and 40°C. Hagon (1976) and Gibbs-Russel and Spies (1988) reported that non-dormant *T. triandra* seed germinated optimally at high alternating temperatures, where germination commenced sooner than at lower alternating temperatures. In the present study it was observed that the optimum temperatures for germination decreased with after-ripening. In both study populations the loss of dormancy through after-ripening coincided with the onset of spring when temperatures were still relatively low.

The breaking of dormancy in grass and cereal seeds by stratification is common (Groves *et.al.* 1982) and represents a natural mechanism to ensure germination in spring (Probert and Langley 1989). Seed dormancy in *T. triandra* may be overcome by stratification (Gibbs-Russel *et.al.* 1990). Evans and Knox (1969) and Mott (1978), however, reported that stratification had no effect on *T. triandra* seed dormancy and suggested that physiological ecotypes of *T. triandra* occur. The two populations under investigation differed in response to stratification. This variation is attributed to the difference in depth of seed dormancy and may reflect adaptation to different regional environmental conditions.

Although inter- and intra-specific differences in chilling requirements may be of ecological importance, this is not always the case. In ecotypes of *Artemisia tridentata* (Meyer, Monsen, Durrant and McArthur 1990), as found in *T. triandra*,

depth of dormancy correlates with mean winter temperature at the collection site and more dormant ecotypes require a longer period of chilling at for release from dormancy. In contrast, species of *Dioscorea* from sites in Japan experiencing colder climates were less dormant and required less chilling for release from dormancy than species from warmer sites (Okagami and Kawi 1982). The exposure of hydrated seed to low temperatures (stratification) can also lead to induction of dormancy, as occurs in winter annual species which are adapted for autumn germination (Baskin and Baskin 1986). Furthermore, prolonged stratification may lead to the induction of secondary dormancy in seed of spring germinating species in which dormancy is overcome by short periods of chilling (Willemsen 1975). The trend observed in *T. triandra* seed from the Zululand population, in which increasing the period of stratification in excess of ten days results in a lower level of germination than occurs in the after-ripened controls, may mark the commencement of induction of secondary dormancy. This possibility warrants further investigation.

A small proportion of Drakensberg seed had the ability to germinate during summer, following seed shed. During autumn and winter temperatures in the natural habitat lie outside the optimum required for germination. This strategy will prevent seedling emergence immediately before winter, thereby limiting seedling mortality during the harsh winter period. A combination of after-ripening and stratification, during winter, removes dormancy in the remaining seed. After-ripened, stratified seed has the ability to germinate over a wide range of temperatures. Furthermore, an increase in the rate of germination and a decrease in optimum temperatures for germination facilitate maximum germination in spring. Seed collected in Zululand differed from that from the Drakensberg in that a large proportion of seed (60 %) had the ability to germinate in late summer following seed shed, should favourable conditions have prevailed. It is unclear why the majority of seed became dormant five weeks after being shed. The Umfolozi region in Zululand does not experience a severe winter. Mean minimum winter

temperatures are in the region of 12°C, short periods of low temperatures (5°C) are, however, not uncommon and could break dormancy of imbibed seed. Low soil temperatures would, however, prevent germination. The behaviour of non-dormant Zululand seed resembled that of non-dormant Drakensberg seed. Over-wintering facilitated germination in spring. To substantiate this hypothesis, the seed burial experiments reported in Chapter 6 were carried out.

As was observed for seed germination response to temperature, after-ripened seed of *T. triandra* germinated at a broader range of water potentials than dormant seed. It is tempting to speculate that the ability of *T. triandra* seed from Zululand to germinate at lower water potentials than seed from the Drakensberg population reflects adaptation to the hotter, more arid Zululand site. Such an assumption needs to first be supported by additional research to quantify soil available moisture in the respective sites. The ability to complete successful germination under conditions of water stress may confer a competitive advantage on a species, but only if the subsequent seedling is also able to grow successfully at low water potentials. The growth of *T. triandra* seedlings at low water potentials was not investigated in this study, but is worthy of further research particularly in light of Everson (1994) reporting that field seedling establishment in *T. triandra* is limited by water stress.

Predation of dormant *T. triandra* seed may play a major role in the germination ecology of the grass in high altitude grasslands. The density (<1.2 %) of *T. triandra* seed in the soil seed bank at the onset of spring is low (Everson 1994). High levels of *T. triandra* seed predation by ants and rodents (45 - 97 %) have been reported in savanna and grasslands in South Africa (Capon and O'Conner 1990; Everson 1994). As a result of deep dormancy at seed shed, characterised by a specific high temperature requirement for germination, the majority of seed produced is unlikely to germinate at seed shed and it is probable that seed predation during late summer accounts for the loss of a large percentage of

T. triandra seed produced in the high altitude Drakensberg grasslands. As a consequence little seed enters the soil seed bank thereby limiting the potential for seedling recruitment. These results partly explain the failure of *T. triandra* to re-establish in disturbed and degraded veld in the absence of sown seed.

CHAPTER 6: THE EFFECT OF FIELD BURIAL OF SEED IN SOIL ON THE RATE OF AFTER-RIPENING, SEED VIABILITY AND SEED RESPONSE TO TEMPERATURE AND LIGHT

6.1 Introduction

Endogenous cycles of dormancy, which coincide with regional climatic patterns, have been reported in buried seed (Baskin and Baskin 1985; 1987; 1991) and are most commonly associated with annual and weedy plant species which form a persistent seed bank and have evolved to capitalize on either disturbance within a community or a short growing season as found in sub-arctic environments (Baskin and Baskin 1985). In contrast, *T. triandra* is a sub-climax species of stable grassland communities which forms a transient seed bank with little seed carry over through successive seasons (Everson 1994). Marked differences in the level of seed dormancy, duration of the after-ripening period and the requirement for stratification occur in *T. triandra* seed from altitudinally and geographically distinct sites (Baxter, Van Staden and Granger 1993). Freshly shed seed of *Themeda triandra*, collected at Cathedral Peak, is deeply dormant and has a limited capacity for germination with less than 10 per cent of seed able to germinate. Germination is also only possible at a narrow range of high (25°C to 30°C) temperatures. As the seed ages, however, dormancy is lost and the range of temperatures at which germination occurs increases. Furthermore, the optimum temperature at which maximum germination occurs decreases (Baxter *et.al.* 1993). The loss of seed dormancy in dry storage coincides with the onset of spring leading these authors to suggest that an endogenous cycle of seed dormancy has evolved which limits seed germination prior to the onset of the harsh winter period experienced in this mountainous region. This hypothesis is supported by germination data from a coastal population of *T. triandra* (Zululand; South Africa) in which seed is shed later

in the season and has a lower level of dormancy (40 % germination) following seed shed. In addition a shorter period of after-ripening is required before germination, in excess of 80 percent, is possible. Maximum germination coincides with the onset of spring. The same trend in seed germination response to temperature, as observed for the Drakensberg population, is evident. In addition, seed from the Drakensberg population has a considerably greater requirement for stratification to overcome dormancy, than is required by seed of the Zululand population (Baxter *et.al.* 1993). The authors again contend that the difference in stratification requirements between populations may reflect adaptation to the climate experienced by the parent population.

A field seed burial experiment was initiated to test the hypothesis that overwintering of seed in the soil results in a loss of dormancy which coincides with the onset of spring. In addition the seed burial experiment was designed to test two further hypotheses.

Burial of seed in soil may alter the seed light requirements for germination. Wesson and Wareing (1969) report that dark dormancy may be induced by burial of seed in soil. Conversely, Pons (1991a) reports that seeds overwintering in soil may lose a light requirement for germination. Similar reports have been made concerning the seed of *T. triandra*. Lock and Milburn (1971) suggest that burial of *T. triandra* seed in soil for six weeks leads to the induction of a degree of dark dormancy. In contrast, dormant freshly harvested (Mott 1978) and non-dormant after-ripened (Hagon 1976) seed of *T. triandra* germinates equally well in both the light and the dark. The second hypothesis under investigation was therefore that burial of T. triandra seed in soil would induce a light requirement in seed which had no light requirement at the outset of the experiment.

Results obtained in Chapter 3 indicate that *T. triandra* seed from the Drakensberg could be successfully held in dry storage at room temperature (25°C ± 3°C) for

three years with a gradual and consistent decline in seed viability (from ± 80 % to 50 %) over the storage period. Everson (1994), however, reports significant loss of *T. triandra* seed viability within 10 months of seed harvest (80 % to less than 40 %) for *T. triandra* seed held in dry storage at room temperature at Cathedral Peak in the Drakensberg. Unfortunately, Everson (1994) did not report the range of temperatures to which stored seed was exposed, nor did she elaborate on seed storage conditions. The range of variables to which the seed was exposed is therefore unknown. This discrepancy in the longevity of *T. triandra* seed emphasises the importance of the storage environment during storage of dry seed, as identified in Chapter 3. The third hypothesis investigated was that loss of viability of T. triandra seed increases when seed is buried in soil.

6.2 Methods

The field seed burial experiment was conducted at Cathedral Peak in the Natal Drakensberg. A site was located adjacent to the Brotherton burning trial at an altitude of 1800 metres. Burning of grassland and the associated removal of aboveground vegetation and the accumulation of blackened material on the soil surface, affects the microclimate at the soil surface (Everson 1994) and may result in elevated temperatures at the soil surface (Mott 1978). Consequently it was decided to duplicate the seed burial experiment in recently burnt and unburnt grassland. In addition, at each site seed was buried in both nylon mesh bags (Swiss Silk Bolting Cloth Mfg. Co. Ltd. Zurich; NYBOLT 18 S) and glass "U-tubes" (Figure 6.1). A mesh of 0.39 mm was selected to exclude large insect predators. The open end of each "U-tube" was covered with the same material to exclude large insects. Use of two different seed burial containers enabled the effect of groups of variables under investigation to be differentiated. Seed stored in mesh bags was in direct contact with the soil and, it is assumed, closely approximated the natural condition enabling seed contact with water moving through the soil

profile, and with soil microbes. In contrast, seed stored in glass "U-tubes" was isolated from direct contact with soil microbes and soil water. Each storage vessel was, however, open at one end and was therefore in contact with the soil atmosphere and was able to equilibrate to changes in soil humidity. It was not feasible to record temperature at the depth of burial at regular intervals during the experiment, but at each site a maximum / minimum thermometer was buried at equivalent depth to the seed containers to record the extremes in temperature experienced.

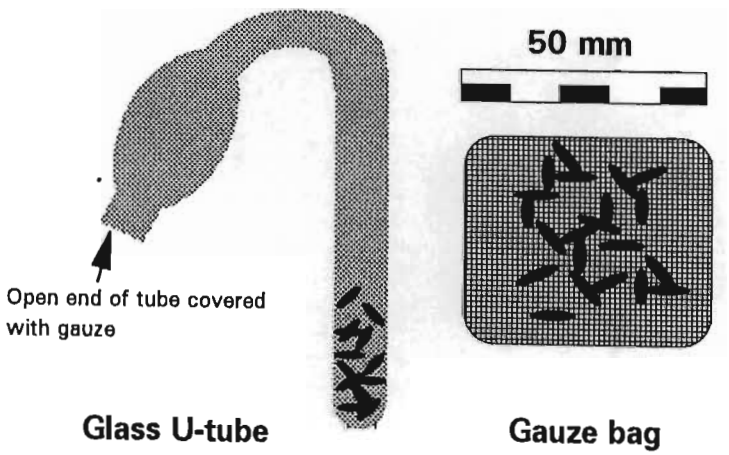


Figure 6.1: Diagrammatic representation of the containers in which *Themeda triandra* seed was buried during the winter of 1991 at Cathedral Peak.

In both burnt and unburnt grassland 2000 dormant seeds of *T. triandra* (eight-weeks-old) were buried in 10 lots of 200 seeds. Seeds were buried on 16 June 1991 and retrieved on 17 September 1991, a period of 90 days. Seeds and the thermometers were buried at a depth of 40 mm below the soil surface. Prior to, and on completion of, the experiment seed response to a range of constant and alternating temperatures and light was assessed. The protocol followed is as recorded in Chapter 5. Similarly, viability, moisture content and seed germination response to light were determined prior to and on termination of the experiment. Seed stored in a laboratory at 25°C ± 3°C, as described in Chapter 3, constituted

the control.

6.3 Results

During the burial period the maximum soil temperatures recorded at the depth of seed burial were 20.2°C and 20.5°C in burnt and unburnt grassland respectively. Minimum temperatures of 11.3°C and 2.3°C were recorded in burnt and unburnt grassland respectively.

Seed viability in the control seed population decreased from 82.3 % to 76.7 % during the 90 day experimental period (Table 6.1). In contrast, viability of seed buried in the field decreased to 67 % in unburnt grassland with no difference between seed storage method. Similarly, in burnt grassland viability of seed stored in mesh bags decreased to 69 % and in glass U-tubes to 54 %. Differences between storage methods were not significant ($P \leq 0.05$).

Table 6.1: Viability of *Themeda triandra* seed prior to and after burial in soil in burnt and unburnt grassland areas in the KwaZulu / Natal Drakensberg. Seed was buried for 90 days during the winter of 1991. Data represent mean viability ± Standard Error. Data followed by a different letter is significantly different ($P \leq 0.05$)

Storage conditions	Viability (%)
Control [25°C±3°C]	
Initial viability	82.3 (±4.1) a
After 3 months	76.7 (±3.7) ab
Burnt Grassland	
Glass tubes	54.0 (±1.2) c
Gauze bags	68.7 (±3.7) abc
Unburnt Grassland	
Glass tubes	67.3 (±3.7) abc
Gauze bags	67.3 (±4.1) abc

Germination of *T. triandra* seed under constant light did not differ significantly from seed germination in the dark for any treatment following burial for 90 days (Table 6.2), but the level of germination differed significantly ($P \leq 0.05$) between seeds which overwintered in direct contact with the soil and those isolated in glass U-tubes.

Table 6.2: Germination of seed of *Themeda triandra* in the light or dark following burial of seed at Cathedral Peak for 90 days during the winter of 1991. Data represent mean germination ± Standard Error. Data are reported as a percentage of viable seed. Within a column data followed by different letters are significantly different ($P \leq 0.05$). An * in column B or UB indicates a significant difference ($P \leq 0.05$) in germination, between storage vessels, in either burnt and unburnt grassland.

	Burnt Grassland			Unburnt Grassland		
	Glass U-tube	Mesh bags	B	Glass U-tube	Mesh bags	UB
Light	29.6 (4.3) a	69.6 (8.6) a	*	20.8 (7.9) a	68.3 (7.4) a	*
Dark	35.2 (1.9) a	81.5 (7.6) a	*	17.8 (4.9) a	68.3 (10.4) a	*

Burial of *T. triandra* seed in soil promoted dormancy loss. Following 90 days of burial, seed germination was possible at a wider range of constant (Figure 6.2) and alternating (Figure 6.3) temperatures, and to a greater extent than in seed which was stored in the laboratory for 90 days at 25°C±3°C. Higher levels of germination were recorded for seed stored in mesh bags than for seed stored in U-tubes, at all constant and alternating temperatures tested (Figure 6.2 and 6.3). In addition germination was recorded over a wider range of temperatures in field aged seed than was recorded for seed stored at 25°C±3°C with germination taking place at lower temperatures. In the field aged control (glass U-tubes) maximum germination occurred at 40°C (24 % for seed buried in unburnt, and 37 % for seed buried in burnt areas of grassland) with a consistent decrease in the level of germination as temperature decreased. Germination was recorded at 15°C, but not at lower temperatures. No germination took place at 10°C. In contrast, in seed

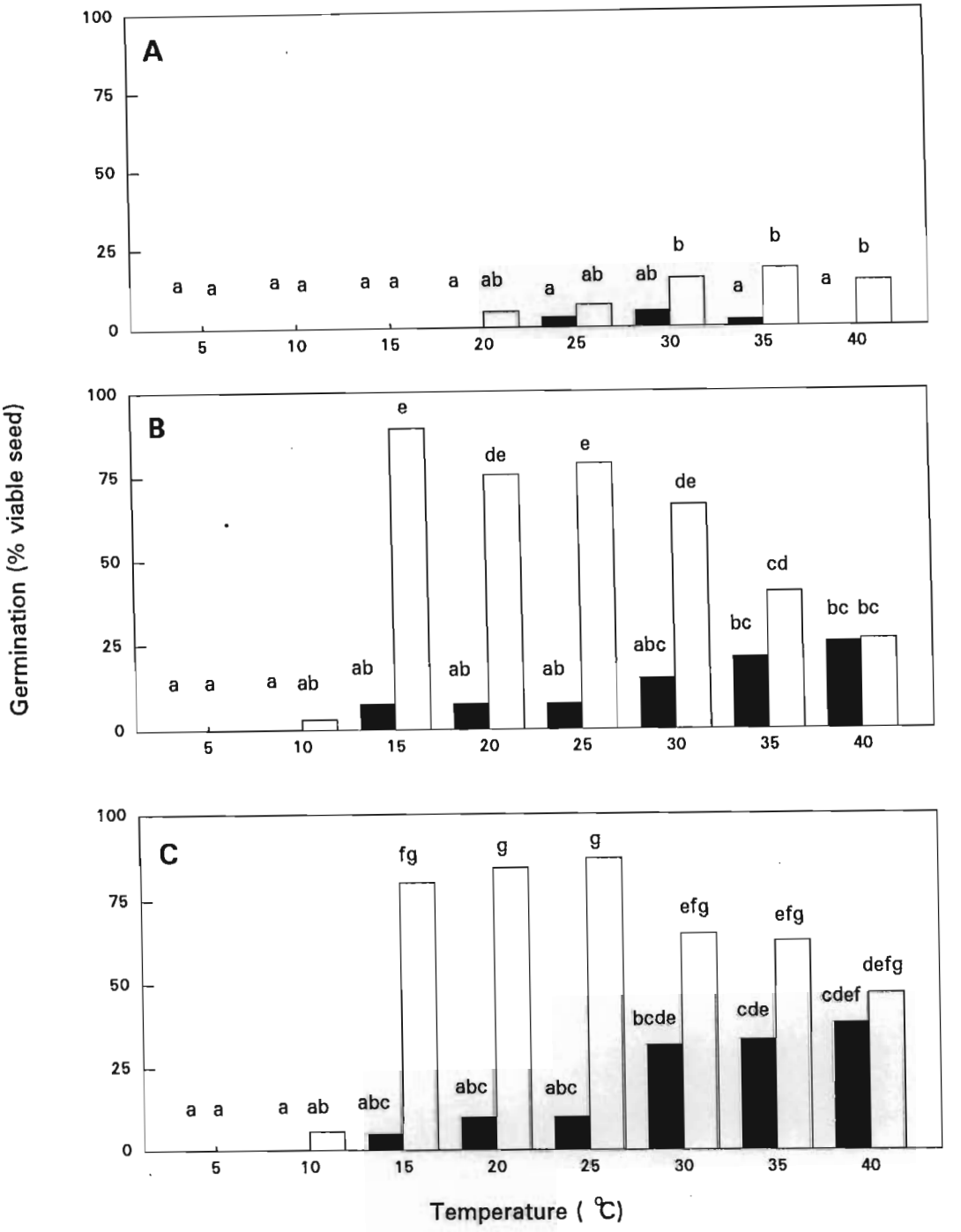


Figure 6.2: Germination of seed of *Themeda triandra* at a range of constant temperatures following a 90 day period of field burial. **A** control, after-ripened at 25°C±3°C, **B** burnt or **C** unburnt grassland at Cathedral Peak (1800 m) during the winter of 1991. Solid columns represent seed buried in glass U-tubes while open columns represent seed buried in nylon mesh bags. Columns bearing different letters are significantly different ($P \leq 0.05$).

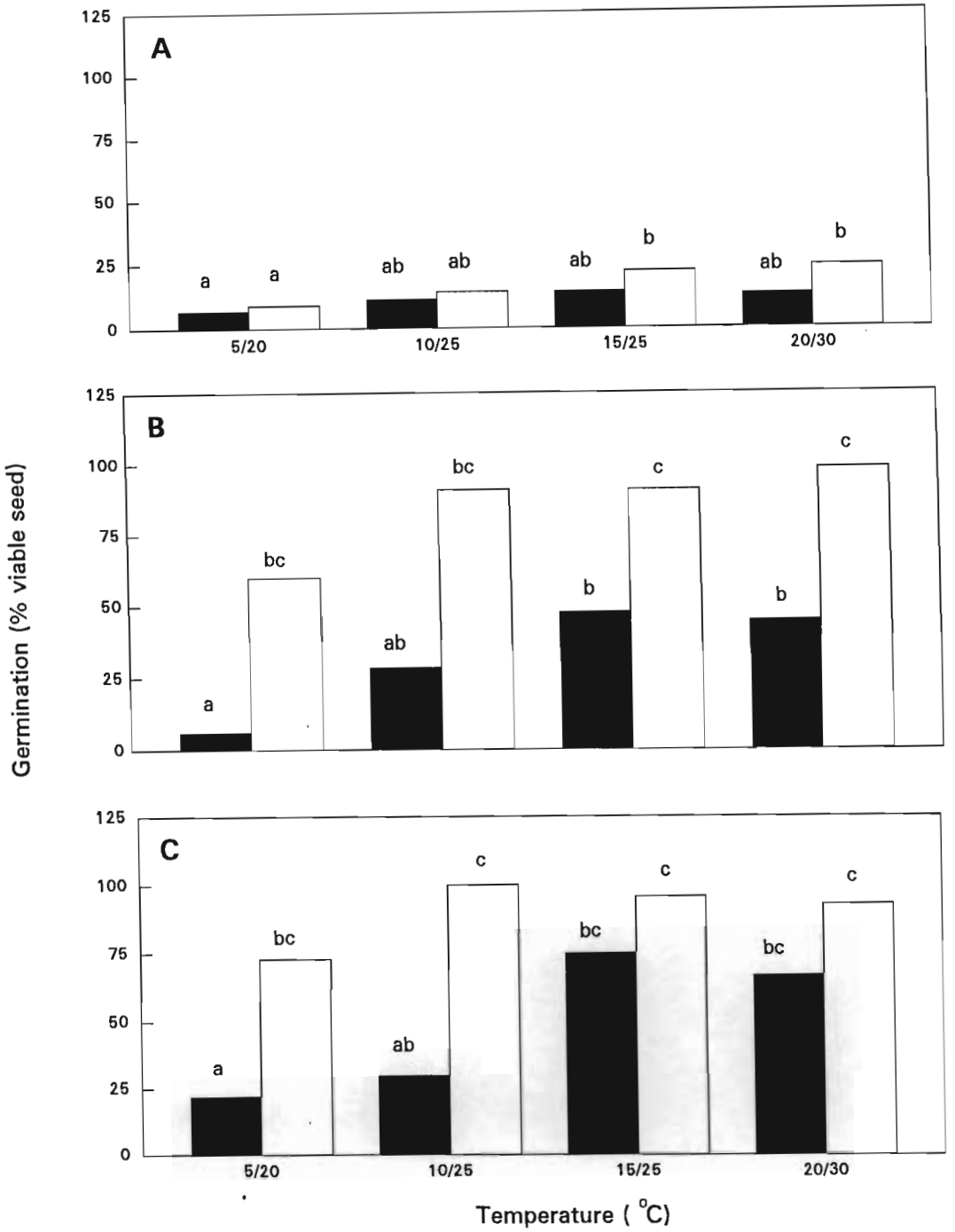


Figure 6.3: Germination of seed of *Themeda triandra* at a range of alternating temperatures following a 90 day period of field burial. **A** control, after-ripened at 25°C±3°C, **B** burnt or **C** unburnt grassland at Cathedral Peak (1800 m) during the winter of 1991. Solid columns represent seed buried in glass U-tubes while open columns represent seed buried in nylon mesh bags. Columns bearing different letters are significantly different ($P \leq 0.05$).

which overwintered in direct contact with the soil (mesh bags), germination in excess of 75 % was achieved at 15, 20 and 25°C with a consistent decline in germination as temperature increased. Furthermore, overwintering in contact with the soil facilitated limited seed germination at temperatures as low as 10°C. A similar trend was evident, although not as pronounced, for seed germination at alternating temperatures.

It is of interest to note that at most temperatures tested the level of seed germination was higher for seed which had been buried in burnt grassland, when compared with the level of seed germination following burial in unburnt grassland. This trend held for both seed stored in mesh bags and for seed stored in U-tubes. Moreover, the level of germination in seed buried in U-tubes in burnt grassland was significantly higher at 30°C, 35°C, 5/20°C and 15/25°C (Figure 6.3).

6.4 Discussion

Contrary to the report by Lock and Milburn (1971), seed burial in soil failed to induce a light effect in *T. triandra* seed. No other records of burial induced changes in seed response to light could be found in the literature although seasonal changes in the light requirements of buried seeds do occur (Baskin and Baskin 1980; Pons 1991b). As discussed in Chapter 5 and disproving the second hypothesis, the seed of *T. triandra* is insensitive to light as is expected of a perennial climax species which persists predominantly by vegetative means in a stable community.

Loss of seed viability during dry storage follows a negative cumulative normal distribution (Harrington 1972) in which the probability of seed mortality is dependant on seed age (Roberts 1972; Rice 1989). In contrast, the rate of seed bank depletion appears to be constant and displays a negative exponential

distribution for seed loss (Roberts 1970; Roberts and Feast 1973; Warnes and Anderson 1984), suggesting age-independent seed loss through germination, granivory and pathogenic decay (Rice 1989). Thus, seed longevity during dry storage provides a poor indication of the potential field longevity of buried seed, as demonstrated in *Bromus tectorum*. Little loss of viability occurs in *B. tectorum* seed over ten years of dry storage (Hull 1973), but few seeds persist in the soil for more than one year (Mack and Pyke 1983). The results presented in this Chapter provide confirmation that *T. triandra* seed behaves in a similar manner. Loss of viability is predictable and slow under controlled seed storage conditions, but loss of viability increases under field conditions. Moreover seed dormancy is lost within one winter cycle thereby ensuring maximum germination of buried viable seed. It is probable that the rate of viability loss in dry *T. triandra* seed in the field approximates that reported by Everson (1994) where dry seed was stored at ambient temperature and therefore subjected to diurnal fluctuations in temperature and moisture content.

Just as the rate of viability loss was accelerated by burial of *T. triandra* seed so the rate at which seed dormancy was lost increased. Germination in excess of 75 % was obtained after only 90 days of burial, while a period of almost 8 months (240 days) is required to attain the same level of germination in seed dry after-ripened at 25°C ± 3°C (See Figure 3.3A). Associated with this rapid loss of dormancy was the ability of seed to germinate at considerably lower temperatures, particularly in seed which was buried in mesh bags. The failure of *T. triandra* seed buried in glass U-tubes to germinate to the same extent following field overwintering remains unexplained. However, laboratory studies (Hagon 1976; Baxter *et.al.* 1993) have shown that *T. triandra* seed dormancy can be overcome by stratification, a term which describes the chilling of imbibed seed. It is likely that seed buried in mesh bags, being in direct contact with the soil, was better hydrated than seed stored in U-tubes. Similarly, changes in soil moisture content would have directly impacted on seed stored in mesh bags. Repeated cycles of

hydration and dehydration are widely reported to overcome dormancy in grass seed (Simpson 1990) although this has not been conclusively demonstrated for *T. triandra*.

Irrespective of which process causes loss of dormancy in buried *T. triandra* seed, the rate of dormancy loss is important. Baxter *et.al.* (1993) suggest that after-ripening of *T. triandra* seed maximises spring germination, possibly through stratification of buried seed during the winter period. As established in this Chapter, field after-ripening of buried *T triandra* seed does take place and germination is maximised at lower temperatures which approximate the early spring environment. A flush of *T. triandra* seedlings should therefore be evident in early spring in Drakensberg grasslands. Everson (1994), however, recorded a flush of *T. triandra* seedlings in late summer, prior to the winter period. Furthermore, *T. triandra* seed does not persist in the soil seed bank beyond one season indicating that the seedlings observed in late summer are derived from dormant seed shed in December (approximately 2 months previously). The data presented by Everson (1994) therefore indicate that after-ripening, and hence loss of dormancy, of buried *T. triandra* seed may take place at a far more rapid rate in the field than recorded under controlled conditions (see Chapter 3). The rate at which dormancy is lost in buried seed of *T. triandra*, immediately after seed shed, was not investigated in this study, but warrants further investigation in light of the apparently contradictory results of this study and that of Everson (1994).

CHAPTER 7: OVERCOMING SEED DORMANCY IN *Themeda triandra*

7.1 Introduction

Geographically distinct populations of *T. triandra* display different levels of seed dormancy (Groves, Hagon and Ramakrishnan 1982). Seed from all *T. triandra* populations investigated to date has to some extent been dormant, following seed shed (West 1951; Cresswell and Nelson 1971; Ndwula-Senyimba 1972; Lock and Milburn 1971; Martin 1975; Hagon 1976; Mott 1978; Groves *et.al* 1982; McDougall 1989; Baxter, Granger and van Staden 1993). *Themeda triandra* seed dormancy involves both a physical restraint by the seed coat (Martin 1975; Baxter and van Staden 1992; Chapter 4) and a dormant embryo (Martin 1975; Groves *et.al.* 1982). This type of primary seed dormancy is referred to as combinational dormancy and involves a fully formed, dormant embryo (Baskin and Baskin 1989). The mechanisms governing dormancy in *T. triandra* seed have yet to be fully unravelled. Gibberellins have widely been shown to promote germination of *T. triandra* seed (Cresswell and Nelson 1971, 1972; Martin 1975; Hagon 1976; Mott 1978; Groves *et.al* 1982), and it has been suggested that endogenous gibberellin synthesis, under the control of free boron, governs *T. triandra* seed germination (Cresswell and Nelson 1972). The promotion of RNA synthesis and stimulation of ∝-amylase activity in caryopses of *T. triandra* following application of boron has been used to strengthen this argument and to suggest that boron may also fulfil an independent role in promotion of *T. triandra* seed germination (Cresswell and Nelson 1973). No other reference to boron promoting seed germination could be located. In contrast, boron impedes the action of GA_3 in promoting germination of soybean seed (De la Haba, Roldán and Jiménez 1985) and inhibits the germination of, and reduces ∝-amylase activity in, sunflower seed (Jiménez and Barea 1979). Furthermore, boron exerts no effect on germination of seed of the grasses *Agropyron elongatum* and *Elymus cincereus* (Roundy 1985).

The role of boron in promotion of *T. triandra* seed germination requires confirmation.

In addition to investigation into the dormancy mechanism operative in *T. triandra* seed, previous studies have focused on seed response to environmental variables (see Chapter 5) and on ways of overcoming or breaking primary seed dormancy. No single treatment has been shown to completely break seed dormancy in *T. triandra*. Success in partially overcoming seed dormancy in *T. triandra* has, however, been achieved by application of gibberellic acid [GA_3] (Cresswell and Nelson 1971, 1972; Martin 1975; Hagon 1976; Mott 1978 and Groves *et.al.* 1982), storage of seed at 62/24°C for 30 days (Hagon 1976) and short duration exposure of dormant seed to high temperatures of 90-100°C for 4 to 8 hours (West 1951). Application of KNO_3 (Hagon 1976; Mott 1978), GA_3 in combination with kinetin (Hagon 1976) and increased periods of imbibition followed by re-drying (Mott 1978) have proved ineffective in overcoming dormancy or promoting germination. Increased periods of imbibition and re-drying, may also lead to a reduction in the final level of seed germination (Mott 1978), possibly due to the induction of secondary dormancy. In the absence of applied treatments *T. triandra* seed naturally undergoes dry after-ripening, a process in which the rate of dormancy loss is largely governed by storage temperature (Chapter 3).

Given the key role that gibberellins play in promoting *T. triandra* seed germination, the gibberellin response of seed of *T. triandra*, from different populations and of different ages, was evaluated. In addition, assessment of endogenous levels of gibberellin-like activity was completed, making use of the Dwarf-rice bioassay of Murakami (1968: 1970). Furthermore, the presence of compounds inhibitory to seed germination (Black 1959), the effect of applied abscisic acid on germination of non-dormant *T. triandra* seed and the ability of high temperature to overcome *T. triandra* seed dormancy (Hagon 1976) were investigated. Research reported in this Chapter thus deals largely with confirmation and identification of the

mechanisms active in maintenance of primary seed dormancy, treatments to overcome dormancy and the promotion of *T. triandra* seed germination. Secondary dormancy is not covered.

7.2 Methods

The effect of boron on germination of dormant *T. triandra* seed was assessed at a range of boron concentrations (0 to $5x10^{-4}$ mg $B.l^{-1}$). This concentration range spanned the range of boron concentrations used in previous studies (Cresswell and Nelson 1971, 1972, 1973). In addition, a range of boron sources were used. Glass-distilled water was used in preparation of both treatment and control solutions. Additional boron may, therefore, be unaccounted for in treatment and control soultions. In keeping with the studies of Cresswell and Nelson (1971, 1972, 1973) the effect on *T. triandra* seed germination of boron, in combination with GA_3, was also assessed. Dormant seed from the Drakensberg population was germinated in the presence of combined solutions of boron and GA_3.

Loss of dormancy in *T. triandra* seed during dry after-ripening is accelerated at high temperatures (Chapter 3; Hagon 1976). Short duration exposure of dormant seed to high temperatures has, however, also been shown to break seed dormancy (West 1951; Taylorson and Brown 1977). To investigate the effect of high temperature on loss of dormancy in *T. triandra* seed, deeply dormant seed (eight-weeks-old) from the Drakensberg population was exposed to a temperature of 70°C for 0, 7, 14 or 28 days prior to germination in distilled water. Control seed lots were allowed to undergo dry after-ripening (25°C ± 2°C) for the duration of the treatment periods prior to germination. Seeds were stored in open glass containers during treatment. To ensure that seeds were not exposed to unknown volatile compounds released from the glass containers during heating, all treatment containers were pre-heated to 100°C for 24 hours and allowed to cool prior to use. A 7 day treatment period produced the most successful results. The effect

of a 7 day heat treatment at 70°C on loss of dormancy in *T. triandra* seed of increasing age was assessed. Dry after-ripened seed, from 8 to 68 weeks in age, was exposed to heat treatments prior to germination in distilled water. Seeds were heat-treated as described above.

Early studies have identified the presence of compounds which inhibit seed germination, present in caryopses or coats of seeds (Black 1959). To investigate the possible presence of such compounds in the caryopses of *T. triandra*, aqueous extracts were prepared. Extract preparation followed the method described in Chapter 4, with the exception that the caryopses of 100 seeds (ie. from which the glumes were first removed) were used. Lettuce seed (*Lactuca sativa* var. Great Lakes Mesa) and excised non-dormant *T. triandra* caryopses were used in a germination bioassay to assess the level of inhibitor in chromatographically separated fractions of the crude extract and ABA standards. The effect of extracts prepared from dormant *T. triandra* caryopses on growth of germinated seedlings was assessed.

As abscisic acid (ABA), a known inhibitor of germination, co-eluted under the same chromatographic system with one area of inhibition obtained from the crude caryopses extract, the ability of ABA to inhibit germination of non-dormant *T. triandra* seed was assessed. ABA solutions were prepared in the concentration range 0.1 to 10.0 $mg.l^{-1}$ ABA. Non-dormant seed was germinated in the presence of ABA solutions. The ability of GA_3 to counteract the inhibitory effect of ABA was also assessed by joint application of ABA and GA_3 at the effective concentrations graphed.

Gibberellic acid (GA_3) and GA_{4+7} solutions were prepared in the concentration range 0-1000 $mg.l^{-1}$. Dormant and non-dormant seed from both Zululand and Drakensberg populations was tested for germination response to gibberellin. In addition, Drakensberg seed of different age (8, 32 and 78 weeks) was germinated

in the presence of GA_3 solutions of different concentration. A positive *T. triandra* seed germination response was detected in the presence of gibberellins. Endogenous gibberellin levels in *T. triandra* seeds were determined for dormant and after-ripened seed.

Determination of gibberellin-like activity: Before determining endogenous gibberellin levels it is essential to know at what point embryo growth is initiated ie. when germination has taken place. This is to avoid confusion caused by detection of hormone changes arising from post germination growth of the developing seedling. Dormant and non-dormant seeds (Drakensberg population) were imbibed in distilled water for increased periods of time from 0-96 h. Wet and dry seed masses were determined for 10 replicates of 3 seeds per time interval. An increase in dry mass indicated the onset of embryo growth. In addition the levels of gibberellin-like activity were determined in dormant and non-dormant seeds subjected to increasing periods of imbibition from 0-48 h. Seeds were allowed to imbibe for 48 h before being frozen in liquid nitrogen and extracted for gibberellin-like activity as described below.

Extraction of gibberellin-like substances: All extractions were carried out on intact *T. triandra* seeds (caryopses and glumes) to eliminate the time consuming process of glume removal. The efficiency with which gibberellins can be extracted from plant material is difficult to determine, but is a function of the association of gibberellins with impurities such as lipids, protein, tannin and phenolics (Hedden 1987). In addition, phenolics may form stable complexes with gibberellins resulting in reduced biological activity (Corcoran, Geissman and Phinney 1972). Simple tests described by Harbourne (1986) to detect the presence of phenolics and tannins were conducted.

To test for tannins three 0.1 g samples of ground, intact, seeds were placed into separate test tubes and covered with 2 M HCl. The tubes were placed into a

boiling water bath to allow hydrolysis to proceed. After 30 minutes the tubes were removed and allowed to cool. A red colour indicated the presence of tannin. Tannins were not present in *T. triandra* seed extracts.

To test for phenolics three 0.1 g samples of ground, intact, seeds were leached by soaking in distilled water for 7 hours, with continuous shaking. The leachate was obtained by filtration and tested for phenolics with the addition of 1 percent ferric chloride solution. A black, purple or blue colour indicated the presence of phenolics. No phenolics were present in *T. triandra* seed extracts.

Gibberellin extraction is frequently initiated in an ethanolic or methanolic solution to remove excess protein, followed by liquid-liquid partitioning to separate gibberellins into polar, acidic and neutral fractions on the basis of polarity (Hedden 1987). A number of different extraction media have, however, successfully been used to extract gibberellin-like substances. These include direct partitioning of acidified liquid *Cucurbita* endosperm (Beale, Bearder, Hedden, Graebe and MacMillan 1984), extraction of plant material in phosphate buffer (Jones 1968) and extraction of plant material in the detergent Triton-X100 (Browning and Saunders 1977).

The purpose of this research was not to separate and identify specific gibberellins active in promoting seed germination, but rather to investigate the presence and levels of gibberellin-like activity in wild seed. Consequently, the objective was to obtain seed extracts free of impurities to enable bioassay of crude gibberellin extracts to detect gibberellin-like activity. Four extraction methods, adapted from those of Reeve and Crozier (1980), Hedden (1987) and Jones (1968) were compared using *T. triandra* seed samples spiked with known amounts of GA_3. Extracts were bioassayed to determine the level of recovery of gibberellin. Each extraction and bioassay was conducted twice with repeatable results.

For each extraction *T. triandra* seeds were flash frozen in liquid nitrogen and freeze dried before being ground to a coarse powder in a Thomas Wiley intermediate mill (mesh size 0.4 mm). One gramme of ground seed material, approximately 200 seeds, was used per extraction. In all extractions the temperature of the extract was kept below 40°C as gibberellins are known to be unstable at higher temperatures (Hedden 1987).

Extraction 1: The first extraction method followed was that of Reeve and Crozier (1980) and Hedden (1987), as adapted by Drewes (1989). Freeze dried, ground, seed material was extracted in 80 percent ethanol for 6 h at 5°C. After extraction, seed debris was removed by filtration through Whatmann's no. 1 filter paper, rinsing the filtered residue thoroughly with cold ethanol. The filtrate was reduced to the aqueous phase under vacuum. The aqueous phase was diluted to double its volume with 0.2 M phosphate buffer (pH 8.1) and partitioned five times against half volumes of petroleum ether to remove lipid material, discarding the upper ether phase each time. The aqueous phase containing polar, acidic and neutral gibberellins was reduced to dryness under vacuum. The residue was redissolved in 2 ml methanol and stored at -15°C until use.

Extraction 2: The extraction differed from that described above in that the final aqueous phase containing gibberellins was not dried down, but acidified to pH 2.5, by the addition of HCl. This solution was partitioned three times against equal volumes of ethyl acetate. The upper ethyl acetate fractions were retained, combined and reduced to dryness under vacuum. This fraction contained the bulk of the free gibberellins, excluding the very polar gibberellins (Durley and Pharis 1972). The dried residue was redissolved in methanol and stored at -15°C until use.

The third and fourth extraction methods were adapted from the method of Jones (1968).

Extraction 3: Powdered, freeze dried seed material was extracted for 6 h at 5°C in acidic phosphate buffer (pH 2.5). After extraction seed debris was removed by filtration, as described above, and the residue thoroughly rinsed with cold acidic phosphate buffer. The filtrate was partitioned three times against equal volumes of ethyl acetate, retaining the upper ethyl acetate phase each time. These gibberellin containing fractions were combined and taken to dryness under vacuum. The residue was redissolved in methanol and stored at -15°C until use.

Extraction 4: This exaction differed from extraction 3 in that the initial extraction was carried out in phosphate buffer at pH 8. After filtration the pH of the filtrate was reduced to pH 2.5 by addition of HCl, prior to partitioning against ethyl acetate. Thereafter, the extraction proceeded as described for extraction 3.

Based on results obtained from the dwarf rice bioassay for gibberellin-like activity (described below), extraction 1 was adopted (Table 7.1). Although extraction method 4 yielded a similar level of gibberellin recovery, and was less time consuming than extraction 1, the ability of phosphate buffer to extract lipophilic gibberellins has been questioned (Hedden 1987).

Table 7.1: Dwarf rice bioassay for gibberellin-like activity to compare four possible methods for extracting gibberellins from *Themeda triandra* seeds. Data represent mean leaf sheath lengths (n = 20). Within a column data sharing the same letter are not significantly different ($P \leq 0.05$).

Extraction	Leaf sheath length (mm)	
Control (water)	15.1	a
Control (50 % acetone)	16.5	a
Method 1	25.7	d
Method 2	21.6	bc
Method 3	20.4	b
Method 4	23.9	cd

Dwarf rice micro-drop bioassay for gibberellin-like activity: The dwarf rice bioassay for gibberellin-like activity (Murakami 1968, 1970) was used with slight modification. The Tan-ginbozu cultivar of *Oryza sativa* L. was selected because it is sensitive to a wide range of gibberellins (Crozier, Kuo, Durley and Pharis 1970) and lacks endogenous gibberellin activity (Suge and Murakami 1968). Dwarf rice seed var. Tan-ginbozu was kindly supplied by Dr. M. Koshioka (National Research Institute of Vegetables, Ornamental Plants and Tea, Ministry of Agriculture, Forestry and Fisheries, Japan).

Dwarf rice seed was sterilized for twenty minutes in 0.3 % sodium hypochlorite to which 0.05 % of the surfactant Tween twenty was added. Sterilized seed was thoroughly rinsed in running tap water for 8 hours before being placed in a beaker of distilled water to germinate at 32°C in the dark. The germination period lasted 60 h with the distilled water being changed after the first 24 h. At 60 h, germinated seeds were selected for uniform coleoptile emergence and planted onto 0.9 % agar in plastic Repli dishes. Two seeds were planted per Repli dish compartment and alternate rows used to facilitate handling. Repli dishes were then placed into deep plastic trays lined with moistened paper towel and covered with clear plastic to prevent desiccation. After planting, trays were returned to a growth chamber at 32°C with constant light for a further 60 h before treatment of the seedlings. Gibberellin samples were taken to dryness under a stream of air and redissolved in 0.3 ml 50 % aqueous acetone prior to application. Gibberellin A_3 and A_{4+7} standards were prepared in 50 % acetone. The dwarf rice bioassay produces a log dose-log response relationship in the range 0.1-1000 ng GA_3. All standards were prepared such that 2 μl droplets contained 0.1 to 1000 ng gibberellin. A control solution of 50 % acetone was also applied. All solutions contained the surfactant Tween 20. Treatment involved placing a 2 μl droplet of test solution in the axil of the first leaf of a dwarf rice seedling. After treatment, seedlings were returned to the growth chamber at 32°C. The bioassay was terminated after 72 hours and the length of the second seedling leaf sheath

measured as an indicator of the presence of gibberellin-like activity. Twenty seedlings were treated for each test solution. For each *T. triandra* seed treatment, the extraction and bioassay of gibberellin-like activity was repeated twice with repeatable results.

7.3 Results

Germination of dormant Zululand and Drakensberg *T. triandra* seed was not promoted in the presence of boron within the concentration range tested, irrespective of boron source (Table 7.2). Similarly, *T. triandra* seed germination in the presence of combined solutions of boron and GA_3 failed to promote germination to a level greater than that of GA_3 alone (Figure 7.1).

Table 7.2: Germination of dormant *Themeda triandra* seed in the presence of boron derived from a range of different boron sources. Data represents mean germination ± SE. For each seed population, data within a column followed by a different letter is significantly different ($P \leq 0.05$).

Boron concentration ($mg.l^{-1}$)	Boric acid		Boric acid PO_4 buffer (pH 7.0)		Sodium borate		Boric acid / Sodium borate buffer (pH 6.4)	
Drakensberg population								
0	7.4 (2.5)	a	7.4 (2.5)	a	7.4 (2.5)	a	7.4 (2.5)	a
5	2.5 (1.5)	a	0	a	3.9 (2.3)	a	3.9 (1.2)	a
5×10^{-1}	1.2 (1.2)	a	4.9 (2.0)	a	3.9 (2.3)	a	1.2 (1.2)	a
5×10^{-2}	7.4 (2.6)	a	9.9 (4.1)	a	6.2 (3.1)	a	2.5 (1.5)	a
5×10^{-3}	4.9 (3.2)	a	3.7 (2.3)	a	3.9 (2.3)	a	1.2 (1.2)	a
5×10^{-4}	3.7 (3.7)	a	6.2 (1.2)	a	4.9 (2.0)	a	0	a
Zululand population								
0	36.6 (4.1)	a	36.6 (4.1)	a	36.6 (4.1)	a	36.6 (4.1)	a
5	17.6 (2.6)	a	13.6 (2.7)	a	42.1 (5.6)	a	42.1 (6.0)	a
5×10^{-1}	25.8 (1.4)	a	40.7 (2.7)	a	46.1 (3.5)	a	40.7 (5.7)	a
5×10^{-2}	23.1 (2.6)	a	44.8 (6.4)	a	32.6 (9.4)	a	33.9 (5.7)	a
5×10^{-3}	32.6 (3.8)	a	42.1 (9.8)	a	32.6 (5.0)	a	50.2 (6.0)	a
5×10^{-4}	42.1 (2.6)	a	36.6 (2.6)	a	33.9 (5.2)	a	36.6 (4.6)	a

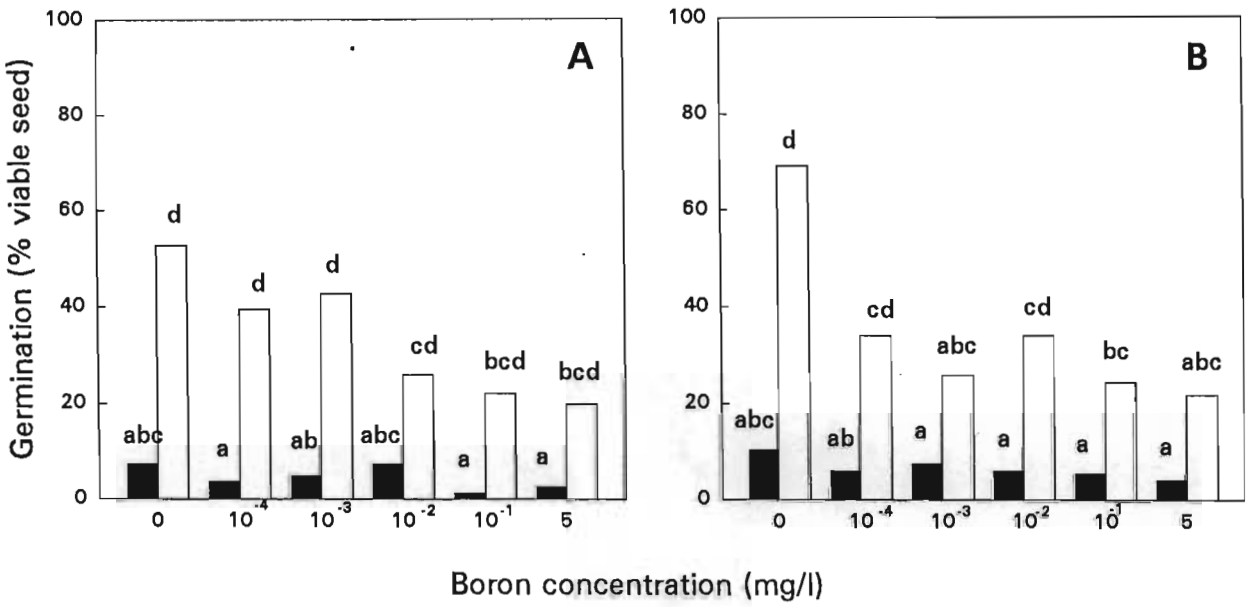


Figure 7.1: The effect of boron, applied individually (open bars) or applied in combination with GA_3 (closed bars), on germination of eight-week-old *T. triandra* seed from the Zululand (A) and Drakensberg (B) populations.

Loss of *T. triandra* seed dormancy was significantly promoted following storage at 70°C for 7 days (Table 7.3). Following longer treatment periods, however, no germination took place. Similarly, high temperature treatments were only effective when applied to *T. triandra* seed soon after seed shed. Once after-ripening had rendered 50% of the viable seed population germinable (seed age ± 21 weeks) heat treatment was no longer effective, while in seed greater than ± 32 weeks old heat treatment reduced the final level of seed germination (Table 7.4).

An endogenous compound capable of inhibiting the germination of lettuce seed was detected in aqueous extracts of dormant *T. triandra* caryopses. This endogenous inhibitory compound extracted from dormant *T. triandra* caryopses co-eluted with applied ABA. This compound (or compounds) did not, however, inhibit

the germination of non-dormant *T. triandra* caryopses (Figure 7.2), but were effective in retarding radicle elongation of germinated seeds (Table 7.5). In the presence of extracts prepared from dormant caryopses, radicle elongation was more severely retarded than in the presence of extracts prepared from non-dormant caryopses.

Table 7.3: Germination of eight-week-old *Themeda triandra* seed following increased pre-treatment of dry seed at 70°C. Control seed lots were stored at 25°C±2°C for the duration of the pre-treatment period prior to germination. Data represent mean germination (± S.E.). Within a column data followed by different letters are significantly different ($P \leq 0.05$)

Treatment time	Germination (% viable seed)			
(days)	Control		Pre-treated	
0	13.2 (3.3)	a	13.2 (3.3)	b
7	13.5 (2.3)	a	46.7 (7.0)	c
14	15.0 (1.6)	a	0	a
28	21.3 (4.7)	a	0	a

Table 7.4: Germination of *Themeda triandra* seed following pre-treatment of dry seed, of different age, at 70°C for 7 days. Data presented represent mean germination (±S.E.).

Seed age	Germination (% viable seed)		ANOVA [1]
(weeks)	Control	Pre-treated	
8	13.2 (3.3)	47.6 (7.0)	*
21	50.5 (5.1)	64.1 (7.6)	ns
32	78.5 (2.9)	52.0 (8.5)	ns
68	100.0 (3.4)	89.3 (8.0)	ns

[1] Statistical comparison of control vs. pre-treated seed germination data for each seed age cohort (* significant difference $P \leq 0.05$; ns not significant)

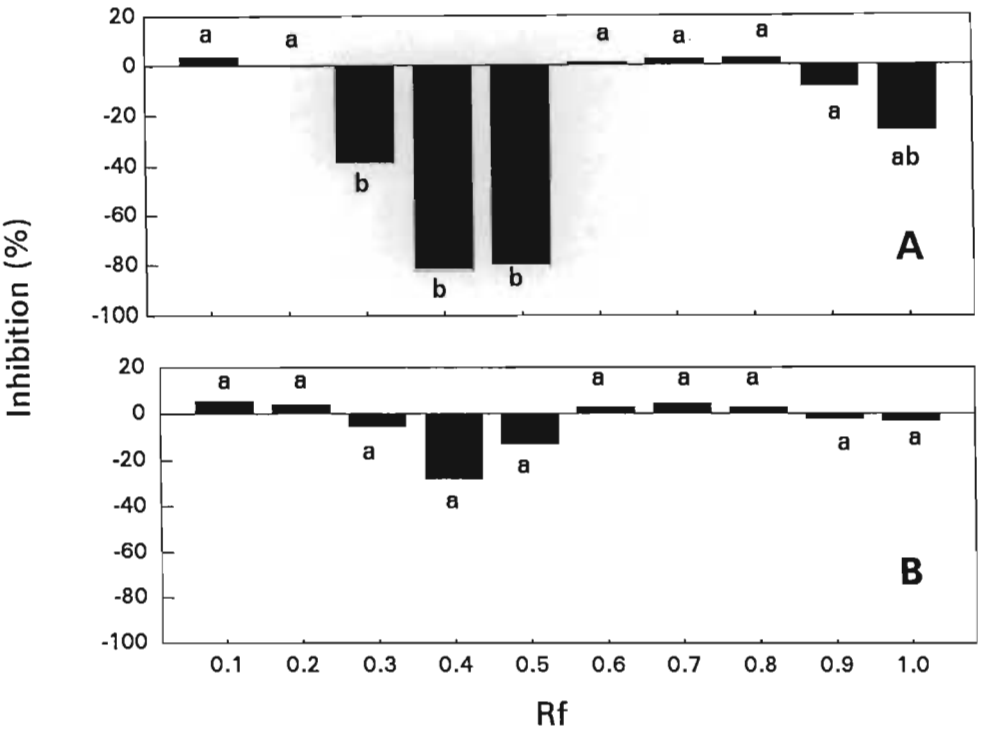


Figure 7.2: The level of inhibition caused by extracts, prepared from dormant *T. triandra* caryopses, on A lettuce and B non-dormant *T. triandra* seed from the Drakensberg population. Columns bearing different letters are significantly different ($P \leq 0.05$).

Table 7.5: The effect of aqueous extracts, prepared from dormant and non-dormant *Themeda triandra* caryopses, on germination of non-dormant *Themeda triandra* seed and elongation of the plumule and radicle after the first 48 hours of seedling growth. A caryopsis refers to an excised embryo with associated endosperm, while seed refers to a caryopsis enclosed by the inner and outer glumes. Within a column different letters indicate statistical significance ($P \leq 0.05$). Data presented are means ± (S.E.)

Extract	Germination (% viable seed)	Plumule length (mm)	Radicle length (mm)
Control	88.0 (4.3) a	5.3 (0.6) a	9.1 (0.7) a
Dormant caryopses	78.9 (5.9) a	5.2 (0.5) a	4.5 (0.5) b
Non-dormant caryopses	84.3 (8.0) a	5.9 (0.5) a	5.1 (0.6) b

Applied ABA significantly inhibited germination of non-dormant *T. triandra* seed from both Drakensberg and Zululand populations, at all concentrations tested. The inhibitory effect of applied ABA could be partially overcome by joint application with GA_3, particularly at higher ABA concentrations (Figure 7.3).

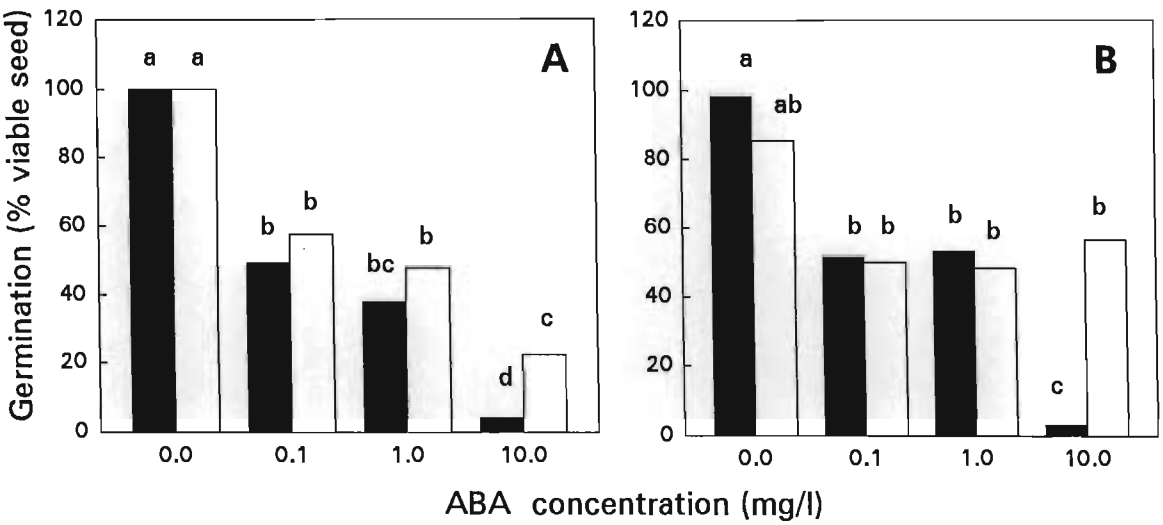


Figure 7.3: The influence of abscisic acid on germination of non-dormant A Drakensberg and B Zululand *T. triandra* seed (solid bars), and the ability of applied GA_3 (closed bars) to overcome ABA induced inhibition. Columns bearing different letters are significantly different ($P \leq 0.05$).

Dormant *Themeda triandra* seed from both the Drakensberg and Zululand populations responded positively to applied GA_3 and GA_{4+7} (Figure 7.4). For both seed populations maximum germination was recorded at a concentration of 10 $mg.l^{-1}$ GA_{4+7}, while maximum germination of both Drakensberg and Zululand seed was recorded at 500 $mg.l^{-1}$ GA_3 and 100 $mg.l^{-1}$ GA_3 respectively. The requirement for higher GA_3 concentrations to achieve maximum germination of Drakensberg seed, in comparison to Zululand seed, reflects the deeper level of primary dormancy of Drakensberg seed. This link between depth of primary seed dormancy and the GA_3 concentration required to achieve maximum *T. triandra* seed

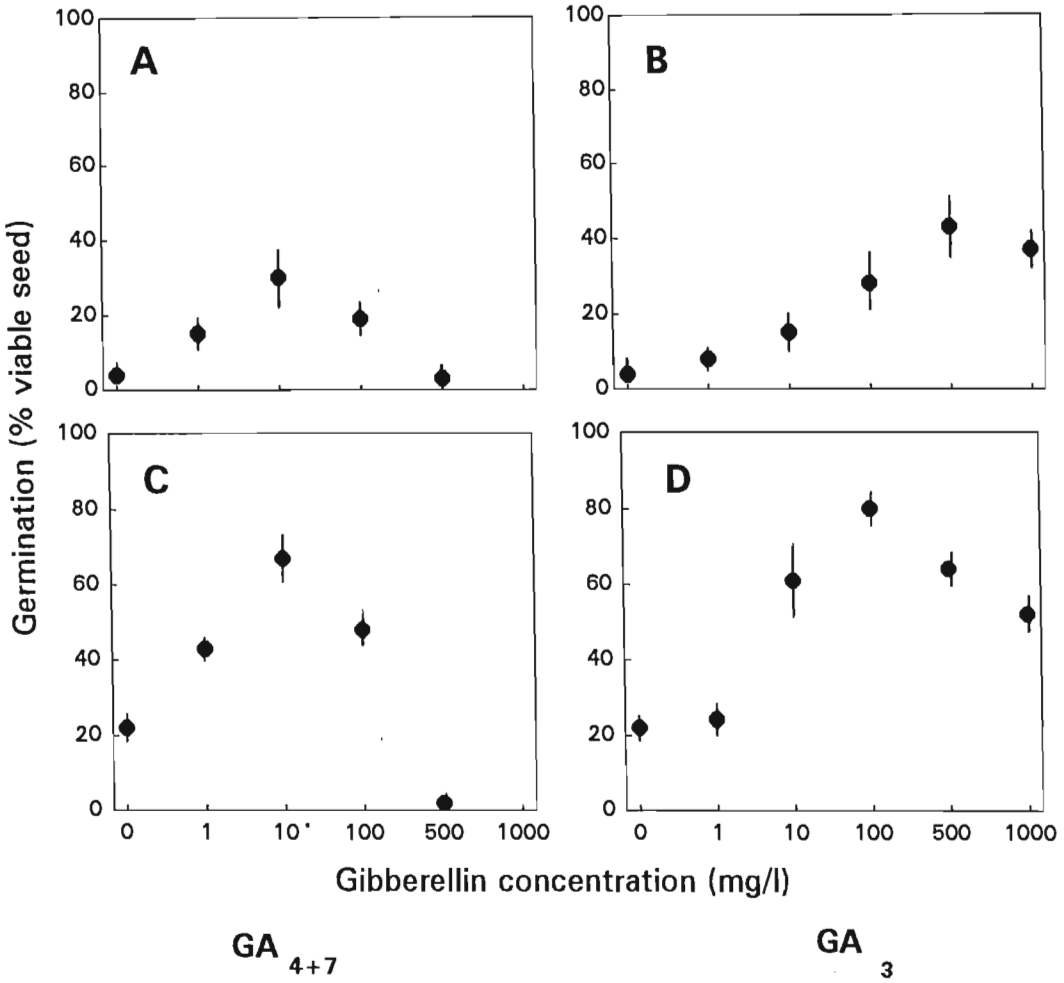


Figure 7.4: Germination of dormant Drakensberg and Zululand *T. triandra* seed in the presence of dilution series of GA_3 and GA_{4+7}. A and B represent Drakensberg seed, while C and D represent Zululand seed.

germination is reinforced by comparison of the decreasing GA_3 concentrations required to achieve maximum germination in *T. triandra* seed (Drakensberg population) of increasing age and hence decreasing seed dormancy level (Table 7.6). The GA_3 concentration at which maximum germination of *T. triandra* seed which had after-ripened seed for 8, 32 and 78 weeks at (25°C±3°C) was realised, decreased from 500 to 100 to 50 mg $GA_3.l^{-1}$ respectively. In the absence of exogenous GA_3 the level of germination of seed of different ages, was 4.0, 74.4

and 86.3 per cent respectively.

Table 7.6: The influence of seed age on the germination response of Drakensberg *Themeda triandra* seed to Gibberellic acid (GA_3). Data are mean germination percentages (±S.E.). Within a column germination percentages followed by different letters are significantly different ($P \leq 0.05$).

GA_3 concentration ($mg.l^{-1}$)	Seed age (weeks)					
	8		32		78	
0	4.0 (1.0)	a	74.4 (2.6)	a	86.3 (5.1)	a
1	8.0 (1.6)	ab	76.7 (8.6)	a	83.8 (7.6)	a
10	17.3 (3.3)	b	-		-	
50	-		89.7 (5.4)	ab	100 (9.9)	a
100	25.3 (2.5)	bc	100 (1.5)	b	93.9 (19.5)	a
500	42.7 (3.7)	d	84.6 (6.2)	ab	93.9 (8.6)	a
1000	37.3 (1.5)	cd	82.1 (4.2)	a	100 (14.5)	a

The end of the germination process and the commencement of seedling growth is marked by the onset of embryo growth and is characterised by an increase in seed dry weight. This point differs for dormant and non-dormant *T. triandra* seed (Figure 7.5) with embryo growth commencing after ± 48 hours in non-dormant seed, but only after ± 72 hours in dormant seed. Similarly, the levels of endogenous gibberellin-like substances begin to increase at different times in dormant and non-dormant *T. triandra* seed (Figure 7.6). A peak in endogenous GA-like activity occurs 12 hours after the commencement of imbibition of non-dormant seed, but is only evident 24 hours after commencement of imbibition of dormant seed. From standard curves characterising the elongation response of the second leaf sheath of dwarf rice seedlings to applied GA_3 and GA_{4+7} (Figure 7.7) the gibberellin concentrations present in seed extracts were estimated. The response of dwarf rice seedlings to GA_3 and GA_{4+7} standard solutions of equivalent GA concentration differed slightly. In the concentration range 10 to 100 ng GA a consistently greater elongation response was obtained in the presence of GA_3 than GA_{4+7}. The levels of GA-like activity detected are reported as either ng $GA_3.g^{-1}$ air

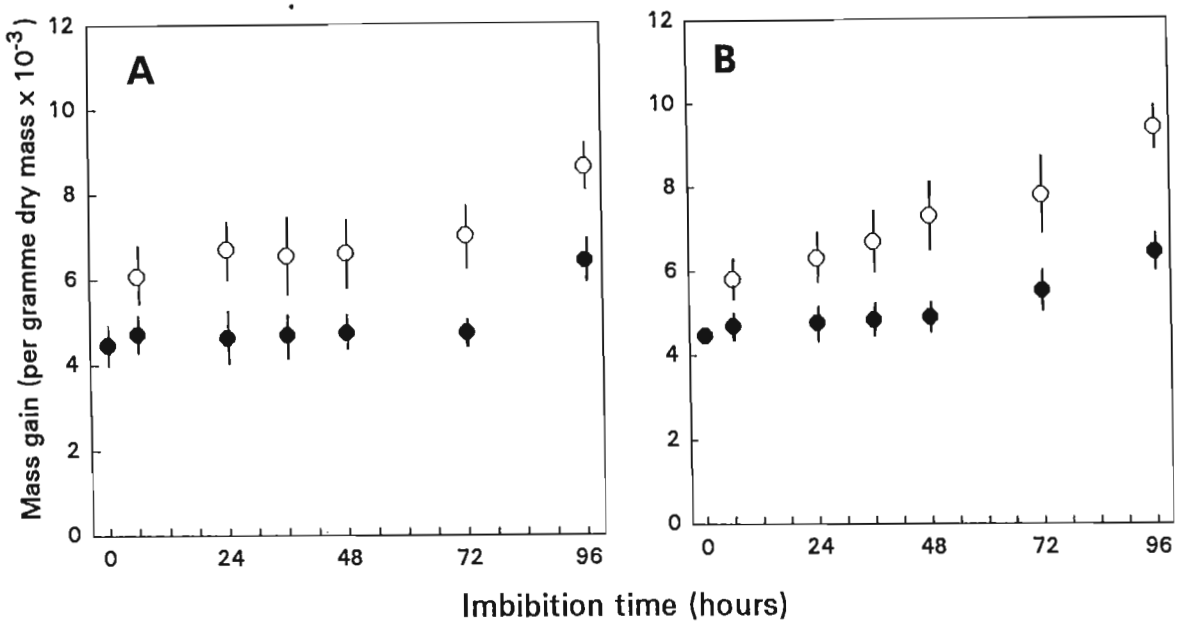


Figure 7.5: The onset of *T. triandra* embryo growth, as represented by an increase in seed dry mass. Data are standardised to 0.005 g seed fresh mass and represent mean seed mass (n = 20). Open circles represent wet mass and closed circles represent dry mass of A dormant and B non-dormant *T. triandra* seeds. [Bars represent SE]

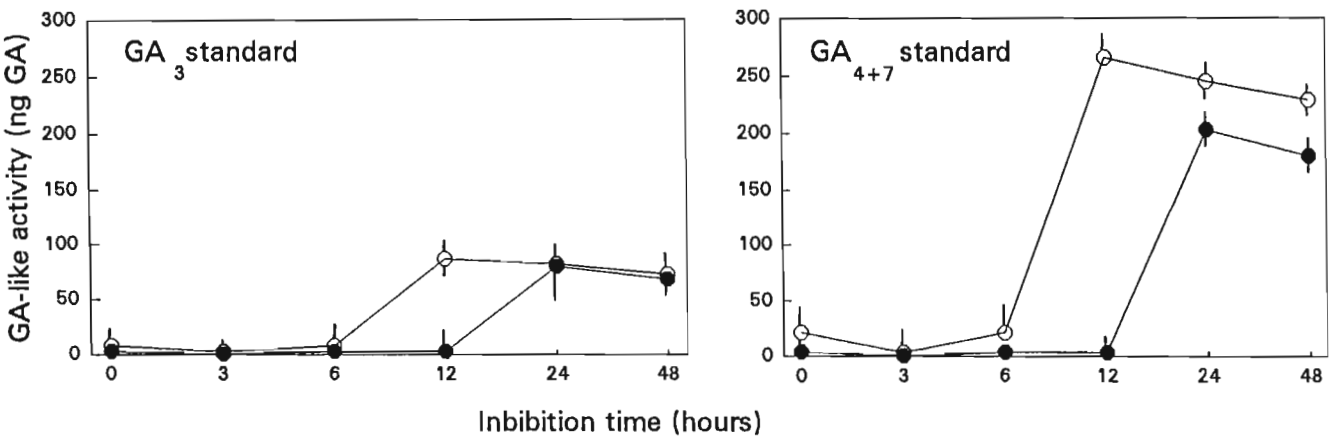


Figure 7.6: Changes in the level of gibberellin-like substances in dormant (closed circles) and non-dormant (open circles) *T. triandra* seed with increasing imbibition time.

dry seed, or ng $GA_{4+7}.g^{-1}$ air-dry seed. The peaks of GA-like activity detected in dormant and non-dormant seed reflect a gibberellin concentration of 80 and 87 ng $GA_3.g^{-1}$ air-dry seed respectively. While the corresponding GA_{4+7} equivalent concentrations are 203 and 266 ng $GA_{4+7}.g^{-1}$ air-dry seed for dormant and non-dormant seed respectively. The levels of GA-like activity also differed markedly in dry seed and for the first 6 hours of imbibition with non-dormant seed extracts containing approximately four times the level of GA-like activity recorded in dormant seed extracts.

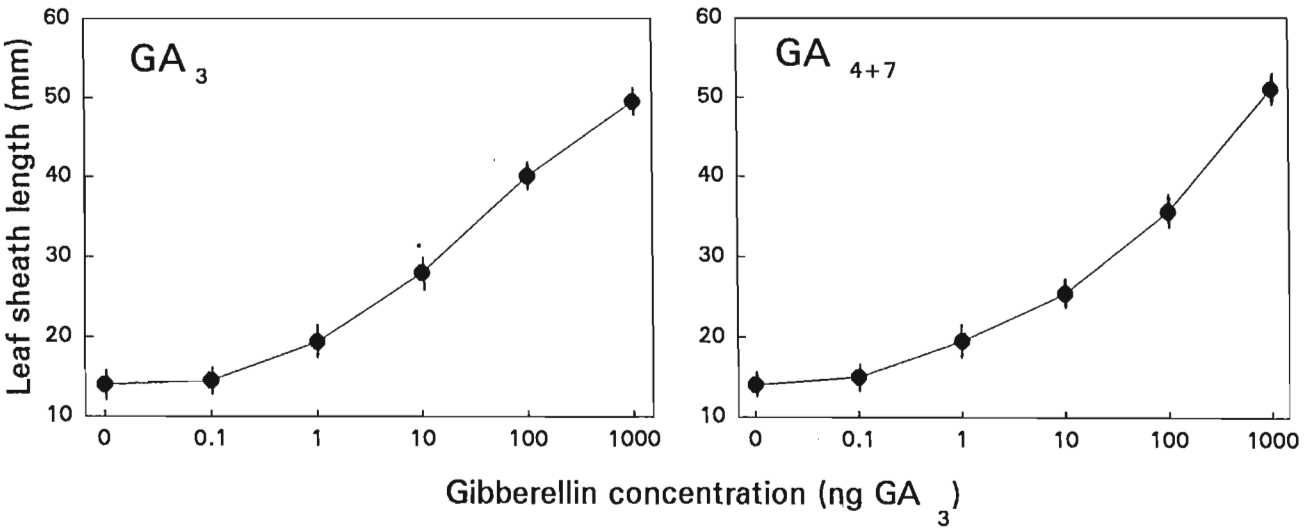


Figure 7.7: Standard curves characterising the response of gibberellin deficient dwarf-rice seed (*Oryza sativa* var. Tan-ginbozu) to a dilution series of applied GA_3 and GA_{4+7}.

7.4 Discussion

Boron has previously been shown to significantly improve the level of seed germination in dormant (eight-week-old) *T. triandra* seed, raising the percentage germination from ± 20% to > 80% (Cresswell and Nelson 1971; 1972). The promotive effect of boron on *T. triandra* seed germination was further enhanced

by joint application of boron and GA_3 (Cresswell and Nelson 1972). These authors suggest a role for boron as an intermediary in the synthesis of gibberellins in *T. triandra* seeds, but further report (Cresswell and Nelson 1973) that boron exerts a positive influence on RNA levels and increases ∝-amylase activity in dormant seed suggesting an independent role in promotion of seed germination. Contrary to previous reports (Cresswell and Nelson 1971; 1972) applied boron failed to promote germination of eight-week-old *T. triandra* seed from either the Drakensberg or Zululand populations under investigation. Furthermore, joint application of GA_3 and boron reduced the final level of *T. triandra* seed germination in comparison to that obtained in the presence of GA_3 alone. A similar result has been reported for soybean seed in which the antagonistic effect of boron on applied GA_3 negatively influenced both protein and carbohydrate metabolism (De la Haba, Roldán and Jiménez 1985). Similarly, as reported in this Chapter for *T. triandra*, boron has no effect on germination of the seed of the grasses *Agropyron elongatum* and *Elymus cincereus* (Roundy 1985). No other reports of boron promoted seed germination could be located. Applied boron does, however, inhibit the germination of soybean (De la Haba, Roldán and Jiménez 1985) and sunflower (Jiménez and Barea 1979) seeds and, in contrast to the results of Cresswell and Nelson (1973), inhibits ∝-amylase activity (Jiménez and Barea 1979). The role of boron in promoting germination of *T. triandra* seed is thus doubtful and should be viewed with caution. The response of *T. triandra* seed collected at Frankenwald Research Farm (26°08'S, 28°14' E; University of the Witwatersrand) should be re-evaluated for seed germination response to boron. In the event that *T. triandra* seed re-collected at Frankenwald responds positively to boron when re-evaluated, the impact of parental soil boron status on sibling (seed) response to applied boron would be worth investigating further.

The significant promotion of germination of dormant *T. triandra* seed by short duration (7 days) exposure to high temperature may have practical application in breaking dormancy in large volumes of seed. This technique, which is evaluated

as a seed pre-treatment in Chapter 9, should, however, be used with caution because exposure of seed, greater than 21 weeks in age, to high temperature proved detrimental to germination and reduced the final germination level. Whether this reduced germination was caused by induction of secondary dormancy or embryo mortality was not quantified.

The identification of germination inhibiting compounds within seeds has received moderate research attention (Black 1959; Gutterman, Evenari, Cooper, Levy and Lavie 1980; Thomas, Dearman and Biddington 1986; Pinfield, Stutchbury, Bazaid and Gwarazimba 1989), but has yielded few conclusive results. The results presented in this Chapter can be viewed in a similar light. Using simple chromatographic and extraction techniques the presence of two potential groups of extracts which significantly inhibited germination of lettuce seed were identified, but these extracts failed to significantly inhibit the germination of excised non-dormant *T. triandra* caryopses. Although the germination of non-dormant *T. triandra* caryopses was not significantly inhibited in the presence of extracts prepared from dormant *T. triandra* caryopses, it is of interest that one of the areas of inhibition (Rf 3 and 4) co-eluted with ABA standards separated using the same chromatographic system.

Abscisic acid has long been implicated in control of the onset of primary seed dormancy (Pinfield *et.al.* 1989) but, the role of ABA in direct control of primary dormancy in seeds has been questioned (Thomas *et.al.* 1986). Although the role and site of action of ABA in maintenance of dormancy is equivocal (McWha and Hillman 1974; Ruedinger 1982), of interest in the context of this investigation, is the ability of applied ABA to inhibit radicle elongation (Gallie, Miracca and Sparvoic 1980). In the presence of extracts prepared from dormant *T. triandra* caryopses, radicle growth of newly germinated *T. triandra* seedlings is inhibited. It is of interest that extracts prepared from non-dormant caryopses, although inhibitory to radicle elongation, do not limit radicle length to the same extent as extracts from

dormant caryopses. These data provide an indication that ABA, or other compounds inhibitory to seed germination, may be present in dormant *T. triandra* caryopses. The reduced inhibitory action, on radicle growth of non-dormant *T. triandra* caryopses extracts, may be due to a decrease in the level of such an endogenous inhibitor, as suggested for other species. In *Acer plantoides* seed endogenous ABA levels decrease during seed storage and stratification with decreasing seed ABA content corresponding to increased seed germinability (Pinfield *et.al.* 1989). The well documented ability of gibberellins to overcome ABA induced inhibition of seed germination (Gray and Thomas 1982) lends weight to this argument given the key role played by gibberellins in promotion of *T. triandra* seed germination. Furthermore, biomolecular engineering of cereal crops has revealed that interaction between ABA and gibberellins controls gene conscription. Two major ∝-amylase gene families have been recognised in wheat and barley which reside on different chromosomes and code for isozymes that can be resolved by electro-focusing into two groups, ∝-amy1 and ∝-amy2 (Lazarus, Baulcomb and Martienssen 1985). These two isozyme groups display different responses to endogenous ABA and GA hormone status, with ∝-amy1 transcription dependant on a low ABA threshold concentration and high sensitivity to low GA concentration, while ∝-amy2 transcription appears to be more dependant on increasing GA concentration or more responsive to inhibition of transcription at low ABA concentration. Thus, differential response to ABA and GA hormone status within wheat embryos affords differential ∝-amy1 and ∝-amy2 gene transcription (Lenton and Appleford 1990), providing support for the suggestion that GA stimulates and ABA inhibits transcription of ∝-amylase genes in the aleurone of cereals (Jacobsen and Chandler 1987; Fincher 1989; Huttly and Baulcomb 1989). Furthermore, determination of endogenous GA and ABA levels in wheat seed has revealed that during the first 48 hours of imbibition and germination the content of ABA in embryo and endosperm tissues decreases from 176 to 92, and 354 to 49 pg per seed part, respectively as detected using GC-SIM [selected ion monitoring] (Lenton and Appleford 1990). Over the same time period the embryo

and endosperm tissue content of GA's increases substantially with endogenous GA_{19} content (a precursor for GA_{20}, GA_1 and GA_8) in embryo tissue reaching 732 and 1311 pg / seed part after 48 and 72 hours respectively. Quantification of the levels of endogenous hormones, other than gibberellins, present in *T. triandra* caryopses was regarded as being outside of the scope of this investigation and not assessed. Hormone status within seeds is not static, as discussed above, and the balance and interplay of different hormones is likely to control dormancy and the onset of germination. Detailed investigation of changes in endogenous hormone levels within *T. triandra* caryopses following increasing imbibition and with increasing after-ripening would thus be relevant. Interaction between applied ABA, GA, kinetin and plant-derived smoke are discussed further in Chapter 8.

The level of endogenous gibberellins in *T. triandra* caryopses has previously been determined by Martin (1975) who reports equivalent GA concentrations in non-dormant *T. triandra* seed of 5.94 ng $GA.g^{-1}$ air-dry seed 4 hours after commencement of imbibition and 18.8 ng $GA.g^{-1}$ air-dry seed after 48 hours of imbibition. The level of endogenous gibberellin detected in dormant *T. triandra* seed (Martin 1975) was less than 3.75 ng $GA.g^{-1}$ air-dry seed and did not differ following 4 or 48 hours of imbibition. These levels of endogenous gibberellin are substantially lower than the peak gibberellin levels of 80 and 87 ng GA_3, and 203 and 266 ng GA_{4+7}, per gram air-dry dormant and non-dormant *T. triandra* seed respectively. Although this discrepancy is significantly large to necessitate debate, the levels of endogenous GA-like activity reported are within the same order of magnitude and can be accounted for. Martin (1975) did not justify the selection of the specific imbibition times allowed prior to GA extraction. Data presented in this Chapter, however, clearly indicates that endogenous gibberellin synthesis takes place at a slower rate, and seedling growth commences later, in dormant *T. triandra* seed than in non-dormant seed. Detection of endogenous gibberellin activity 48 hours after the start of imbibition is therefore unlikely to be representative of the same physiological state in dormant and non-dormant seed.

Moreover, the maximum levels of GA-like activity were detected in dormant and non-dormant *T. triandra* seed 24 and 12 hours after commencement of imbibition. Similarly, in wheat embryos GA-conjugates are present as free acids 12 hours after the start of imbibition (Thomas, Khan and O'Toole 1978). Following 48 hours of imbibition the levels of GA-like activity recorded in extracts of dormant and non-dormant *T. triandra* seed had decreased slightly to 0.53 and 6.30 ng GA_3 equivalent per gram air-dry seed and 0.60 and 8.83 ng GA_{4+7} equivalent per gram air-dry seed. These values remain above those reported by Martin (1975) but, this discrepancy may be attributed to differences in the methods of GA extraction employed. The method of gibberellin extraction followed by Martin (1975) involved freezing imbibed caryopses overnight, placing caryopses in a water bath at 50°C for 20 minutes, allowing the extract to stand at 4°C overnight followed by a boiling of the supernatant for two minutes prior to use in a barley half seed bioassay. Subsequent research (Hedden 1986) has, however, revealed that gibberellins become unstable at temperatures in excess of 40°C. The low gibberellin levels reported by Martin (1975) may therefore be due to thermally induced gibberellin breakdown. It would thus appear that the earlier levels of endogenous GA detected in dormant and non-dormant *T. triandra* seeds (Martin 1975) reflect an under representation of the actual seed state.

Previous studies also report that both the level of endogenous gibberellins and the ability to synthesize gibberellins is greater in non-dormant *T. triandra* seed than in dormant seed (Nelson 1971; Martin 1975). Results presented in this Chapter are in partial agreement with the first statement as endogenous levels of GA-like activity were approximately four times higher in non-dormant than in dormant *T. triandra* seed extracts during the first 6 hours of imbibition. Thereafter, however, the levels of endogenous GA-like activity peaked at similar levels in extracts from dormant and non-dormant *T. triandra* seed with maximum levels of endogenous GA-like activity in dormant seed reaching ± 75% of that recorded for non-dormant seed. It must also be noted that the levels of *T. triandra* seed

germination in the dormant and non-dormant seed populations, at the time of these experiments, were < 10% and > 75% respectively, with viability in both populations exceeding 83%. It is unlikely that GA synthesis takes place in the ± 90% of eight-week-old (dormant seed population) seed which fails to germinate. The concentration of gibberellins synthesized by the germinable eight-week-old seed is therefore likely to be substantially higher than that synthesized by the bulk of the non-dormant seed population to yield comparable peak levels of detectable GA-like activity. This argument is supported by the germination data obtained following exposure of *T. triandra* seed, of increasing age, to applied GA_3 solutions of equivalent GA concentration (Table 7.6). The GA_3 concentration required to maximise germination decreased as seed age, and hence the proportion of germinable seed, increased. Similarly, eight-week-old *T. triandra* seed from the high altitude Drakensberg and low-altitude Zululand populations possess different levels of primary dormancy with only 5% of Drakensberg and 24% of Zululand seed germinable. In the presence of applied GA_3 maximum germination of Drakensberg seed was recorded at a concentration of 500 mg $GA_3.l^{-1}$ while in the less dormant Zululand population maximum germination was recorded at 100 mg $GA_3.l^{-1}$, suggesting that seed sensitivity to GA may increase during after-ripening.

As evident from the preceding discussion, results have been presented in this Chapter which demonstrate the ability of dormant (eight-week-old) seed to synthesize gibberellins, possibly at higher concentrations per metabolically active seed, than synthesized by non-dormant *T. triandra* seed. This conclusion is in conflict with that of previous studies (Nelson 1971; Martin 1975) in which dormant seed (seed of low germinability which has yet to undergo after-ripening) is regarded as unable to synthesize gibberellins. Martin (1975) reports endogenous gibberellin levels within dormant *T. triandra* seed to be < 3.75 ng GA per gram of air-dry seed, and estimates the actual level of gibberellins to be 2.25 ng GA per gram air-dry seed suggesting that the low gibberellin levels present in dormant

T. triandra seed could not accurately be detected, which may account for the failure to identify GA synthesis in dormant *T. triandra* seed.

In conclusion of this Chapter, the role of boron in promoting germination of *T. triandra* seed has been questioned, the presence of a compound/s capable of inhibiting germination of lettuce seed, but not *T. triandra* seed, has been reported and the possibility that this inhibitory compound may be ABA discussed. Furthermore, short duration exposure of dry *T. triandra* seed to high temperature has been shown to partially overcome primary dormancy. The level of endogenous gibberellin-like activity present in dormant and non-dormant *T. triandra* seed has been determined and, in contrast to previous studies (Nelson 1971; Martin 1975), dormant *T. triandra* seed is shown to have the ability to synthesize gibberellins, within 24 hours of the start of imbibition. On the basis of the data reported it is probable that primary seed dormancy and subsequent seed germination in *T. triandra* is controlled by the balance of an endogenous inhibitor (possibly ABA) and gibberellins. The increased germinability of after-ripened seed, and freshly shed seed of high germinability, is attributed to increased gibberellin sensitivity. Similarly, the increased germination rate of non-dormant *T. triandra* seed can be attributed to a reduction in the time to commencement of gibberellin synthesis in non-dormant seed. The interaction of cytokinins and plant-derived smoke in facilitation germination of *T. triandra* seed are discussed in Chapter 8.

CHAPTER 8: THE EFFECT OF PLANT-DERIVED SMOKE ON *Themeda triandra* SEED GERMINATION

Section 8.1 General Introduction

Seed from a wide range of plant species common to fire dominated communities has recently been shown to germinate in response to plant-derived smoke (de Lange and Boucher 1990; Brown 1993a). Brown (1993a) cites unpublished results which indicate that the germination of seed of *Themeda triandra* may also be promoted by smoke. As *T. triandra* is a fire-climax species which is favoured by regular burning, every two or three years (Tainton 1984; Everson 1985), a possible association between *T. triandra* seed germination and smoke warranted further investigation.

The field of smoke-stimulated seed germination is a young one and many basic questions are unanswered. Five key questions identified are listed below;

1. *Is the germination of* Themeda triandra *seed promoted by plant-derived smoke?*
2. *Does the nature of the plant material burned affect the level to which* T. triandra *seed germination is promoted?*
3. *By what mechanism does plant-derived smoke act to stimulate* T. triandra *seed germination?*
4. *Is "smoke-stimulated germination" of practical relevance to understanding field germination of, or facilitating germination of* T. triandra *seed?*, and
5. *What is, or are, the bio-active constituent/s of plant-derived smoke ?*

Only the first four of these questions have been addressed in this Thesis and, for

clarity of presentation, are introduced, reported and discussed individually in Sections 8.2, 8.3, 8.4 and 8.5 of this Chapter. Sections 8.2 and 8.3 represent papers which were published during the course of the investigation. The papers are reported in entirety, although the numbering of figures and tables and the style of each paper has been modified to conform with the rest of this Thesis. Figure 8.2.1 has also been added for clarity. However, as these papers were not published in series a certain amount of repetition is unavoidable and does occur.

Investigation of the bio-active constituents of plant-derived smoke (the fifth question listed) was regarded as being outside of the scope of this investigation, but is under investigation by another research team in the Department of Botany at the University of Natal, Pietermaritzburg.

Section 8.2 The impact of smoke on *T. triandra* seed germination

This Section comprises the publication:

Baxter, B.J.M., van Staden, J, Granger, J.E. and Brown, N.A.C. (1993) Plant-derived smoke and smoke extracts stimulate seed germination of the fire-climax grass *Themeda triandra* Forssk. ***Environmental and Experimental Botany 34: 217-223***

8.2.1 Introduction

The role of fire in stimulation of seed germination has long been recognised. Germination cues associated with fire may be direct, as high temperature (Purdie 1977; Jeffrey, Holms and Rebelo 1988; Musil and de Wit 1991;) or plant-derived smoke (de Lange and Boucher 1990; Brown 1993a), or indirect by modified micro-environments (Ruyle, Roundy and Cox 1988) or exposure of seed to the leachate of charred wood (Keeley, Morton, Pedrosa and Trotter 1985; Keeley and Pizzorno 1986). Furthermore, ethylene is released during burning of vegetation (Russel, Fraser, Watson and Parsons 1974) and is known to stimulate seed germination (Adkins and Ross 1981; Van de Venter and Esterhuizen 1988). Studies on the stimulatory effects of plant-derived smoke on seed germination have thus far been limited to investigating the effect of smoke from burning sclerophyllous fynbos vegetation on germination of seed of species common to the fynbos (de Lange and Boucher 1990; Brown 1993a). Fynbos being a community in which fire has played an integral part in determining community structure (Cowling 1987). Similarly, studies investigating the effect of ash leachates on seed germination (Lock and Milburn 1971; Keeley and Pizzorno 1986) have favoured woody and chaparral vegetation. No reports concerning the stimulation of germination by smoke or ash leachates, derived from grass material, were found.

Themeda triandra is a dominant, palatable species of grassland and savanna regions in Africa and Australia. After a disturbance, *T. triandra* is replaced by less palatable, and hence undesirable, species and does not re-establish naturally

(Tainton 1984). Recent research has, however, highlighted the potential to re-establish *T. triandra* artificially from seed (McDougall 1989), but the artificial re-establishment of this important grass is limited by factors such as low seed viability (McDougall 1989) and dormant seed (Baxter *et.al.* 1993; Groves *et.al.* 1982). A better understanding of the cues which trigger the breaking of seed dormancy in *T. triandra* is required. In many areas *T. triandra*-dominated grasslands exist as a sub-climax community long maintained by the frequent occurrence of fire and regarded as fire-climax communities (Tainton and Mentis 1984). After fire, a marked increase in the number of *T. triandra* seedlings has been recorded in burnt areas relative to unburnt grassland (Lock and Milburn 1971; Ndawula-Senyimba 1972; Trollope 1984a). Mowing of unburnt grassland, followed by removal of the cut debris, failed to increase *T. triandra* seedling emergence (Ndawula-Senyimba 1972; Trollope 1984a), suggesting that fire promotes *T. triandra* seed germination by a means other than modification of the seedbed microclimate. As possible support for this hypothesis, the work of Brown (1993a), who cites unpublished results which indicate that *T. triandra* seed germination is stimulated by exposure to plant-derived smoke, merits further consideration in regularly fire prone grassland areas of southern Africa. As in fynbos, in fire-climax grasslands fire plays an integral part in the maintenance of community structure (Tainton 1984).

Experiments were initiated to determine the effect of plant-derived smoke, smoke extracts, ash leachates, ethrel and ethylene on the germination of seed of the fire-climax grass *T. triandra.*

8.2.2 Methods

Themeda triandra seed was collected at Cathedral Peak in the Natal Drakensberg at an altitude of 1800 m.a.m.s.l. Seed was air dried for 8 weeks and then stored at - 15° C until required. *Themeda triandra* leaf material used to generate smoke

and ash leachates was collected in a *T. triandra* dominated grassland in Pietermaritzburg. Material used was mostly dry leaf matter collected prior to spring growth.

Seeds were smoked in a wooden chest (80 x 50 x 40 cm). Smoke was generated by burning *T. triandra* leaf material in a standard beekeepers smoker. Smoke was introduced at the base of the chest and could escape slowly past a loose fitting lid (Figure 8.2.1). Dormant *T. triandra* seed (eight-weeks-old) was exposed to grass smoke for 5, 15, 45 and 90 min. Dry or pre-imbibed seeds were exposed to smoke. To investigate whether the degree of seed imbibition affected the germination response to smoke, *T. triandra* seeds were pre-imbibed for 0-36 hr, at 6-hr intervals, prior to exposure to smoke for 5 min. After smoke exposure, seeds were removed from the substrate on which they were treated and prepared for germination tests. Unless otherwise stated, germination was assessed at 30° C under constant light. Seeds were placed on two layers of Whatmans No. 1 filter paper in 9-cm diameter disposable plastic Petri dishes and moistened with 4.5 ml of distilled water. In all experiments, four replicates of 25 seeds were used. A seed was considered germinated, and removed, when the emerged radicle exceeded 1 mm in length. Germination was assessed at two-day intervals and seldom occurred after the sixth day. All experiments were terminated on the tenth day.

In a second experiment, an aqueous smoke extract was prepared by continuously bubbling smoke, from burning *T. triandra* leaf material, through a column of water (500 ml) for 45 min. A second smoke extract, derived from burning fynbos material, was provided by Dr N. Brown (Brown 1993a). From each extract, a dilution series was prepared to concentrations of 20, 10, 2 and 1 percent of the initial extracts. Eight-week-old *T. triandra* seed was germinated in the presence of these solutions.

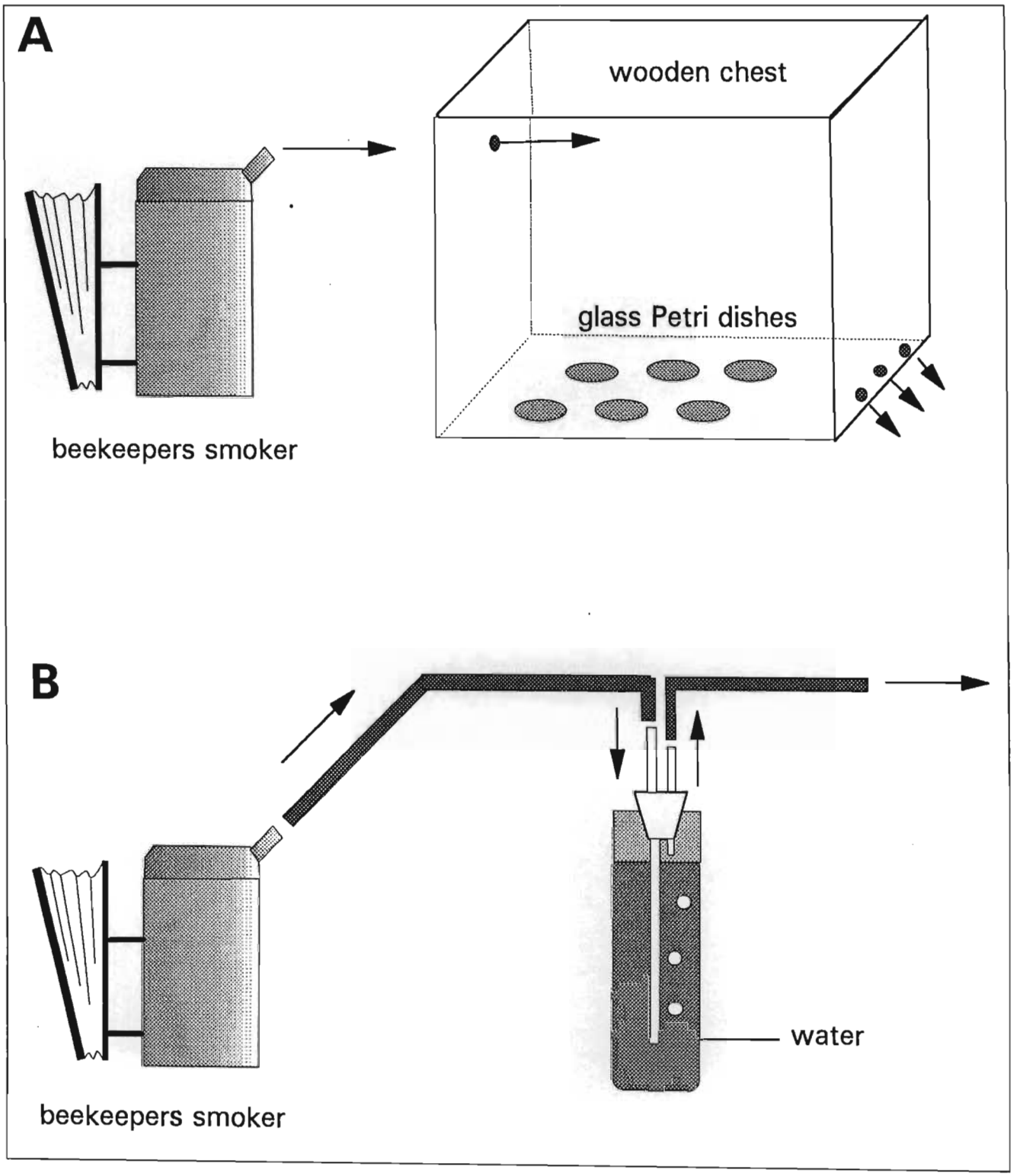


Figure 8.2.1: Apparatus used for A smoking seeds and B preparation of aqueous smoke extracts.

A 2 % dilution of *T. triandra* smoke extract provided the greatest increase in germination for that extract. Germination of eight-week-old seed in the presence of a 2 % solution of *Themeda* smoke extract was assessed at constant temperatures of 15, 20 and 30° C. At each temperature, seed in the presence of distilled water constituted the control.

The effect of ethylene on germination of eight-week-old *T. triandra* seed was assessed in 120 ml vaccine bottles sealed with gas-tight rubber stoppers held in place by crimped aluminium caps. Seeds were placed onto two layers of 4.25 cm diameter Whatmans No. 1 filter paper moistened with 1.2 ml of distilled water. A range of ethylene concentrations (10^3, 10^2, 10, 1.0, 10^{-1}, 10^{-2} and 10^{-3} $\mu l.l^{-1}$) was tested. Ethylene stocks were first prepared in gas mixing bottles at sufficient concentration so that 1 ml of stock injected into each germination vial provided the desired ethylene concentration. Before injection of the ethylene stock into each germination vial, an equal volume of air was removed. Ethylene and an aqueous smoke extract was jointly applied to dormant seed to test for possible interactive effects on *T. triandra* seed germination. Ethylene and the *Themeda* smoke extract were applied at effective optimum concentrations (i.e. ethylene 1.0 $\mu l.l^{-1}$ and aqueous smoke extract 2 % of stock). The effect of ethrel on *T. triandra* seed germination was assessed at the same concentrations used in the ethylene experiment. Seeds were germinated in the presence of ethrel for the full germination period.

An aqueous extract of *T. triandra* ash was prepared by soaking 25 g ash in 500 ml distilled water for 1 hr. Extracts were either used directly or filtered prior to use. Eight-week-old seed was germinated in the presence of these extracts. Seeds on filter paper moistened with distilled water were also covered with dry ash prior to germination.

All results are presented as a percentage of viable seed. Viability was determined

using a standard tetrazolium test for respiratory activity (Anon 1985b). Viability in the seed lot sampled for this experiment was found to be 83 % (± 0.9 %). Prior to statistical analyses all percentage data were subjected to arcsine square root transformation of percentages to degrees (Sokal and Rolf 1981). Treatment differences were detected using Tukey's Multiple Range test.

8.2.3 Results

Exposure of dry or imbibed *T. triandra* seed to plant derived smoke resulted in increased germination. The stimulatory effect of plant derived smoke on *T. triandra* seed germination was greatest after short periods of exposure of 5 or 15 min (Table 8.2.1). The germination response to plant-derived smoke increased with an increased state of imbibition (Fig. 8.2.2). A bioactive compound/s capable of stimulating *T. triandra* seed germination was present in aqueous extracts of smoke derived

Table 8.2.1: The effect of different lengths of exposure to plant-derived smoke on germination of dry and pre-imbibed *Themeda triandra* seed. Within a column, germination percentages followed by a different letter are significantly different ($P \leq 0.05$).

Exposure time (minutes)	Germination (% of viable seed)	
	Dry seed	Pre-imbibed seed
0	5.0 (1.9) a	5.0 (1.9) a
5	17.9 (5.3) a	22.7 (5.3) a
15	13.1 (3.6) a	26.3 (3.1) b
45	15.5 (4.5) a	16.7 (5.9) ab
90	8.4 (3.6) a	10.8 (2.3) ab

from both fynbos and grass material (Fig. 8.2.3). However, direct comparisons cannot be made between extracts owing to different concentrations of the smoke extract stocks. Maximum germination (53 %) was attained from a 20 % dilution

of the fynbos extract. Germination was significantly increased at dilutions as low as 2 % of the stock fynbos solution. Germination in the presence of a *T. triandra* smoke extract was greatest at a 2 % dilution of the stock solution. At higher concentrations the extract proved inhibitory to seed germination.

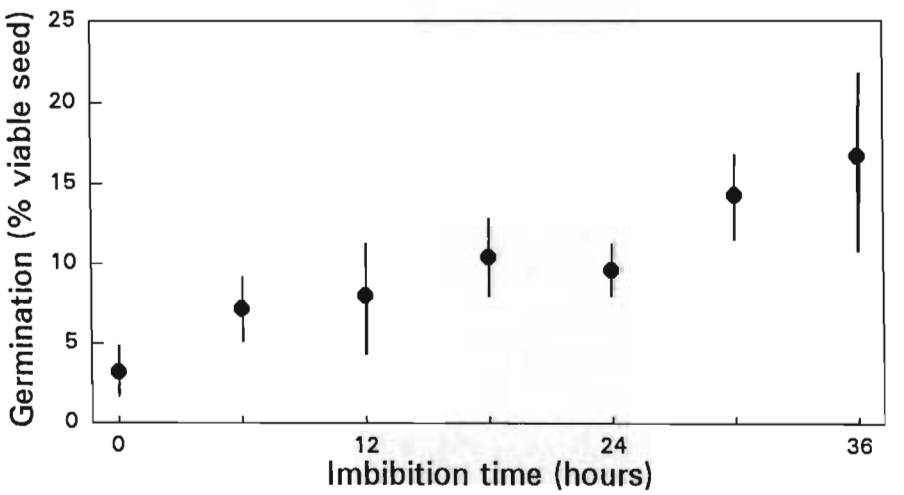


Figure 8.2.2: The effect of the degree of seed imbibition on the germination response of *Themeda triandra* seed to plant-derived smoke. (Mean ± SE).

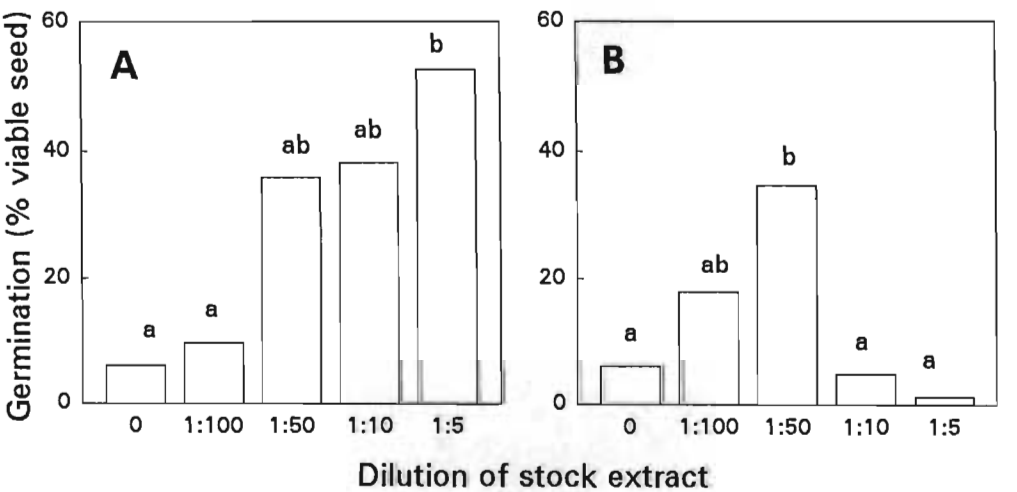


Figure 8.2.3: The effect of aqueous extracts prepared from plant-derived smoke, on the germination of eight-week-old *Themeda triandra* seed. A aqueous smoke extract prepared from burning sclerophyllous fynbos vegetation, B aqueous smoke extract prepared from burning *T. triandra* leaf material. Bars bearing different letters are significantly different, $P \leq 0.05$.

In the presence of a *T. triandra* smoke extract, germination increased at all temperatures tested (Table 8.2.2). At 15° C, a sub-optimal temperature for the germination of eight-week-old *T. triandra* seed, germination increased from 2 to 22 %, while at 30° C, the optimum germination temperature for this population (Baxter *et.al.* 1993), germination increased from 6 to 36 %. Germination of eight-

Table 8.2.2: The effect of an aqueous smoke extract, derived from burning *T. triandra* leaf material, on the germination of eight-week-old *T. triandra* seed at a range of constant temperatures. For each temperature treatment, germination percentages bearing a different letter are significantly different , $P \leq 0.05$.

	Temperature		
	15°C	20°C	30°C
Control	2.4 (1.4) a	10.8 (6.3) a	6.0 (2.3) a
Smoke extract	21.5 (1.4) b	31.1 (8.4) a	35.8 (3.1) b

week-old seed was not increased significantly in the presence of ethrel or gaseous ethylene and no interactive effect between ethylene and an aqueous smoke extract was detected (Table 8.2.3). Germination of *T. triandra* seed in the presence of the

Table 8.2.3: The effect of ethylene, ethrel and plant-derived smoke applied in combination with ethylene, on germination of eight-week-old *T. triandra* seed. Within a column, germination percentages followed by the same letter are not significantly different ($P \geq 0.05$).

Ethylene concentration ($\mu l.l^{-1}$)	Germination (% viable seed)		
	Smoke	Ethylene	Ethrel
0	20.3 (6.3) a	3.2 (1.6) a	3.2 (1.6) a
10^{-3}	-	2.4 (1.1) a	3.2 (1.6) a
10^{-2}	-	3.2 (1.0) a	3.9 (1.4) a
10^{-1}	-	2.4 (1.7) a	3.2 (1.6) a
1.0	21.5 (4.5) a	3.9 (1.4) a	3.2 (1.3) a
10^{1}	-	2.4 (1.1) a	2.4 (1.1) a
10^{2}	-	2.4 (1.1) a	1.6 (1.0) a
10^{3}	-	2.4 (1.7) a	0.8 (0.8) a

ash from burnt leaf material, or in the presence of the filtrate prepared from such material, failed to significantly increase seed germination (Table 8.2.4).

Table 8.2.4: Germination of *Themeda triandra* seed (eight-weeks-old) in the presence of ash or aqueous ash extracts prepared from burnt *T. triandra* leaf material. [24.5 g ash / 500 ml distilled water]. Germination percentage followed by the same letter are not significantly different ($P \geq 0.05$).

Treatment	Germination (% viable seed)		
distilled water control	6.0	(2.3)	a
ash	15.5	(3.6)	a
unfiltered ash extract 100%	9.6	(1.9)	a
unfiltered ash extract 50%	8.4	(5.6)	a
filtered ash extract 100%	9.6	(3.3)	a
filtered ash extract 50%	10.8	(2.3)	a

8.2.4 Discussion

Smoke derived from *T. triandra* leaf material significantly increased the germination of eight-week-old dormant seed of the fire climax grass *T. triandra*. In contrast, exogenous application of ethylene or ethrel failed to stimulate *T. triandra* seed germination, suggesting that ethylene is not the active component of plant-derived smoke. This conclusion is in agreement with that of de Lange and Boucher (1990) who reported increased germination of *Audouinia capitata* seed following exposure to plant-derived smoke, but no germination response following seed treatment with ethrel. The stimulatory effect of seed exposure to smoke increased relative to the state of seed imbibition, suggesting that smoke may act on an enzyme system or on phytohormone metabolism. Germination of *T. triandra* seed in the presence of an aqueous smoke extract increased relative to controls at all temperatures tested. That the bioactive compound/s in an aqueous smoke extract did not increase germination at 15° C to the same extent as at 30° C lends weight to an argument

that smoke acts on an enzyme system. Low temperatures retard the rate of enzymatic reactions.

Dormant seed of *T. triandra* possesses a specific requirement for high temperature to germinate (Baxter *et.al.* 1993). The stimulation of *T. triandra* seed germination by smoke at sub-optimal temperatures is therefore of ecological importance and may increase the likelihood of seed germination in the field. Smoke-stimulated seed germination may be of particular importance in tufted grasslands characterised by high summer rainfall and low winter temperatures (around 0°C) where fire is used as a management tool to maintain existing grassland community structure. Fire stimulates *T. triandra* seedling emergence (Lock and Milburn 1971; Ndawula-Senyimba 1972; Trollope 1984b) but the effect of fire on seedling emergence cannot be simulated in unburnt grassland by cutting and removing above ground biomass (Ndawula-Senyimba 1972; Trollope 1984b). Storage of dormant *T. triandra* seed at high temperatures accelerates after-ripening (Hagon 1976; Groves *et.al.* 1982). It is probable that increased *T. triandra* seedling emergence after fire results from the combined influence of smoke and high temperature on soil-stored seed. The effect of short exposure (i.e., less than 30 minutes) of *T. triandra* seed to high temperatures is under investigation. Ash plays no direct role in stimulation of *T. triandra* seed germination. The failure of ash to stimulate seed germination was not unexpected as Wicklow (1977) has shown that seed germination of the pyrophyte of *Emmenanthe penduliflora* was stimulated by charred, but not ashed, wood.

Brown (1993a) reported a positive response to plant-derived smoke in a wide range of fynbos species. In this investigation, germination of *T. triandra* seed collected in a montane grassland in Natal was stimulated by plant-derived smoke and aqueous smoke extracts derived from *T. triandra* leaf material and sclerophyllous fynbos material. This result suggests that stimulation of seed germination by plant-derived smoke may be common in fire dependant floral communities. Furthermore,

the bioactive compound/s present in smoke, which stimulate seed germination, may originate from a compound/s common to both sclerophyllous fynbos vegetation and grasses. The bioactive compound/s in aqueous extracts of charred wood of the chaparral shrub *Adenostoma fasciculatum* could also be detected following dry heating of the wood at 175° C for 30 min (Keeley and Pizzorno 1986). These authors postulate that the bioactive component present in charred wood of *A. fasciculatum* may be an oligosaccharide type molecule which is a thermal breakdown product of xylan or other hemicelluloses having glucuronic acid side chains. The bioactive compound/s present in fynbos smoke can also be obtained by dry heating the plant material at temperatures as low as 80° C (N. Brown, personal communication), indicating that the bioactive component of plant-derived smoke may also be a thermal breakdown product. The authors are currently investigating whether the bioactive component present in smoke derived from grass leaf material can be obtained by charring the leaf material. The possibility that hemicelluloses and/or cellulose may be the source of the bioactive compound/s present in plant-derived smoke warrants further investigation.

Section 8.3 Importance of the nature of the burning material

This Section comprises the publication:
Baxter, B.J.M, Granger, J.E. and van Staden, J. (1995) Plant-derived smoke and seed germination: Is all smoke good smoke, that is the burning question? ***South African Journal of Botany 61: 275-277***

8.3.1 Introduction

Plant-derived smoke stimulates the germination of seed from a wide range of plant species common to fire-dependant floral communities (De Lange and Boucher 1990; Baxter, Van Staden, Granger and Brown 1993: Brown 1993a). The promotive effect of smoke on *T. triandra* seed germination is retained during seed storage, providing an effective means of seed pre-treatment for seed which proves difficult to germinate (Baxter and Van Staden 1994). In addition, smoke has been shown to stimulate germination of soil-stored seed (De Lange and Boucher 1990). Neither the mechanism by which smoke acts to stimulate seed germination, nor the active ingredients in plant-derived smoke is known. The use of smoke directly, or as a seed pre-treatment, may be of use in promoting seed germination for species from fire-dependant floral communities, on disturbed sites. In all studies thus far, smoke has been generated by burning leaf material from a single species, or a limited number of species. A question which needs to be addressed is whether all plants, when burnt, produce smoke which is equally effective in the promotion of seed germination. This question was investigated for 27 species common to the fire-climax grasslands of the Natal Drakensberg. The potential of smoke, produced independently from each species sampled, to stimulate germination of dormant seed of *Themeda triandra* was tested.

8.3.2 Plant material and methods

Themeda triandra seed was collected at Cathedral Peak (1800 m above mean sea level) in the Natal Drakensberg. Seed was air dried for eight weeks prior to storage

in glass bottles at -15°C until use. Dormancy is retained under these storage conditions (see Chapter 3). At seed shed *T. triandra* seed from this population is deeply dormant and requires an eight month period of dry after-ripening, at ambient temperature, to lose dormancy (Baxter, Van Staden and Granger 1993). All germination trials were conducted in disposable plastic Petri dishes on filter papers moistened with equal volumes of distilled water. Germination was assessed at 30°C under constant light, the optimum germination conditions for dormant seed from this seed population (Baxter, Van Staden and Granger 1993).

Leaf material from plants sampled was collected in montane grassland at Cathedral Peak in February 1993. All material was carefully cleaned to ensure that it was species specific. Leaf material was air dried for four days before being burnt. Leaf moisture content was determined, on a wet mass basis, at the time of burning. For each species tested smoke was generated by burning 50 g of leaf material in a standard Beekeepers smoker. Difficulty was experienced in getting leaf material of certain species to burn. Consequently, the trial was repeated and, for each species tested, 10 g of *T. triandra* leaf material was first set alight in the Beekeepers smoker to provide a large, hot flame used to ignite material of the species being screened. Smoke was only introduced into a seed-smoking bag once the leaf material of the test species was burning and never within 90 seconds of the addition of leaf material from the species being screened to the burning *T. triandra* leaf material. Smoke generated following this protocol was regarded as being species specific. For those species which burnt readily no difference in *T. triandra* seed germination response was obtained irrespective of whether 50 g of leaf material of the test species only was burnt, or whether 10 g of *T. triandra* leaf material was used as a "starter".

Smoke generated was introduced into a plastic bag containing six replicates of 25 *T. triandra* seeds. Seeds were exposed to smoke prior to seed imbibition. Smoke was introduced into the plastic tent for three minutes, after which the bag was

sealed for a further two minutes. Petri dishes containing treated seeds were removed after five minutes and the filter papers moistened with distilled water for germination. After each test all equipment was thoroughly cleaned and the used plastic bag discarded and replaced with a new one.

Radicle emergence was the criterion for germination. Germinated seeds were removed at two day intervals. Germination seldom occurred after the sixth day and germination assays were terminated after 10 days.

All germination data were analysed using ANOVA and Tukey's range test. Before statistical analysis percentage data were subjected to arcsine square root transformation (Sokal and Rolf 1981).

8.3.3 Results and Discussion

Smoke generated from 18 of the 27 species screened significantly increased the germination of *T. triandra* seed (Table 8.3.1). Furthermore, the smoke from four species namely *Aristida junciformis*, *Diospyros austro-africana*, *Erica woodii* and *Sopubia cana* promoted seed germination of *T. triandra* to a greater extent than smoke generated from *T. triandra* leaf material. Smoke generated from a further 8 species promoted *T. triandra* seed germination, but this effect was not statistically significant. Smoke from only one species, *Leontonyx squarrosus*, did not promote *T. triandra* seed germination. No species screened produced a smoke detrimental to the germination of *T. triandra* seed.

That smoke from 26 of the 27 species screened promoted *T. triandra* seed germination to some extent confirms that the active component of plant-derived

Table 8.3.1: The effect of smoke, generated from burning leaf material of different plant species, on the germination of *Themeda triandra* seed. For each germination trial 40 g of leaf material, of a single species, was burnt to generate smoke. Within a column data sharing the same letter are not significantly different ($P < 0.05$).

Species	Germination (%)		Moisture content of leaf material (% Wet mass)
Control (unsmoked)	6.0 (2.0)	a	-
Themeda triandra (50 g)	20.0 (2.8)	b	22.0
T. triandra (10 g)	18.0 (1.5)	b	22.0
Loudetia simplex	14.0 (1.2)	b	17.3
Miscanthus capensis	18.0 (6.0)	b	13.5
Trachypogon spicatus	16.0 (5.9)	b	12.6
Cymbopogon excavatus	11.0 (1.9)	ab	14.0
Aristida junciformis	25.0 (4.4)	b	15.0
Monocymbium ceresciforme	18.0 (3.5)	b	25.0
Buddleia salviifolia	16.0 (1.6)	b	13.8
Diospyros austro-africana	23.0 (4.4)	b	15.2
Sebaea sedoides	16.0 (3.3)	b	25.4
Athanasia punctata	13.0 (3.4)	b	21.9
Erica woodii	22.0 (4.2)	b	19.7
Erica spp.	15.0 (4.1)	b	14.3
Helichrysum aureo-nitens	19.0 (1.9)	b	13.8
H. adenocarpum	12.0 (2.8)	ab	18.7
H. ecklonis	20.0 (5.9)	b	20.7
Metalasia muricata	11.0 (6.4)	a	14.1
Lotononis eriantha	12.0 (3.7)	ab	20.8
Leucosidea sericea	14.0 (5.8)	ab	17.3
Pteridium aquilinum	19.0 (1.9)	b	14.4
Rubus ludwigii	17.0 (5.5)	ab	14.9
Protea caffra	12.0 (3.0)	ab	11.6
Crassula vaginata	19.0 (3.0)	b	33.9
Leontonyx squarrosus	6.0 (1.2)	a	16.1
Acalypha spp.	9.0 (1.9)	a	14.3
Buchenroedera lotononoides	17.0 (2.5)	b	18.3
Sopubia cana	21.0 (2.5)	b	14.1

smoke is widespread and strengthens the argument that the active compound/s

may originate from a commonly occurring component in plants. The assertion of Roche, Dixon and Pate (1994) and Smith and Van Staden (1994) that the plant source of smoke appears to be unimportant should, however, be treated with caution. These data indicate that although the majority of species screened produce a germination promoting smoke, the effectiveness of this smoke in promoting *T. triandra* seed germination varied considerably. This variation in the effectiveness of smoke from different species to promote seed germination may reflect interspecific differences in the abundance of the unidentified component from which smoke is derived. This variation may also, however, reflect differences in the temperature at which smoke was generated. Keeley and Pizzorno (1986) report that the unidentified compound in charred wood which promotes chaparral seed germination can also be produced by dry heating plant material at 175°C for 30 min. The bioactive compounds present in fynbos smoke, generated by burning *Passerina vulgaris* material, can also be obtained by dry heating stem and leaf material of the same species at temperatures as low as 80°C (unpublished results cited by Brown 1993b). Similarly, the bioactive component of *T. triandra* smoke has been obtained by dry heating leaf material at temperatures between 175°C and 225°C. At lower temperatures, however, dry heating of *T. triandra* leaf material did not yield a germination promoting component (B.J.M. Baxter; unpublished results). The relationship between temperature and the level of activity of germination promoting compounds in plant-derived smoke warrants further investigation.

It must be noted that in this experiment the effect of a range of smoke sources was screened against seed of a single species (*T. triandra*). Conversely, previous studies have screened smoke, from a limited source, against seed from a wide range of different species (Brown 1993a; Brown *et.al.* 1993). During a natural fire it is probable that the smoke produced from many different species would promote germination of seed from an equally broad range of species. It would be of considerable interest, however, to determine whether a species specific relationship

has evolved within fire-dominated plant communities between parent plant and seeds with respect to smoke cued germination.

In early reports on the germination promoting effect of plant-derived smoke (de Lange and Boucher 1990; Brown 1993a), smoke was generated by burning a mixture of wild species. Given the marked variation in the effectiveness of smoke produced from different species in promoting seed germination, workers in the field of smoke stimulated seed germination are urged to follow the lead of Baxter *et.al.* (1993) and Brown *et.al.* (1993) in burning a single species to generate smoke. Furthermore, it is suggested that researchers also determine and report the moisture content and total mass of plant material burnt. These data should also be incorporated in the data bases of smoke-responsive species initiated by the National Botanical Institute at Kirstenbosch, South Africa and by the staff of Kings Park and Botanic Garden, Australia.

Section 8.4 The mechanism by which smoke promotes germination

Plant-derived smoke and *Themeda triandra* seed germination : smoke interaction with plant hormones.

8.4.1 Introduction

Plant derived smoke and aqueous extracts of smoke have been shown to promote seed germination in a wide range of species from fire-dominated plant communities (de Lange and Boucher 1990; Baxter *et.al.* 1993; Brown 1993a). Early indications are that the active component of smoke is not species specific (Brown 1993; Baxter *et.al.* 1993), although not all species from fire dominated communities produce seed which germinates in response to smoke (Brown *et.al.* 1993; Brown 1993b).

The promotive effect of smoke on *T. triandra* seed germination increases in accordance with an increased level of seed imbibition (Baxter *et.al.* 1993). Furthermore, *T. triandra* seed can be pre-treated with aqueous smoke extracts, dried and stored for up to 21 days with no loss in the level of *T. triandra* seed germination on re-imbibition (Baxter and van Staden 1994), leading these authors to suggest that smoke acts at a metabolic level to promote seed germination. It is not known, however, whether imbibed dormant *T. triandra* seed (ie. seed which has attained full imbibition is water, but which failed to germinate) will germinate when exposed to smoke.

It is of interest that germination of *Audouinia capitata* seed can be improved by exogenous treatment with smoke or gibberellic acid. Brown *et.al.* (1993) cite unpublished results which indicate that seed dormancy of *Syncarpa vestitum* is also overcome by smoke or gibberellins. Similarly, germination of dormant seed

of *T. triandra* can be improved by exogenous treatment with gibberellins (Groves *et.al.* 1982; Baxter and Van Staden 1992) or smoke (Baxter *et.al.* 1993; Baxter and van Staden 1994). Van de Venter and Esterhuizen (1988) provided indirect evidence that ethylene and ammonia, which are released during burning, may trigger seed germination of *Erica hebecalyx*. Seed germination of *A. capitata* and *T. triandra*, however, is not promoted by either ethrel (de Lange and Boucher 1990; Baxter *et.al.* 1993) or ethylene (Baxter *et.al.* 1993), although seed germination in both species is promoted by smoke. The mechanism by which smoke acts to promote seed germination is not known.

In this section of Chapter 8 the interactive effects of smoke and the plant hormones gibberellic acid, kinetin and abscisic acid on germination of *T. triandra* seed are reported.

8.4.2 Plant material and methods

The source and handling of *T. triandra* seed material prior to experimentation has been described (Baxter *et.al.* 1993).

In all experiments reported, aqueous extracts of smoke, generated by burning only *T. triandra* leaf material, were used. The method of preparation of aqueous smoke extracts has been described (see section 8.2.2). Unless otherwise stated, germination was assessed at 30°C under constant light. Six replicates of 25 seeds were used. Seeds were germinated in 9 cm diameter sterile plastic Petri dishes on two layers of Whatman's No.1 filter paper, moistened with 4.5 ml of distilled water or treatment solution. Radicle emergence was the criterion for seed germination and germinated seeds were counted and removed at two day intervals. Germination seldom occurred after the sixth day and experiments were terminated after 10 days, unless otherwise indicated.

In all reports to date smoke treatments (gaseous or aqueous) have been applied to either dry seed, or during the early stages of seed imbibition and germination. If smoke acts at a metabolic level to facilitate germination, the positive effect of smoke on seed germination should be evident irrespective of when smoke treatment commences. To test this hypothesis, *T. triandra* seeds were germinated on filter papers moistened with distilled water for 6 days. After 6 days ungerminated seeds were transferred to filter papers moistened with a 2 % aqueous smoke extract. Controls involved seeds germinated in either distilled water or a 2 % aqueous smoke extract. Germination was allowed to proceed for a total of 14 days.

To further the level of understanding of how smoke might act to promote *T. triandra* seed germination a number of experiments involving treatment of seed with smoke in combination with plant hormones, were initiated. Seed germination was assessed in the presence of gibberellic acid (GA_3 100 $mg.l^{-1}$) and kinetin (50μM). Treatment solutions were applied individually, or in combination with an aqueous smoke extract. When a treatment solution and smoke extract were applied in combination the concentration of each constituent, in the combined solution, equalled the concentrations listed above for each constituent in isolation (GA_3 100 $mg.l^{-1}$ and kinetin 50μM).

Abscisic acid (ABA) is known to inhibit germination of non-dormant *T. triandra* seed (see Section 7.3). But, ABA induced inhibition of *T. triandra* seed germination can be overcome by application of GA_3 (Chapter 7). Similarly, ABA induced inhibition of lettuce seed germination can be overcome by applied kinetin (Thomas 1977). As seed germination was promoted in the presence of GA_3, smoke and smoke applied in combination with GA_3 or kinetin, the ability of these substances to overcome ABA inhibited germination of non-dormant *T. triandra* seed was investigated. ABA was applied, individually or in combination, at an effective concentration of 10 $mg.l^{-1}$. To confirm results obtained for *T. triandra*, the same

treatments were applied to seeds of radish, wheat and sunflower. Germination conditions were as for *T. triandra* seed.

Germination data are presented as a percentage of viable seed. *Themeda triandra* seed viability equalled 83 %. Viability of other seed types exceeded 90 per cent. All percentage data were arcsine square root transformed before statistical analyses (Sokal and Rolf 1981). Treatment differences were detected using Tukey's multiple range test.

8.4.3 Results

Germination of dormant *T. triandra* seed, which had failed to germinate in water, was significantly promoted ($P \leq 0.05$) on transfer of seed to smoke solutions (Figure 8.4.1). The level of seed germination attained following transfer of seeds to smoke solutions approximates that of *T. triandra* seed germinated directly in aqueous smoke extracts.

In the presence of combined solutions of an aqueous smoke extract and gibberellic acid (GA_3), the final level of germination of *T. triandra* seed (Figure 8.4.2) is significantly ($P \leq 0.05$) greater (63%) than the level of germination attained in the presence of either smoke (aq) or GA_3 in isolation (27 and 22 percent respectively). *Themeda triandra* seed germination in combined solutions of smoke (aq) and kinetin was also greater than the level of germination in smoke (aq) alone (39 and 27 percent respectively). Kinetin, in isolation, failed to promote *T. triandra* seed germination.

It was initially assumed that the significant synergism evident in the action of smoke (aq) and GA_3 on *T. triandra* seed germination may be indicative of smoke mimicking, or substituting for, gibberellins and thereby promoting seed germination. This hypothesis was discounted by testing a dilution series of smoke extract for

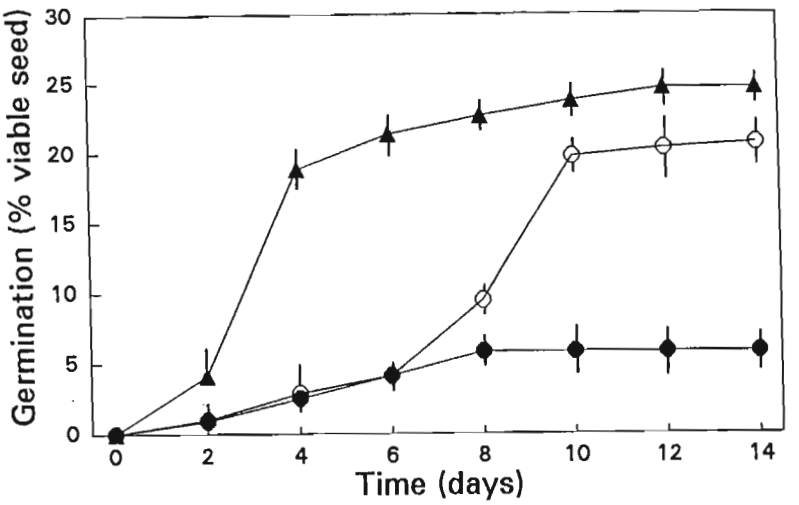


Figure 8.4.1: The effect on *Themeda triandra* seed germination of transfer of ungerminated *T. triandra* seeds to an aqueous smoke solution, six days after commencement of germination in distilled water. Closed circles represent seed germinated in distilled water only; open circles mark germination following transfer to smoke on the sixth day; closed triangles represent the level of germination of *T. triandra* seed held in the presence of aqueous smoke for 14 days.

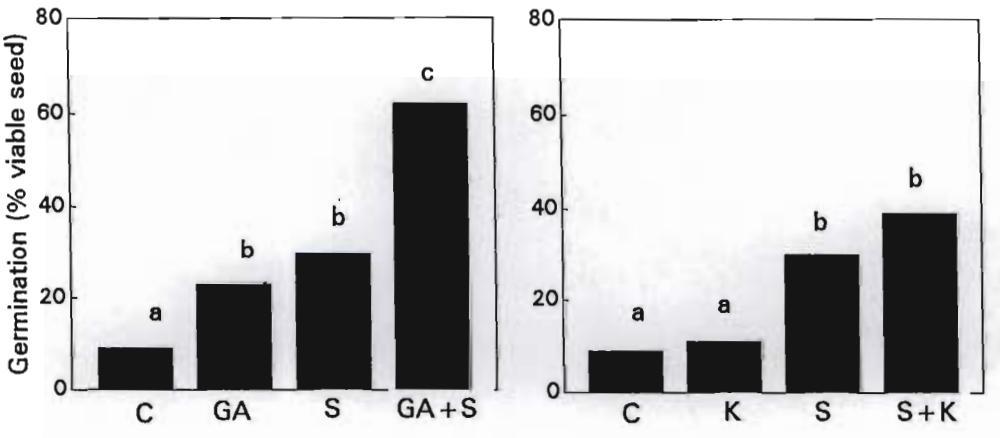


Figure 8.4.2: The effect of A GA_3 and B kinetin on *T. triandra* seed germination when applied individually or in combination with an aqueous smoke solution. Bars bearing different letters are significantly different ($P \leq 0.05$). C = control, GA = GA_3 (100 $mg.l^{-1}$), K = kinetin (50 μM) and S = smoke (2 % aq.).

gibberellin-like activity using the dwarf-rice bioassay of Murakami (1970). In addition, extracts were prepared from *T. triandra* seeds, imbibed in the presence of smoke. These extracts were also tested for levels of gibberellin-like activity using the dwarf-rice bioassay. This second test was carried out to determine whether smoke facilitates the promotion of gibberellin synthesis. Both tests proved to be negative. Results are not included in this Chapter, but are appended for reference by future researchers (Appendix 1).

Abscisic acid (ABA) inhibits the germination of non-dormant *T. triandra* seed, reducing germination by 67 percent. But, ABA induced inhibition of *T. triandra* seed germination is overcome when seeds are transferred from ABA to aqueous smoke extracts, GA_3 or kinetin (Table 8.4.1), indicating that smoke may promote *T. triandra* seed germination by counteracting the inhibitory effect of ABA. These results were validated by repeating the experiment using non-dormant wheat, radish and sunflower seed. For all three additional species tested ABA inhibited the germination of seed. In wheat and radish seed, ABA induced inhibition of seed germination was overcome by smoke (aq), GA_3 and kinetin. The same trends were obtained using sunflower seeds, but results were not statistically significant ($P \leq 0.05$).

Table 8.4.1: The effect of gibberellic acid (100 $mg.l^{-1}$), kinetin (50 μM) and aqueous smoke (2 %) in overcoming ABA induced inhibition of non-dormant seed of *T. triandra*, wheat, radish and sunflower. Within a column data bearing the same letter are not significantly different ($P \leq 0.05$).

Treatment	Species			
	Themeda triandra	Wheat	Radish	Sunflowers
Water	84.4 (± 6.4) d	91.7 (± 1.7) c	98.7 (± 1.3) b	76.7 (± 6.1) b
ABA	17.3 (± 2.9) a	46.7 (± 1.7) a	36.0 (± 8.3) a	53.3 (± 1.7) a
ABA + Smoke	57.3 (± 4.7) b	83.3 (± 3.3) c	90.7 (± 3.5) b	66.7 (± 1.3) ab
ABA + GA_3	64.3 (± 5.9) bc	80.0 (± 2.9) bc	86.7 (± 1.3) b	65.0 (± 5.8) ab
ABA + Kinetin	75.6 (± 4.0) cd	68.3 (± 4.4) b	84.0 (± 6.1) b	66.7 (± 1.3) ab

8.4.4 Discussion

The mechanism by which plant-derived smoke enables seed germination remains elusive. These results confirm, however, that smoke acts at a metabolic level as suggested by Baxter, van Staden, Granger and Brown (1993). Moreover, smoke counteracts the inhibitory effect of exogenously applied abscisic acid suggesting a role in removing a block to seed germination. The discrepancy in the final level of *T. triandra* seed germination in the presence of smoke applied in combination with GA_3 or kinetin is worth noting. The role of gibberellins in promoting $\propto$-amylase synthesis in grass and cereal seeds is well known (Simpson 1990) and GA_3 is known to promote germination of *T. triandra* seed (Groves *et.al.* 1982; Baxter and van Staden 1992). In contrast, exogenous application of cytokinins rarely improves seed germination, (Hurtt and Taylorson 1978; Chancellor and Parker 1972; Sharma, McBeath and Vanden Born 1976) and applied cytokinins do not improve the level of germination of dormant *T. triandra* seed (Hagon 1976). Cytokinins do, however, counteract the effect of compounds which inhibit seed germination, particularly abscisic acid [ABA] (Khan 1975) and are therefore regarded as playing a permissive role in seed germination, allowing other promoters [eg: Gibberellins] to act (Khan 1971; Gray and Thomas 1982).

The role of ABA, and other inhibitors in maintenance of seed dormancy remains equivocal, with high levels of ABA reported in both dormant and non-dormant (McWha and Hillman 1974) seeds. In addition no difference in the level of ABA in dormant and non-dormant seed of *Avena* (Berrie, Buller, Don and Parker 1979; Ruedinger 1982) and *Pyrus* (Dennis, Martin, Gaskin and MacMillan 1980) has been detected. Similarly, the mechanism by which ABA enforces dormancy is under debate. Exogenous application of ABA is known to inhibit both nucleic acid (Walbot, Clutter and Sussex 1975) and protein (Fountain and Bewley 1976) synthesis, metabolic processes which take place soon after commencement of imbibition (Bewley and Black 1984). Yet, exogenous ABA also inhibits radicle

elongation (Schopfer, Bajracharya and Plachy 1979; Gallie *et.al.* 1980), a process which takes place after other endogenous germination processes have been completed. Notwithstanding debate on the equivocal role for ABA in seed dormancy, numerous studies show a correlation between breaking of dormancy and reduction in the level of endogenous ABA (Le Page-Degiury and Garello 1973; Bewley and Black 1984). It is also know that ABA exerts control over ∝-amylase gene expression in *Avena fatua* (Zwar and Hooley 1986) and that applied ABA inhibits ∝-amylase synthesis in wheat (Varty, Arreguin, Gomez, Lopez and Gomez 1983) and *Hordeum vulgare* (Leshem 1978). Inhibition of seed germination by ABA is accompanied by reduced nucleic acid synthesis in wheat (Chen and Osborn 1970), Ash (Villiers 1968) and lettuce (Fountain 1974) embryos. Associated with gibberellin and cytokinin mediated overcoming of ABA inhibited seed germination, however, there is an increase in nucleic acid synthesis (Khan and Heit 1969; Keng and Foley 1987).

The possibility that smoke, in a manner similar to that of cytokinins, plays a permissive role in seed germination warrants further investigation. Although speculative at present, if smoke indeed removes an inhibitory block to seed germination, thereby allowing other compounds to act, the marked synergism between smoke and GA_3 would be explained. The effect of GA_3, in combination with kinetin, on germination of dormant *T. triandra* seed should be investigated further. In addition, it would be of interest to determine whether changes in nucleic acid synthesis take place in *Themeda triandra* embryos associated with GA_3 and kinetin mediated reversal of ABA inhibition of germination and whether smoke triggers a similar response in treated embryos.

Section 8.5 Applied aspects relating to smoke-stimulated germination of *T. triandra* seed.

8.5.1 Introduction

Not only does plant-derived smoke stimulate germination of a wide range of species, under laboratory or greenhouse conditions (Brown 1993a, Brown, Kotze and Botha 1993), but smoke promotes germination of seed buried in the soil seed bank (de Lange and Bouche 1990; Roche, Dixon and Pate 1994). Although the germination promoting compound/s have not been identified (van Staden, Drewes and Jäger 1995), Baldwin, Staszakkozinski and Davidson (1994) estimate that the bioactive compounds are active at concentrations of less than 1 pg per seed and remain active in soil for at least 53 days under greenhouse conditions. The response of buried *Themeda triandra* seed to smoke is not known. It is also not known how long aqueous extracts of smoke remain active in promoting seed germination.

Fire, or factors associated with fire, are known to promote field germination of *T. triandra* seed (Trollope 1984b; Everson 1994). Smoke may be one of a number of cues responsible for post fire *T. triandra* germination. Undoubtedly, soil-stored seed is exposed to elevated temperatures during passage of fire over the seed position, but soil attenuation of heat during a grassland fire has not been quantified. In Australian woodland, however, soil temperatures in excess of 250°C have been recorded at a depth of 2.5 centimetres in dry soils containing approximately 7% water (Beadle 1940). In contrast, in "wet" soils (approximately 21% water) soil temperatures recorded at a depth of 2.5 centimetres were in the range 132°C-135°C. In these experiments a fire burning 20 pounds of cut timber, was burned for 2 hours on the experimental site. The effect of smoke, in combination with short duration high-temperature seed treatments, on *T. triandra*

seed germination is reported in this section.

The germination promoting compounds present in charred chaparral wood (Keeley *et.al.* 1985; Keeley and Pizorno 1986) and combusted cellulose (Baldwin and Morse 1994) can be extracted at temperatures of 175°C and between 125-150°C respectively, in the absence of burning. The ability to extract a compound/s, capable of stimulating *T. triandra* seed germination was investigated.

8.5.2 Methods

The effect of smoke in promoting germination of buried seed of *T. triandra* was assessed under laboratory conditions. The methods for smoking seed, as described in Section 8.2.2 were applied with minor modifications. Seed was buried in soil in glass containers to a depth of 5mm. Following exposure to smoke for 5, 15, 45 or 90 minutes containers were removed and the soil wetted. Germination proceeded as described except that emergence of the plumule at the soil surface was the criterion for germination.

Beadle (1940) recorded soil temperatures of 132°C to 135°C at 2.5 cm depth in moist soil during a controlled woodland fire of two hours duration, during which time 20 pounds of cut *Eucalyptus* wood was burned on a site 4 square feet in area (ie. approximately 23 kg of timber per square meter). To place these figures in perspective, a dry matter grassland yield of 5 tons per hectare equates to 1.08 $kg.m^{-2}$ dry grass material, which would be consumed in a natural fire in a matter of minutes. Accordingly, a lower range of temperatures, between 70°C and 125°C were selected to establish whether short-duration exposure of dry seed to high temperatures impacts *T. triandra* seed germination. Dry *T. triandra* seeds were placed in a muffle furnace, in the absence of soil, equilibrated at temperatures from 70°C to 125°C for 1 to 15 minutes. After heat treatment seeds were allowed

to cool gradually prior to germination at 30°C in distilled water or an aqueous smoke extract (2 %).

Extraction of a germination promoting, water-soluble, compound by heating, not burning, leaf material involved heating 50 g of green *T. triandra* leaf material in a muffle furnace for 20 minutes. On removal, 500 ml distilled water was added to the heated leaf material and the solution allowed to stand for 1 hour before the liquid extract was decanted. Dormant *T. triandra* seeds from the Drakensberg population were germinated in the presence of dilution series of these extracts. Extracts were prepared at a range of temperatures from 50°C to 225°C.

All aqueous extracts of smoke were stored at 5°C ± 1°C in glass bottles. The effectiveness of stored smoke extracts, in promoting germination of dormant seed of *T. triandra* was assessed at regular time intervals.

8.5.3 Results

Germination of *T. triandra* seed buried in soil is significantly promoted by plant-derived smoke (Table 8:5.1). Maximum germination of buried imbibed seed was attained following 5 minutes of smoking. In contrast maximum germination was only attained in buried dry seed following 45 minutes of exposure to smoke.

Short-duration exposure of *T. triandra* seed to high temperature decreased germination for all eight temperature / time combinations tested, when *T. triandra* seed was germinated in distilled water (Table 8.5.2.). In contrast, when *T. triandra* seed was germinated in an aqueous smoke solution, after exposure to high temperatures, no significant reduction in the level of germination relative to controls was recorded. Maximum germination was attained following a two minute exposure of seed at 90°C prior to germination in an aqueous smoke extract.

At temperatures of 100°C or greater seed germination was severely impaired. Smoke significantly increased *T. triandra* seed germination for all temperature treatments below 100°C ($P \leq 0.05$).

Table 8.5.1: The effect of plant-derived smoke on germination of buried seed of *Themeda triandra*. Dry and imbibed seeds, buried in soil, were exposed to smoke for different periods of time. Within a column data followed by a different letter is significantly different ($P \leq 0.05$).

Smoking time (minutes)	Germination (% viable seed)	
	Dry when smoked	Imbibed when smoked
0	6.0 a	6.0 a
5	15.0 b	19.5 a
15	14.0 b	19.5 a
45	23.0 b	16.5 a
90	16.5 b	18.5 a

Table 8.5.2: The effect of short periods of exposure of seed of *Themeda triandra* to high temperature, on germination in distilled water or aqueous extracts of plant-derived smoke. Within a column data followed by a different letter is significantly different ($P \leq 0.05$). An * in the final column indicates a significant difference in the level of germination attained in water versus smoke (aq) for a particular treatment ($P \leq 0.05$), ns = not significant.

	Germination (% viable seed)				
	Water		Smoke extract		
CONTROL	9.0 (± 2.3)	c	21.3 (± 2.7)	cd	*
70°C /2 min	4.9 (± 1.2)	bc	21.3 (± 4.3)	cd	*
70°C /5 min	3.3 (± 1.6)	ab	17.2 (± 1.7)	cd	*
70°C /15 min	3.3 (± 1.0)	bc	14.8 (± 2.2)	c	*
90°C /2 min	2.5 (± 1.7)	ab	24.6 (± 4.6)	d	*
100°C /2 min	1.3 (± 0.8)	ab	0	a	ns
100°C /4 min	0	a	0	a	ns
125°C /1 min	3.3 (± 1.2)	bc	2.7 (± 0.8)	b	ns
125°C /1 min	0	a	0	a	ns

A compound capable of significantly ($P \leq 0.05$) increasing the level of germination of dormant *T. triandra* seed was extracted from grass and leaf material heated at temperatures between 175°C and 225°C (Table 8.5.3.). Extracts prepared from leaf material heated at lower temperatures did not promote seed germination.

Table 8.5.3: The level of *Themeda triandra* seed germination in the presence of a dilution series of aqueous extracts prepared from *T. triandra* leaf material heated at a range of temperatures. Data represent germination, expressed as a percentage of viable seed. Within a column germination values followed by a different letter are significantly different ($P \leq 0.05$)

Extract dilution (%)	Temperature (°C)											
	50		100		150		175		200		225	
Control	7.4 (2.4)	a	7.4 (2.8)	a	7.4 (2.8)	a	7.4 (2.8)	ab	7.4 (2.8)	a	7.4 (2.8)	a
100	0	a	8.2 (5.1)	a	9.0 (2.7)	a	18.1 (4.2)	c	21.3 (3.6)	b	14.8 (1.8)	b
10	10.6 (6.0)	a	8.2 (5.1)	a	12.3 (2.8)	a	9.8 (1.8)	bc	20.5 (1.8)	b	20.5 (1.8)	b
1	9.0 (4.1)	a	9.0 (4.1)	a	7.4 (1.7)	a	5.8 (3.7)	a	4.9 (1.2)	a	12.3 (1.7)	ab

Aqueous smoke extracts used in experiments reported in Chapter 8 were stored for up to 36 weeks with no significant loss in germination promoting activity (Table 8.5.4).

Table 8.5.4: Changes in the germination promoting capacity of an aqueous smoke extract stored at 5°C±2°C for 36 weeks. Within a column data followed by a different letter is significantly different ($P \leq 0.05$).

Storage time (weeks)	Germination (% viable seed)	
0	34.6 (± 4.1)	a
18	28.7 (± 3.2)	a
36	28.7 (± 3.2)	a

8.5.4 Discussion

Plant derived smoke promotes germination of buried *T. triandra* seed, as reported for species from the Cape fynbos (de Lange and Boucher 1990) and Australian heathland (Roche *et.al* 1994). Furthermore, the response of imbibed buried *T. triandra* seed to smoke is substantially quicker than that of dry buried seed. A similar result has been reported by Baxter *et.al.* (1993) following the smoking of dry or imbibed seeds in the absence of soil, leading these authors to suggest that smoke acts at a metabolic level to promote seed germination. Irrespective of the "mode of action" of smoke in promoting seed germination, it is worth noting that the promotive effect of smoke is specific to certain species (Brown 1993a), stimulates germination of buried seed (de Lange and Boucher 1990; Roche *et.al.* 1994) and remains active in the soil for at least 7½ weeks promoting germination at concentrations as low as 1 pg per seed (Baldwin *et.al* 1994). Data reported in this section further indicates that smoke enables germination of *T. triandra* seed rendered less germinable by exposure to high temperature. The demonstration of a positive link between seed exposure to smoke and high temperature may better explain the cues responsible for the flush of seedlings of *T. triandra* (Trollop 1984b; Everson 1994) and numerous other species (Meney, Nielssen and Dixon 1994) after fire.

Although extraction of germination promoting substances within a similar range of temperatures to that reported in this section have been reported (Keeley *et.al* 1985; Keeley and Pizorno 1986; Baldwin *et.al.* 1994) and the specificity of the temperature range at which the active compound is liberated undoubtedly provides an indication as to the nature of the active compound/s, no further comment regarding the nature of the active component of smoke will be made because identification of the active ingredient in smoke is outside the scope of this investigation.

The success with which water-soluble germination promoting compounds can be extracted from *T. triandra* leaf material at relatively low temperatures in the absence of burning facilitates use of smoke-stimulation technologies on an applied scale. Subsequent to this investigation in which aqueous smoke extracts were stored for 36 weeks without loss of biological activity, Drewes, Smith and van Staden (1994) report storage of a similar extract for 12 months without loss of biological activity. The stability of the active components of smoke, in aqueous solution, during prolonged storage benefits application of these technologies on a large scale.

Section 8.6 General Discussion

This study contains the first reports where smoke derived from grass promotes seed germination. Previous reports of smoke stimulated seed germination were based on the use of smoke derived from burning of woody chaparral (Keeley *et.al.* 1985; Keeley and Pizzorno 1986) or fynbos (de Lange and Boucher 1990; Brown, 1993) vegetation. The discovery that grassland smoke promotes germination of *T. triandra* seed (Baxter *et.al.* 1993) has importance in broadening the awareness of scientists and land managers to new factors which play a role in facilitating germination of wild seed, particularly in the extensive grassland and savanna regions of Africa and Australia where fire plays an integral part in maintaining community structure (Tainton and Mentis 1984) and where protection from fire results in a deterioration in grassland quality and species composition (Everson and Tainton 1984). Moreover, this study has shown that a wide range of species common to grassland areas produce smoke, when burnt, that promotes germination of *T. triandra* seed, reinforcing the argument that the origin of promotive compounds in smoke may be from a commonly occurring plant component. The results presented in Section 8.3, also strongly suggest that wild-fire smoke, derived from a large number of species in the plant community burnt, will be effective in promoting germination of native seed. Equally important in the context of field germination in response to wild-fire smoke is the failure of any species screened to produce smoke detrimental to seed germination.

An awareness of the role of smoke in promoting seed germination needs to permeate land management decisions. Fire is already used as a powerful land management tool to ensure the presence of dominant fire-climax grass species in grassland and savanna regions. Results from long term burning experiments conducted at Cathedral Peak indicate that the most favourable burning programme, for maintenance of a fire-climax grassland community, is a biennial spring burn. Under this burning regime the dominant grass species persist in a state of

equilibrium with the proportion of plants increasing and decreasing in size within the system being approximately equal (Everson 1994). In contrast, an annual winter burn, although favouring maintenance of established *T. triandra* tufts, leads to an increase in the unpalatable species *Tristachya leucothrix* and *Stiburus alopecuroides* while a five year burning rotation encourages dominance of large tufts at the expense of smaller individuals and seedlings. Everson (1994) concluded that in Drakensberg grasslands competition is the major constraint to seedling establishment, necessitating some form of disturbance to disrupt the established competitive interactions which exist within the grassland community, to allow seedlings to become established. Fire provides this form of disturbance, by removal of the established grass canopy and promotion of vegetative tiller production, allowing seedlings a window of reduced competition in which to become established. But, the timing of a burn must be managed to advantage both seed germination and seedling establishment. Annual burning provides regular disturbance and favours perpetuation of established *T. triandra* tufts, but little *T. triandra* seed set occurs in the first season following a burn. In contrast, protection from burning for longer periods leads to a reduction in grassland diversity (Everson 1994). Biennial spring burning allows optimum *T. triandra* seed set and field establishment (Everson 1994).

It is important to note that maximum emergence of *T. triandra* seedlings follows burning (Ndwula-Senyimba 1972; Trollope 1984b; Everson 1994), and that seedling emergence following spring burning occurs prior to seed shed (Everson 1994). Seedlings must therefore be derived from soil-stored seed. In this Chapter the ability of smoke to promote germination of buried *T. triandra* seed has been reported. Delaying the commencement of biennial burning within a season to maximise smoke generation, by burning when more green leaf material is present, is not recommended because the detrimental impacts of summer burning on grassland diversity and species abundance are well documented (Everson 1985; Everson 1994).

Apart from the beneficial effects of fire in reducing competition, burning may also modify the soil chemical and physical environment. The removal of canopy and litter material containing allelopathic compounds has been reported (Christensen and Muller 1975). In addition, the positive effect of fire in increasing post fire soil temperature is detectable for up to four weeks after a burn in Kwazulu-Natal grasslands (Savage and Vermeulen 1983). Elevated post fire soil temperature enables increased bacterial fixation of nitrogen following low intensity fire (Biswell 1989). Furthermore, the incidence of mycorrhizal infection of grass roots is higher in regularly burned tallgrass prairie than in infrequently burned areas (Wallace and Svejcor 1987; Wallace 1987; Bentivenga and Hetrick 1991). In contrast to the beneficial effects of smoke reported in this Chapter, compounds present in smoke may also inhibit growth of fungi, rusts and certain algae (Pyne 1984). Another possible benefit of smoke and fire may therefore be the cuing of germination into an environment in which soil born pathogens have largely been retarded or killed by smoke associated with fire thereby facilitating seedling establishment. The effect of smoke on beneficial soil mychorrizae should be investigated. Plant-derived smoke is also known to promote shooting of bulbous plants (Hayashi 1971; Imanishi and Fortainer 1983) and improve flower yield (Schipper 1981) and quality (Schipper 1980). The positive effect of smoke on flower yield is evident for up to three years after treatment (Tompsett 1980a, b).

The field of smoke-stimulated seed germination is still young. This research has highlighted the beneficial effects of plant derived smoke on germination of seed of the fire climax grass *T. triandra*. The effects of smoke application on other aspects of plant growth require further investigation. The use of smoke as a seed pre-treatment and the effects of smoke treatments on seedling growth are reported in Chapter 9.

CHAPTER 9: PRE-TREATMENT OF *Themeda triandra* SEED TO IMPROVE SEED GERMINATION

Section 9.1 Introduction

Pre-treatment of seed to improve, or standardise the timing of, germination and seedling emergence is routinely used in the vegetable and horticultural industries (Owen and Pill 1994; Pill, Evans and Krishnan 1994; Zheng, Wilen, Slinkark and Gusta 1994). Within the agricultural industry this practice is not favoured. Seed pre-treatment may, however, have merit in improving the level of germination of rare or difficult to germinate indigenous plants. Similarly, for species such as *T. triandra* where seed is in short supply (Sindel *et.al.* 1993) maximisation of germination of available seed is advantageous. The response of *T. triandra* seed to pre-treatment has not previously been investigated.

In this Chapter the effects of pre-treating *T. triandra* seed with chemical or physical treatments shown to improve germination is reported. For practical use of any pre-treatment technology, however, the impacts of a seed treatment needs to be evaluated beyond the germination process. Assessment of whether seed pre-treatments exert a positive or negative effect on subsequent seedling growth was consequently also evaluated and is reported. Treatments assessed include the priming of seed with osmotica, pre-treatment of *T. triandra* seed with GA_3 or plant-derived smoke, and short periods of exposure of dry seed to high temperatures.

Results obtained following pre-treatment of *T. triandra* seed with plant-derived smoke have been published (Baxter and van Staden 1994) and the paper is included in entirety in Section 9.2. Remaining treatments are reported in Section 9.3. The practicalities of pre-treating *T. triandra* seed are discussed in Section 9.4.

Section 9.2 *Themeda triandra* seed pre-treatment with plant-derived smoke.

This Section comprises the publication:
Baxter, B.J.M and Van Staden, J. (1994) Plant-derived smoke: an effective seed pre-treatment. ***Plant Growth Regulation*** **14: 279-282.**

9.2.1 Introduction

Plant-derived smoke has been shown to stimulate seed germination directly in species from fire dependant fynbos (Brown 1993a; de Lange and Boucher 1990) and grassland (Baxter, Van Staden, Granger and Brown 1993) communities. Although neither the mechanism by which smoke acts to promote germination nor the nature of the active ingredient(s) are yet known, this knowledge is important and useful in improving the success of propagation of threatened plant species (Brown 1993a). Furthermore, smoke has been shown to stimulate germination of seed in soil seed banks (de Lange and Boucher 1990). This information may be of use in the revegetation of disturbed sites bearing fire dependant floral communities. Questions which need to be asked are whether seed can successfully be pre-treated with plant-derived smoke to improve germination on subsequent planting; can smoke pre-treated seed be stored and thirdly, does smoke effect seedling growth? These questions were addressed for *Themeda triandra*, a dominant fire-climax grass species of fire-dependant grasslands in Natal.

9.2.2 Plant material and methods

Themeda triandra seed used was collected at Cathedral Peak (1800 m above mean sea level) in the Natal Drakensberg. Seed was air-dried for eight weeks before storage at -15°C in sealed glass bottles until use. Seed moisture content at storage was less than 10 percent. At seed shed *T. triandra* seed from this

population is deeply dormant and requires an eight month after-ripening period to lose dormancy (Baxter, van Staden and Granger 1993). The dormant condition is retained under these storage conditions (Chapter 3). All germination trials were conducted in disposable Petri dishes on filter paper moistened with distilled water or aqueous smoke solutions. The method of preparation of aqueous smoke extracts has been reported (Baxter, Van Staden, Granger and Brown 1993). Pre-treatments involved seed imbibition in distilled water or aqueous extracts of plant-derived smoke, following which, treated seed was dried under a stream of air and stored at 25°C±2°C for 3 to 21 days. Pre-treatment imbibition times between 0 and 48 h were assessed. Control seed lots, imbibed in a smoke extract for equivalent time periods, were transferred directly to distilled water to germinate after imbibition. To confirm that seed pre-treatments had no detrimental effect on subsequent seedling growth, 24 h water and smoke pre-treated *T. triandra* seeds, stored for 21 days, were planted in a potted growth trial under greenhouse conditions. Control seed populations were allowed to undergo dry after-ripening for 21 days before exposure to distilled water or smoke solutions at the time of germination. Seed was germinated in Petri dishes as described above and seedlings transferred to pots following germination. The growth medium used was a mixture of potting soil and vermiculite (1 : 1). Seedling height and tiller number were adopted as indicators of seedling growth and recorded after 2, 6 and 12 weeks.

Data were analyzed using one way ANOVA and Tukey's range test. Before statistical analysis percentage data were subjected to arcsine square root transformation (Sokal and Rolf 1981).

9.2.3 Results

Pre-treatment of dormant *T. triandra* seed with aqueous extracts of plant-derived smoke significantly increased seed germination over water treated controls. Furthermore, the promotive effect of smoke on seed germination was retained when pre-treated seeds were dried and stored for up to 21 days (Figure 9.2.1). It is important to note that germination of water pre-treated seeds did not differ from that of after-ripened controls. In contrast, germination of smoke pre-treated seed increased relative to controls with increased time in storage.

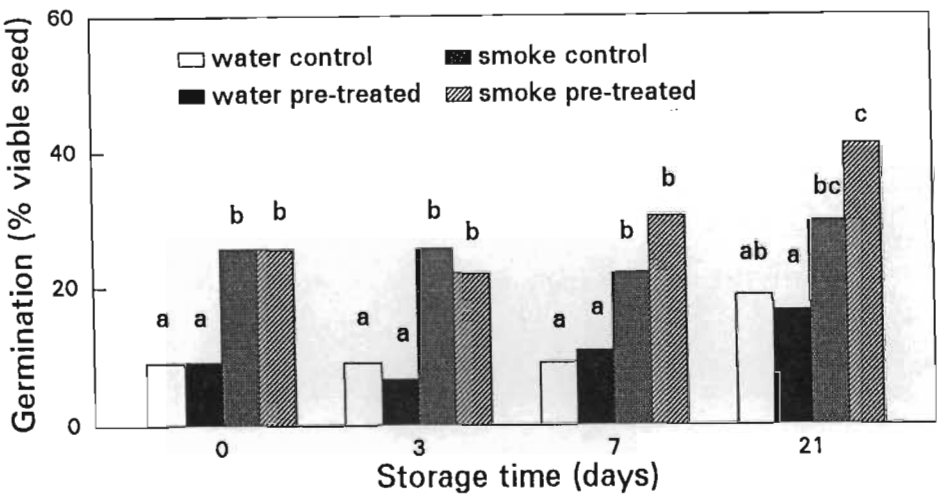


Figure 9.2.1: Germination of pre-treated *Themeda triandra* seed from the Drakensberg population after different periods of seed storage. Control seed lots were subjected to dry after-ripening for the storage period prior to exposure to treatment solutions to account for loss of dormancy by dry after-ripening during storage at 25 °C±2 °C. For each storage time, columns bearing different letters are significantly different ($P \leq 0.05$).

In addition, the rate of germination of smoke pre-treated seed, stored for 21 days, was greater than that of seed pre-treated with smoke and germinated immediately (Table 9.2.1). In contrast, water pre-treated, stored, seed had a slower rate of germination than both smoke pre-treated and water control seed populations. For

T. triandra, a smoke pre-treatment period of 12 h proved most effective in promoting seed germination. All smoke pre-treatments from 3 to 48 h in duration significantly increased germination (Table 9.2.2). Furthermore, for pre-treatment

Table 9.2.1: Germination rate of smoke or water pre-treated *Themeda triandra* seed on re-imbibition, after 21 days in storage at 25°C±2°C. Control seed populations were dry after-ripened for 21 days before pre-treatment, followed by immediate germination. [C = control treatments: PT = pre-treated, stored seed]

Treatment	Germination rate[1]
Water C	0.0146
Water PT	0.0136
Smoke C	0.0164
Smoke PT	0.0176

Germination rate was calculated as the inverse of time taken to reach half maximum germination.

Table 9.2.2: Germination of smoke pre-treated *Themeda triandra* seed after 7 days of storage at 25°C±2°C. Data are mean germination percentages ± SE. Within a column data followed by a different letter are significantly different ($P \leq 0.05$). [C = control treatment, dry seed stored for 7 d before exposure to smoke extracts followed by immediate transfer to distilled water for germination; PT = seed pre-treated with aqueous smoke extracts or water, re-dried and stored for 7 d before germination in distilled water; S = smoke; W = water]

Treatment time (h)	Treatment			Statistical analysis between treatments		
	Water PT	Smoke C	Smoke PT	WC:SC (A:B)	WC:S PT (A:C)	SC:S PT (B:C)
0	9.1 (3.5) a	9.1 (3.5) a	9.1 (3.5) a	ns	ns	ns
3	13.4 (2.5) a	16.1 (4.0) ab	22.5 (3.1) b	ns	ns	ns
6	15.0 (4.3) a	15.9 (4.9) ab	29.1 (6.8)bc	*	ns	*
12	12.5 (3.9) a	15.6 (2.4) ab	36.6 (3.6) c	**	ns	**
24	10.0 (3.4) a	25.9 (4.4) b	29.1 (4.0)bc	ns	**	**
48	7.5 (1.1) a	16.6 (2.1) ab	17.5 (2.5) b	ns	ns	*

The last three columns list the results of statistical analysis (ANOVA) between treatments for each treatment time. (* $P \leq 0.05$; ** $P \leq 0.01$; ns not significant).

periods of 6 to 48 hours germination of pre-treated seed was significantly greater than that of after-ripened seed exposed to smoke. In water pre-treated seed populations imbibition periods exceeding 24 h reduced germination relative to untreated controls.

Germination of *T. triandra* seed in the presence of smoke, or pre-treatment of seed with smoke had no adverse effect on subsequent seedling growth (Table 9.2.3). In contrast, water pre-treatment of fresh *T. triandra* seed significantly reduced seedling height and caused a reduction in tiller number.

Table 9.2.3: Growth of *Themeda triandra* seedlings obtained from smoke pre-treated seed stored for 21 days after treatment. Control populations were subjected to a 21 day dry after-ripening period prior to germination in water or aqueous smoke solutions. Data represent mean ± SE (n = 10). Within a column, data followed by different letters are significantly different ($P \leq 0.05$).

Treatment	Seedling height (mm)						Tiller number			
	2 wks		6 wks		12 wks		6 wks		12 wks	
Water:										
Control	75.8 (9.2)	ab	178.3 (22.5)	b	325.9 (40.9)	b	2.1 (0.5)	a	10.1 (1.6)	ab
Pre-treated	58.9 (11.1)	a	109.5 (15.5)	a	196.1 (32.2)	a	1.4 (0.5)	a	7.4 (1.3)	a
Smoke:										
Control	91.8 (5.5)	b	155.8 (12.0)	ab	311.6 (8.1)	b	1.9 (0.5)	a	9.6 (1.3)	ab
Pre-treated	86.2 (9.9)	ab	156.0 (18.0)	b	362.0 (23.2)	b	1.7 (0.3)	a	12.8 (1.7)	b

9.2.4 Discussion

Short periods of exposure (5-15 min) of dry or imbibed *T. triandra* seed to gaseous smoke promotes germination, with the level of germination increasing in imbibed seed suggesting that smoke initiates a metabolic response in treated seed (Baxter, Van Staden, Granger and Brown 1993). The active component present in smoke can successfully be extracted in water and continues to promote seed germination

(Brown 1993a; Baxter, Van Staden, Granger and Brown 1993). In this study, seed germination was significantly increased following short periods of seed imbibition in aqueous extracts of plant-derived smoke. The stimulatory effect of aqueous smoke on seed germination was retained when seed was re-dried and stored, thereby providing an effective means of pre-treating dormant seed to improve germination. It is not strictly correct to say that smoke pre-treatment increased the rate of seed germination, because the initial imbibition period was not included in the reported germination rates. However, as early metabolic events initiated during pre-treatment are merely arrested, and not reversed, on drying (Bewley and Black 1984), seed pre-treatment with smoke did reduce the time from re-imbibition to radicle emergence and may therefore confer a competitive advantage on emerging seedlings. This may be of particular importance if smoke pre-treated seed is used to revegetate disturbed soil sites where weed seedling competition would be expected. It would be interesting to test whether seedlings arising from smoke pre-treated seed display greater seedling vigour under field conditions given that both seedling height and tiller number were greater in "pre - treated" seedlings than in control seedling populations in the potted trial conducted. A marked increase in *T. triandra* seedlings have been reported in burnt grassland after fire (Lock and Milburn 1971; Ndawula-Senyimba 1972; Trollope 1984b), yet modification of the seedbed microclimate in unburnt grassland failed to significantly increase seedling emergence (Ndawula-Senyimba 1972; Trollope 1984a, b).

Use of smoke as an agent to promote seed germination has a wide application including increasing the efficiency of propagation of threatened or commercially important wild species (Brown 1993a) and the potential re-establishment of wild species from soil-stored seed (De Lange and Boucher 1990). Success has been achieved in re-establishing *T. triandra* from seed by laying cut reproductive tillers over bared ground in summer after seed shed, spraying this hay with a tacking agent to hold it in place and finally burning the hay at the onset of the following

spring by which time natural seed penetration of the soil has occurred (McDougall 1989). Undoubtedly, smoke, in combination with heat, contributes to improve seed germination. The knowledge that smoke can effectively be used to pre-treat seed of the fire-climax grass *T. triandra* to improve germination on planting makes use of this important grass for revegetation more feasible. These technologies may enable seed of difficult to germinate and threatened fynbos species to be made available commercially.

Section 9.3 Osmotic priming, GA_3 and high temperature pre-treatment of *T. triandra* seed.

9.3.1 Introduction

During the early stages of seed hydration, re-drying of seed does not negatively affect subsequent germination (Cocks and Donald 1973; Akalehiywot and Bewley 1980) and may reduce the germination time on rehydration (Crevecoeur, Deltour and Branchart 1976; Hanson 1973). Similarly, priming of seeds in osmotica prior to transfer of seeds to water, or re-drying of seed, reduces the time to maximum seed germination and may result in greater uniformity of germination in comparison to untreated seed (Dell aQuilla and Tritto 1990; Drew and Dearman 1993). Such pre-treatments, used primarily in the vegetable and horticultural industries, may have application in facilitating germination of seed of native species where seed is frequently difficult to germinate and in short supply.

This investigation was undertaken to determine whether treatments which break dormancy, or promote germination, of *T. triandra* seed can be applied as seed pre-treatments and whether these treatments exert a positive or negative effect on subsequent seedling growth. Treatments assessed include the incubation of dry seed at 70°C for 7 days and GA_3 treatment and pre-treatment of *T. triandra* seed. In addition, priming of *T. triandra* seed in polyethylene glycol (PEG 8000) is reported.

9.3.2 Methods

The effect of osmotic priming on germination of *T. triandra* seed was investigated

using Polyethylene glycol (PEG 8000; Sigma). Seed of both dormant and non-dormant Drakensberg and Zululand *T. triandra* populations was incubated for 14 days in solutions of PEG 8000 of increasing concentration. PEG 8000 concentrations represented water potentials of 0, -0.5, -1.0, -1.5 and -2.0 MPa. After the 14 day incubation period seeds were blotted dry and transferred to Petri dishes containing filter papers moistened in distilled water for a further 10 days to germinate. Germination was assessed at 2 day intervals at 30°C under constant light. Germinated seeds were removed.

To optimise seed pre-treatments, dormant *T. triandra* seed was allowed to imbibe in solutions of PEG 8000 or GA_3 for increasing time periods. Pre-treatment imbibition periods were 0, 3, 6, 12, 24 and 48 hours. Following imbibition, seeds were removed from Petri dishes containing filter papers moistened with treatment solutions and blotted dry. Seeds constituting the control were then immediately transferred to Petri dishes containing filter papers moistened with distilled water for commencement of germination. The remainder of pre-treated seed was dried under a stream of air (25°C±3°C) prior to dry storage at 25°C±3°C for 7 days. Following the 7 day storage period, germination of pre-treated seed was assessed.

To assess whether the beneficial effects of pre-treatment, following imbibition in distilled water or GA_3 (100 $mg.l^{-1}$) were retained during storage of dry seed, treated seed was dried under a stream of air and stored at 25°C±3°C for 3 to 21 days. After storage, seed was germinated in distilled water as per normal.

To confirm that seed pre-treatments had no detrimental effect on subsequent seedling growth *T. triandra* seedlings originating from pre-treated seeds, were planted into pots in a number of growth trials under greenhouse conditions. The effect of storing dry *T. triandra* seed at 70°C for 7 days, on the growth of *T. triandra* seedlings, was also investigated. Gibberellin pre-treatments involved storage of treated seed for 21 days prior to germination and planting. In contrast,

seeds exposed to high temperature (70 °C) treatment were germinated immediately after treatment and the emergent seedlings potted. These experiments were conducted in different years and consequently the performance of controls and duration of the experiments vary. The growth medium used in all experiments was a mixture of potting soil and vermiculite (1 : 1). Seedling height and tiller number were adopted as indicators of seedling growth and recorded at regular time intervals. Any indication of seedling abnormality was recorded.

All data were analyzed using one way ANOVA and Tukey's range test. Before statistical analysis percentage data were subjected to arcsine square root transformation (Sokal and Rolf 1981).

9.3.3 Results

Priming of *T. triandra* seed in solutions of PEG 8000 significantly ($P \leq 0.05$) increased germination of eight-week-old seed from the Drakensberg and Zululand populations, at all concentrations tested (Table 9.3.1). Germination of after-ripened seed from both populations was also improved by priming, although this increase in germination was not significant ($P \leq 0.05$) following priming of after-ripened seed from the Zululand population. Furthermore, germination of after-ripened seed from the Zululand population decreased following priming at a water potential of -2.0 MPa. The best levels of *T. triandra* seed germination were achieved at water potentials of -0.5 MPa for Drakensberg seed and -1.5 MPa for Zululand seed.

Partial imbibition in distilled water, followed by drying and storage of seed for 7 days, increased germination of eight-week-old *T. triandra* seed. The increase in germination was, however, not significant ($P \leq 0.05$: Figure 9.3.1). The greatest improvement in germination (9.1 % to 15.0 %) was achieved following imbibition

for 6 hours prior to dry storage. Similarly, imbibition of dormant *T. triandra* seed in solutions of GA_3 for 3 to 48 hours prior to transfer to distilled water improved the level of seed germination (Figure 9.3.1). Drying and storage of seed after treatment further improved subsequent germination, but in contrast to results obtained following smoke pre-treatment (see Table 9.2.2) this increase in germination was not significant. Imbibition of dormant seed in PEG for 3 to 48 hours resulted in a reduced level of germination when seed was immediately transferred to water, but germination increased after dry storage of primed seed for 7 days (Figure 9.3.1). A significant increase in germination was recorded following the 48 hour PEG pre-treatment.

Table 9.3.1: The effect of osmotic priming in polyethylene glycol 8000 (PEG) on germination of dormant and after-ripened *T. triandra* seed from both the Drakensberg and Zululand populations. For each seed population, data within a column followed by a different letter is significantly different ($P \leq 0.05$).

Water Potential (MPa)	Germination following osmotic priming (% viable seed)							
	Drakensberg dormant		Drakensberg after-ripened		Zululand dormant		Zululand after-ripened	
0	5.9 (1.5)	a	73.2 (6.0)	a	23.0 (2.2)	a	85.4 (3.1)	a
-0.5	34.1 (6.1)	b	100 (8.8)	b	36.7 (5.1)	ab	100 (12.2)	a
-1.0	25.0 (4.9)	b	83.2 (9.1)	ab	39.3 (6.3)	b	100 (10.1)	a
-1.5	29.1 (6.9)	b	88.4 (7.8)	ab	42.3 (6.4)	b	100 (13.3)	a
-2.0	33.4 (5.2)	b	100 (8.1)	b	26.8 (3.4)	a	74.0 (8.9)	a

The promotive effect of GA_3 on seed germination was retained when pre-treated seeds were dried and stored for up to 21 days (Figure 9.3.2). It is important to note that germination of water pre-treated seeds did not differ from that of after-ripened controls. In contrast, germination of GA_3 pre-treated seed exceeded that of controls following 21 days post treatment storage.

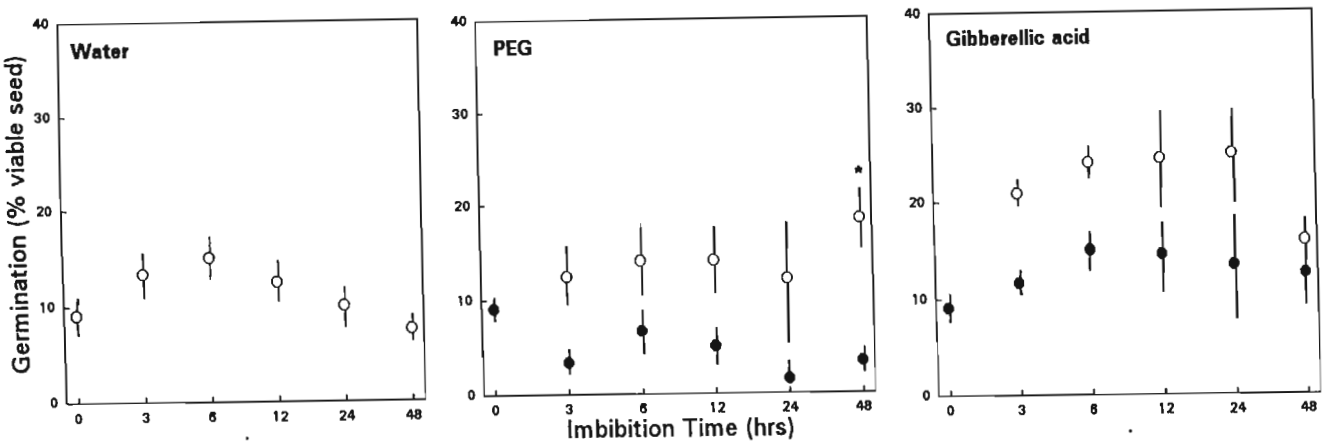


Figure 9.3.1: The effect of pre-treatment imbibition time on germination of eight-week-old (dormant) *Themeda triandra* seed. Solid dots represent the control in which seed was transferred to petri dishes for germination immediately after imbibition in treatment solutions. Open circles represent pre-treated seed which was air dried after treatment and stored for seven days at 25°C±3°C prior to germination. Significant pre-treatment effects are indicated, where * = $P \leq 0.05$

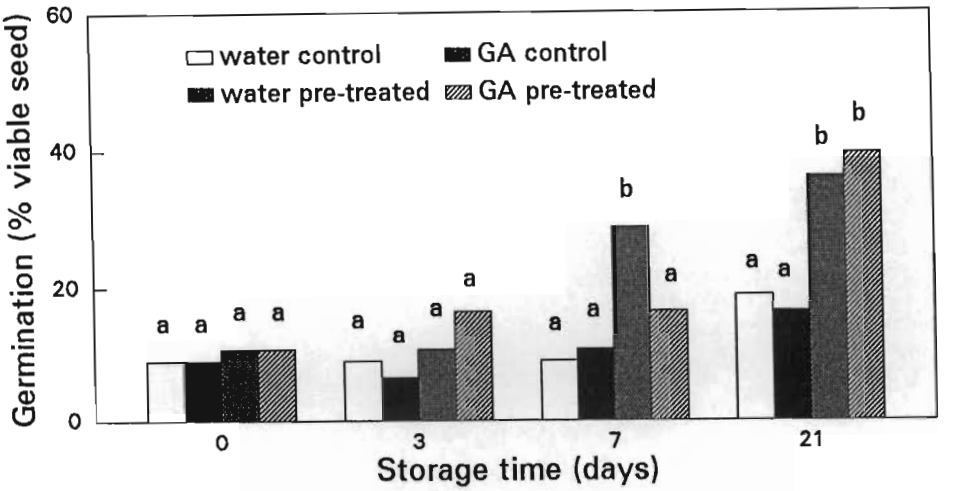


Figure 9.3.2: Germination of GA_3 pre-treated *Themeda triandra* seed after different periods of seed storage. Germination was referenced against that of *T. triandra* seed subjected to an equivalent water pre-treatment. Control seed lots were subjected to dry after-ripening for the duration of the storage period prior to exposure to treatment solutions to account for loss of dormancy by dry after-ripening during storage of pre-treated seed at 25°C±2°C. For each storage time, columns bearing different letters are significantly different ($P \leq 0.05$).

Exposure of dormant *T. triandra* seed to high temperature had no effect on seedling height, but significantly ($P \leq 0.05$) increased the tiller number of eight-week-old seedlings from both Drakensberg and Zululand populations from 2.56 to 3.74 and 1.14 to 1.89 respectively (Table 9.3.2). Germination of *T. triandra* seed in the presence of GA_3, or pre-treatment of seed with GA_3 had no significant effect on seedling height or tiller number (Table 9.3.3). In contrast, water pre-treatment of fresh *T. triandra* seed significantly reduced the height of twelve-week-old seedlings from 325.9 mm to 196.1 mm and caused a reduction in tiller number from 10.1 to 7.4 tillers per seedling. Pre-treatment of dormant *T. triandra* seed with GA_3 caused seedling abnormality by promoting etiolation in 10 percent of seedlings (Table 9.3.4). Neither high temperature, or water pre-treatments caused seedling abnormality. Neither smoke treatment nor smoke pre-treated of *T triandra* seed negatively impacted seedling growth.

Table 9.3.2: The effect of pre-treatment of dry eight-week-old *Themeda triandra* seed at 70°C for 7 days on subsequent growth of seedlings from both the Drakensberg and Zululand populations. Data are reported after eight weeks growth. Within a column data followed by a different letter are significantly different ($P \leq 0.05$).

	Drakensberg				Zululand			
	Height (mm)		Tiller number		Height (mm)		Tiller number	
Control	206.6 (9.7)	a	2.56 (0.26)	a	167.8 (7.0)	a	1.14 (0.09)	a
Pre-treated	229.3 (8.5)	a	3.74 (0.16)	b	180.4 (6.1)	a	1.89 (0.18)	b

9.3.4 Discussion

Having demonstrated that *T. triandra* seed can be successfully pre-treated with smoke, stored and show improved post-storage germination (Baxter and van Staden 1994), this component of the research was designed to evaluate the

Table 9.3.3: Growth of *Themeda triandra* seedlings (Drakensberg population) obtained from GA_3 (100 $mg.l_{-1}$) pre-treated seed stored for 21 days after treatment. Control populations were subjected to a 21 day dry after-ripening period prior to germination in water or GA_3 solution. Data represent mean seedling height or tiller number ± SE (n = 10) of 12 week old seedlings. Data bearing different letters are significantly different ($P \leq 0.05$).

Treatment	Seedling height (mm)	Tiller number
Water:		
Control	325.9 (40.9) b	10.1 (1.6) a
Pre-treated	196.1 (32.2) a	7.4 (1.3) a
GA_3:		
Control	299.4 (26.3) ab	10.7 (1.3) a
Pre-treated	362.4 (49.5) b	8.3 (1.0) a

Table 9.3.4: The effect of high temperature (70°C), GA_3 (100 $mg.l_{-1}$) and smoke (2 %) seed pre-treatments on seedling morphology. Assessment was made at the time of termination of each growth trial. Data represent the percentage abnormal seedlings present.

Treatment	Seedling abnormality (%)	Comment
Water control	0	Normal seedlings
Water pre-treated	0	Normal seedlings
Control	0	Normal seedlings
70°C for 7 days	0	Normal seedlings
Smoke control	0	Normal seedlings
Smoke pre-treated	0	Normal seedlings
GA_3 control	0	Normal seedlings
GA_3 pre-treated	10	Etiolated seedlings

potential of GA_3, high temperature and polyethylene glycol treatments as pre-treatments. Although GA_3 treatments and pre-treatments significantly improve the level of *T. triandra* seed germination, it would be counter productive to use gibberellins as seed pre-treatments because of the negative effect of applied GA_3

on seedling growth. In contrast, as reported for smoke, high temperature pre-treatment of *T. triandra* seed appears to benefit seedling growth, increasing tiller number and seedling height. Although short periods of exposure to high temperature are known to promote germination of seed of some *Erica* species (van der Venter and Esterhuizen 1988), the impacts of such treatments on seedling growth have not been reported. Similarly, drying of *Eragrostis curvula* var. *conferta* and *E. lehmanniana* seed for 24 hours at 70°C prior to germination increases the rate, but not the level, of seed germination (Weaver and Jordan 1985). The effect of high temperature treatments on performance of *E. curvula* and *E. lehmanniana* seedlings was not reported. It is probable, however, that holding *T. triandra* seed at elevated temperatures for a number of days promotes seed after-ripening as it is known that *T. triandra* seed dormancy is lost more rapidly during storage at high constant (Chapter 3) and alternating (Hagon 1976) temperatures. The positive response of seedlings, derived from heat pre-treated seeds, coupled with the ease with which dry seed temperature pre-treatments can be applied makes heat pre-treatment of *T. triandra* seed a useful tool for maximising germination of bulk seed volumes. Although the results obtained from potted trials indicate that heat pre-treatment improves seedling performance, the vigour of seedlings derived from heat treated seed warrants further investigation under field conditions. Caution must be exercised when considering high temperature seed pre-treatment because prolonged exposure of dry *T. triandra* seed to high temperature results in rapid loss of viability.

The use of polyethylene glycol 8000 to prime *T. triandra* seed resulted in increased levels of germination in both dormant and after-ripened seed from both seed populations tested. Although priming results in an increase in the level of germination of dormant *T. triandra* seed, the improvement in germination is not sufficiently marked to make osmotic priming of dormant seed a viable option. The promotion of germination of non-dormant seed, which was approximately 24 months old at the time of testing is, however, of particular importance because

PEG 8000 priming appears to overcome negative effects of ageing. Similar results have been obtained for *Amaranthus cruentus* seeds where priming in PEG increased the level of germination in seed damaged during harvest (Pill, Evans and Krishnan 1994). Priming did not, however, reduce the level of abnormal seedlings which arose from harvest damaged seed indicating that the beneficial effects of osmotic priming are limited to facilitation of the seed germination process alone. Osmotic priming in PEG does, however, significantly improve the competitive ability of the indigenous perennial grasses *Pseudoroegneria spicata, Elymus lanceolata, Leymus cinereus, Festuca ovinia, Poa canbyi, P. sandbergii* and *Sitanion hystrix* which, in the absence of priming, are out-competed by the exotic annual *Bromus tectorum*. Priming reduced the time to half maximum germination at low temperature, for all indigenous grasses, by 4 to 8 days rendering the rate of native seed germination comparable to that of *Bromus tectorum* seed (Hardegree 1994). Priming may thus be of considerable importance in maximising germination of aged *T. triandra* seed, particularly for seed which is to be used in specialist applications such as the rehabilitation of sensitive areas. The use of osmotic priming to maximise germination of aged *T. triandra* seed is discussed further in Chapter 11.

Section 9.4 General discussion

The positive effect of smoke and high temperature pre-treatment of seed on the subsequent growth of *T. triandra* seedlings is of particular interest given that a combination of smoke and high temperature is likely to be experienced by buried seed under natural grassland conditions, where fire is common. It is well documented that a flush of *T. triandra* seedlings is evident in burned veld (Ndwula-Senyimba 1972; Trollope 1984a, b; Everson 1994) and that this seedling flush can not be triggered by removal of the grass canopy by cutting alone (Trollope 1984b). A question which arises is whether a chemical and / or physical cue, associated with the smoke and heat of fire, evolved in this fire-climax grass which confers a competitive advantage on seedlings that emerge in the post-fire landscape, thereby ensuring maintenance of *Themeda triandra* as a dominant species in fire-climax grassland and savanna systems?

A seedling develops through three stages *en route* to becoming established, namely a heterotrophic stage when the seedling is reliant on seed reserves, a transitional stage and finally the seedling becomes autotrophic at which point the seedling has no further reliance on seed reserves (Whalley, McKell and Green 1966). In *Agropyron desertorum* (crested wheatgrass) and *Bouteloua gracilis* (blue gamma) seedlings, seedling autotrophism closely coincides with development of adventitious roots at which point (21 days after emergence) seedlings are considered established (Ries and Svejcar 1991). Prior to the establishment of adventitious roots grass seedlings are not able to survive periods of moisture deficit in the upper soil layer. The effect of smoke and heat pre-treatment of *T. triandra* seed on seedling root development was not evaluated, but could lend weight to the question posed above. If root development of *T. triandra* seedlings shows a positive response to smoke and / or heat treatment of seed, just as seedling height and tiller number are increased, the time to adventitious root development may be reduced and field establishment of seedlings would be accelerated.

Smoke treatments which are applied to overcome dormancy in corms and bulbs (Hayashi 1971; Tompsett 1985) have a beneficial effect on subsequent flower yield (Schipper 1981) and quality (Schipper 1980). The beneficial effect of smoke treatment of bulbs on flower yield lasted for three years after treatment (Tompsett 1980 a, b), raising interesting questions regarding possible long-term promotive effects of smoke treatment on growth of *T. triandra* plants derived from smoke pre-treated or treated seed.

Although *T. triandra* seed is likely to remain in short supply for use in revegetation (Sindel *et.al.* 1993), pre-treatment of *T. triandra* seed is not considered to be practical, nor justifiable, based on performance of pre-treated seed when seed is to be used for revegetation of extensive areas. In contrast, the use of seed pre-treatment techniques identified in this Chapter may be of considerable benefit in maximising germination of *T. triandra* seed that is to be used in intensive revegetation projects. The use of *T. triandra* seed pre-treatment in intensive revegetation practice is discussed further in Chapter 11.

CHAPTER 10: FIELD ESTABLISHMENT OF *Themeda triandra* FROM SEEDLING AND TILLER TRANSPLANTS TO INVESTIGATE GENETIC DIFFERENTIATION BETWEEN ALTITUDINALLY SEPARATE POPULATIONS OF *Themeda triandra.*

10.1 Introduction

Themeda triandra is one of the most widely distributed and economically important grasses in South Africa. The grass is palatable and occurs in abundance in undisturbed veld (Tainton 1984). More importantly *T. triandra* occurs in association with other palatable grass species as a stable community, under correct management. Under mismanagement, however, *Themeda* and associated palatable species are replaced by grasses of low palatability. Even if the veld is spared, the *Themeda*-dominated climax community fails to re-establish (Tainton 1984). Similarly, *Themeda* fails to re-establish in mid- to high-altitude grasslands following disturbance. Tainton (1984) suggests that the reason for this may be that mid and high altitude grasslands originally established and attained dominance under climatic, fire, and grazing conditions different to those present today.

The failure of *T. triandra* to re-establish following mismanagement or disturbance has received increased research attention. Everson (1994) reports that the density of *T. triandra* seed in the seed bank in the montane grassland of the Drakensberg is less than 1.2 %, indicating that following disturbance, the seed bank would contribute little to re-establishment of the grass. Furthermore, the large (5-7 mm), heavy-awned seed of *T. triandra* has a limited dispersal range (Gibbs Russell *et.al.* 1991; Everson 1994), and consequently dispersal of seed into disturbed areas is limited. *Themeda triandra* propagates by vegetative means, with mature tufts fragmenting with age as new vegetative tillers are produced around the periphery of a tuft with the centre dying out (Everson 1994). Lateral spread of *T. triandra* and other tufted grasses by vegetative means is therefore slow and successful natural re-establishment of palatable tufted grasses on disturbed land, in mid to high altitude regions, is not likely to occur rapidly, if at all. Recently, however,

limited success has been achieved with the artificial re-establishment of *T. triandra* from seed in Australia (Lodder, Groves and Wittmark 1986; Groves 1990), and in the Natal midlands (Adams and Tainton 1992).

Caution must be exercised when using native species, however, because genetic and physiological uniformity cannot be assumed throughout the range of a species (Clausen, Keck and Hiesley 1940; Hacker 1984, 1988). Most species are comprised of different ecotypes. As first defined by Turesson (1922), an ecotype is a genetically distinct population within a species which has undergone adaptation to local environmental conditions. Within the widely distributed species *T. triandra* morphologically and cytogenetically different varieties occur (Vickery 1961; Spies and Gibbs Russell 1988). Furthermore, when grown under identical (greenhouse) conditions, geographically separate *T. triandra* populations in Australia showed marked variation in the time taken to reach anthesis prompting Groves (1975) to suggest that differentiation of ecological significance has occurred within the species. Preliminary investigation of the two morphologically different, and geographically distinct, populations under investigation in this research indicated that the small high altitude form of *T. triandra* possesses a slower growth rate and longer time requirement to the commencement of flowering than the robust low altitude variety. These preliminary trends are consistent with results obtained for reciprocally transplanted woody perennials (Clausen, Keck and Hiesey 1940, 1948) and reciprocally sown grass populations (Rice and Mack 1991) from altitudinally distinct sites. In addition, differences in the depth of seed dormancy, and in germination response to environmental stimuli, between geographically separate populations of *T. triandra* occur (Groves *et.al.* 1982; Baxter, van Staden and Granger 1993). The fact that differences were evident following growth under uniform conditions, suggests that the variation present between altitudinally separate *T. triandra* populations may reflect hereditary variation and not morphological adaptation to environmental conditions. The implications of such variation attain importance when seed of native species is selected for use in re-establishment of the species in regions beyond the natural

distribution of an ecotype because limits of plant tolerance of environmental variables may differ between ecotypes. As interest in the use of *T. triandra* for re-vegetation of disturbed sites increases (McDougall 1989; Sindel and Groves 1990) the degree to which morphologically different populations of *T. triandra* can survive and reproduce under climatic conditions found outside their geographic range warrants investigation.

Life history parameters such as survivorship and fecundity can be considered as "a set of co-adapted traits, designed by natural selection, to solve particular ecological problems" (Stearns 1976). Although genetic differences between populations may be demonstrated under uniform conditions, field transplants provide a more realistic assessment of genetic and environmental effects (Antonovics and Primack 1982). A field test must, however, meet certain criteria. The entire life cycle of the species must be measurable within a relatively short period of time, some inter-populational variation in life history parameters must exist for the species, and the demographic investigations of differing populations must be conducted under different selective regimes (Schmidt and Levin 1985). In this Chapter the establishment of *T. triandra* seedlings and tiller transplants in transplant gardens at high (1500 m), intermediate (800 m) and low altitudes (100 m) is reported. Plants from both the local and reciprocal areas were established at each site. This research was not designed to investigate the establishment and survival of seedlings and transplants planted into virgin veld, but to determine whether geographically separated populations of *T. triandra* could survive and reproduce, in the absence of competition, under the climatic conditions at reciprocal sites. The objectives of this study were to (a) provide confirmation that the geographically and morphologically distinct *T. triandra* populations under investigation are genetically distinct ecotypes, and (b) to determine whether the two different varieties of *T. triandra*, drawn from altitudinally extreme habitats, are able to survive and reproduce outside of their geographic range.

10.2 Methods

Three field sites were established across an altitudinal gradient spanning the province of KwaZulu-Natal. Extreme sites were established in the Drakensberg and Zululand, as near to the sites of the parent populations from which seed was harvested (Chapter 2) as possible. In addition, a site at intermediate altitude was established in case any population failed to survive at the opposite extreme of the environmental gradient along which the trial was conducted. To avoid causing unnecessary disturbance, only previously disturbed sites were evaluated as potential field experimental sites.

A Drakensberg field site was established at Cathedral Peak at an altitude of approximately 1550 m.a.m.s.l. Although lower than the site of the parent population (1800 m.a.m.s.l) it was felt that the difference in local climate between parent and field sites would not be significant in relation to climatic differences between sites. A suitable site could not be secured within the Umfolozi Game Reserve and the experimental site was consequently established at Eteza. The field site was located approximately 25 kilometres from the parent seed site at a similar altitude (80 m.a.m.s.l) on the Zululand coastal plain. Differences in local climate were also not considered significant. The intermediate field site was established on the University of Natal research farm, Ukulinga, at an altitude of approximately 760 m.a.m.s.l. The geographic and altitudinal distribution of sites is indicated (Figure 10.1).

At each site two categories of *T. triandra* plants were established in field trials;

(a) Tiller transplants: Groups of 3 to 5 tillers were split off from mature *T. triandra* plants collected in undisturbed *T. triandra* dominated grassland at the parent sites and in Pietermaritzburg. Field-collected tussocks were returned to a greenhouse, potted and watered regularly. After three weeks these tussocks were split into smaller units comprising 3 to 5 tillers and are referred to hereafter as tiller transplants. Tiller transplants derived from

individual tufts were divided equally between field sites to ensure that at each site the same gene pool was reflected in the tiller transplants planted.

(b) Seedling transplants: Seedlings from the Drakensberg and Zululand seed populations under investigation were grown under uniform greenhouse conditions (Botany Department - UNP) for six weeks prior to field planting. Seedlings were planted into seedling trays from 17 to 19 August 1992. The planting medium used was potting soil : vermiculite (1:1).

Seedlings and tiller transplants were planted in the field during the week commencing 7 October 1992. All material planted was lightly watered twice a week for the first two weeks after planting.

Experiments were designed to provide 30 tiller transplants or seedlings per population at each site. High mortality of seedling and tiller transplants was anticipated in response to transplant shock which has been recognised as one of the drawbacks of transplant experiments (Antonovics and Primack 1982; Davy and Smith 1985). Consequently, 60 tiller transplants and 100 seedlings per population were planted at each site. To reduce competition between individuals, all seedlings and tillers were planted 0.5 meters apart and field gardens were regularly cleared of weeds. Plants from each population were planted in separate blocks with a 0.5 meter pathway between blocks. The trial layout adopted at each site was dictated by availability of space and differed slightly between sites. The layout of trials at each site is illustrated in Appendix 2.

Although trials were designed to avoid competitive effects, data were not collected from plants located on the periphery of a block as these individuals would experience a reduced competitive regime in comparison to individuals within blocks. Similarly, data collection from an individual plant within a block was terminated if the neighbouring plant died. After planting, data was collected at six weekly intervals, with a field assessment carried out every third week. At each site data collection took two days to complete and data was collected at all sites

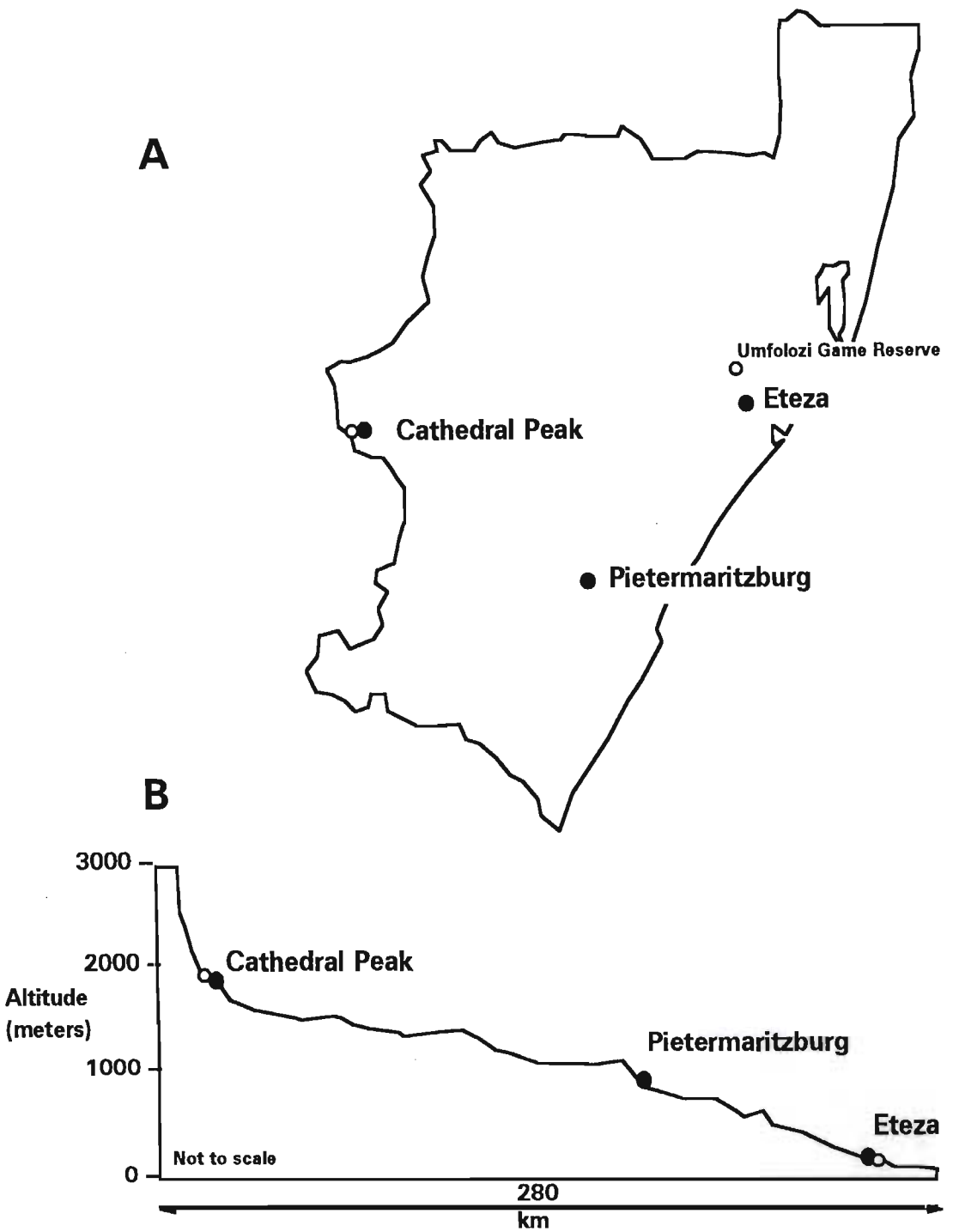


Figure 10.1: Plan indicating A the geographical location and B the altitudinal distribution of the three sites at which field based trials were conducted. Open circles indicate the location of parent seed populations. Closed circles represent the locality of field trial sites.

within the same calender week. For each transplant population a record was kept of mortality, growth (tiller number and rate of tiller initiation) and reproductive effort (plant allocation of tillers to reproductive or vegetative growth; time to anthesis and flowering frequency. Data collection ceased on 3 May 1993 when plants senesced with the onset of winter. A post-winter assessment of plant survival was undertaken on the 6 September and 17 October 1993 to quantify winter seedling mortality.

10.3 Results

Seedling mortality was low at all sites with maximum seedling deaths recorded at the intermediate Pietermaritzburg site (7 %). In contrast, tiller mortality was significantly higher (Chi-square 309.1 : df 6 : $P \leq 0.001$). At all sites more than 60 percent of Zululand tiller transplants died. In contrast, less than 25 percent of Drakensberg tiller transplants died (Figure 10.2). For both Drakensberg and Zululand populations, mortality of transplanted tillers was lowest at the parent site.

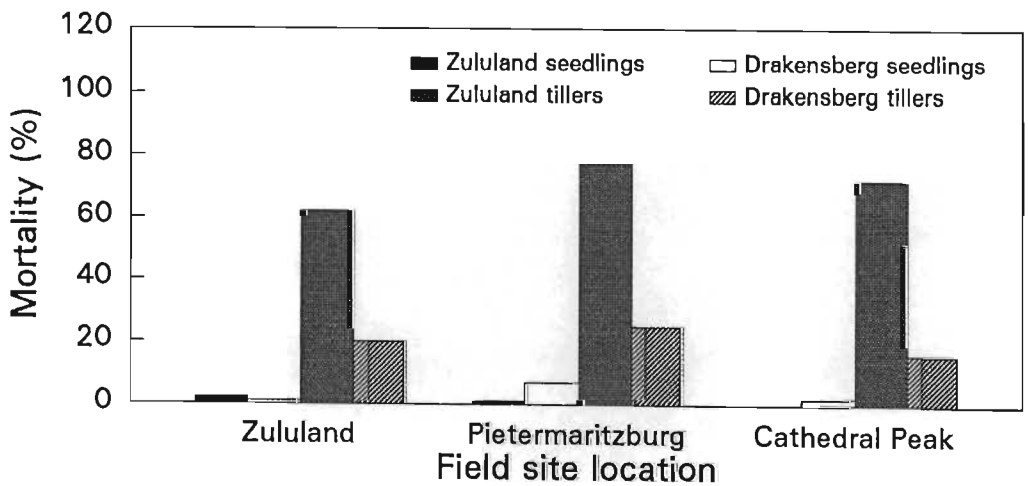


Figure 10.2: Mortality of *Themeda triandra* seedling and tiller transplants, at all field sites, 12 weeks after the time of transplanting.

At all sites, Drakensberg ecotype seedlings produced a greater number of tillers than Zululand seedlings which is consistent with the morphology of the two populations. Total tiller number in seedling transplants increased with altitude (Figure 10.3). Conversely tiller allocation to reproduction declined significantly

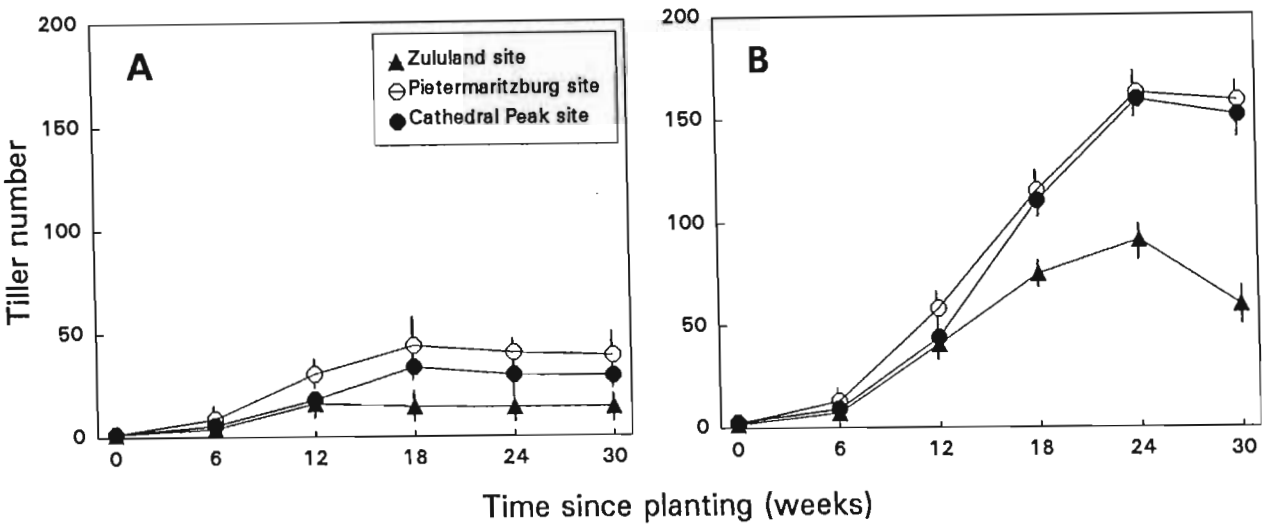


Figure 10.3: Change in the total number of tillers in *Themeda triandra* seedling transplants derived from A Zululand and B Drakensberg populations, at all sites, over time.

with altitude in both seedling populations (Figure 10.4: Chi square 14.3; df 4 ; $P \leq 0.001$). Zululand ecotype seedling transplants had a greater reproductive output at all sites than Drakensberg ecotype transplants (Figure 10.4). In addition, more than 93 percent of Zululand ecotype seedling transplants flowered at all sites. In contrast, site strongly influenced flowering of Drakensberg seedling transplants with flowering decreasing with increasing altitude (Figure 10.5). Furthermore, the time taken to reach anthesis increased as altitude increased (Figure 10.5).

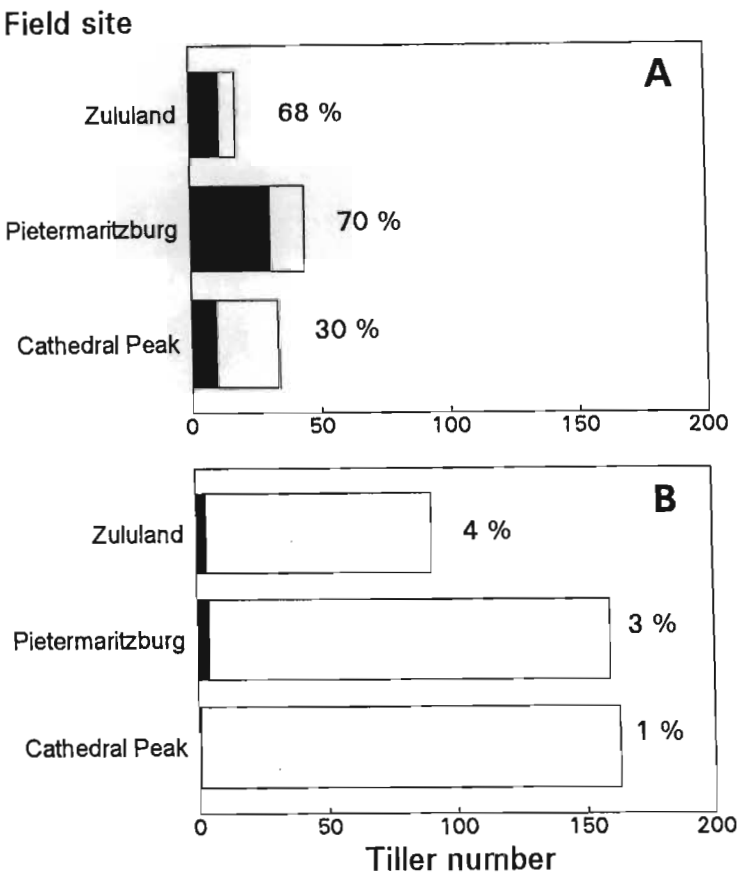
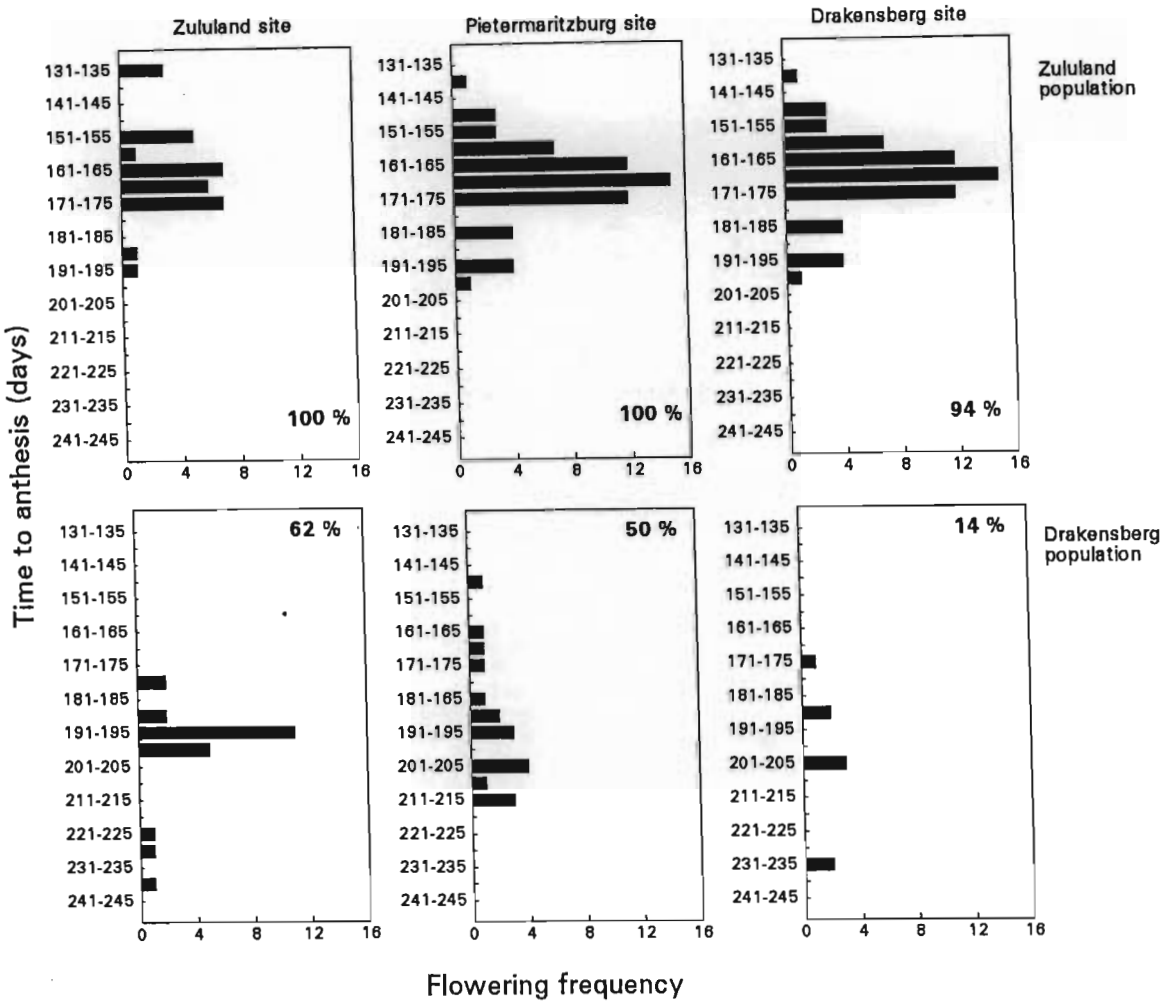


Figure 10.4: Allocation of tillers to reproductive or vegetative growth in seedling transplants of *Themeda triandra*, from A Zululand and B Drakensberg parent populations, grown at geographically and altitudinally separate sites. Numbers at the head of each column represent the percentage of reproductive tillers per seedling population per site. Solid columns represent reproductive tillers and open columns represent vegetative tillers.

10.4 Discussion

Themeda triandra seedling and tiller populations planted into field gardens at reciprocal sites displayed significant, and consistent, phenotypic variation between populations, irrespective of the location of field sites. Moreover, at each site, individuals from high and low altitude parent populations differed in flowering frequency, time to anthesis and time to seed ripeness confirming that genetic



A Sites compared	Significance level	
	Drakensberg	Zululand
Drakensberg - Pietermaritzburg	ns	* *
Drakensberg - Zululand	ns	* *
Pietermaritzburg - Zululand	ns	ns

B Ecotypes compared	Significance level
Drakensberg	* *
Pietermaritzburg	* * *
Zululand	* * *

(* P ≤ 0.05: * * P ≤ 0.01: * * * P ≤ 0.001: ns = Not significant)

Figure 10.5: The frequency of flowering of Drakensberg and Zululand seedling transplants at all field sites. The percentage of plants which flowered per population is recorded for each site. Kolmogorov-Smirnov two sample analyses of flowering frequency data are tabulated for comparisons A between sites within ecotypes, and B within sites between ecotypes.

differentiation of ecological importance has occurred between the populations studied. The two *T. triandra* populations investigated can therefore be regarded as true ecotypes. This conclusion is consistent with that reached by Groves (1975) following growth of geographically distinct *T. triandra* populations from Australia under uniform conditions. These field data also support reports by Liebenberg (1986) that cytogenetic differences exist between collections of the *T. triandra*. Genetic characterisation of the parent populations under investigation would provide confirmation of the conclusions drawn from these field experiments. Genetic analysis of putative ecotypes has generally reinforced interpretations based on transplant garden experiments (Clausen and Hielsey 1958; Heslop-Harrison 1964), confirming that differences in parental climate, which find expression in the genome of the local population, outweigh the effects of local climate on field transplanted individuals. The results presented in this Chapter are also consistent with unpublished observations of *T. triandra* plants from the Drakensberg and Zululand populations studied, grown from seed under uniform conditions in a greenhouse (UNP. Pietermaritzburg) for a period of four years which indicate that *T. triandra* progeny are strongly influenced by maternal environment. Under uniform conditions, individual *T. triandra* plants from the Drakensberg and Zululand study populations retained the distinctive morphological characters of height, growth habit and tiller form which distinguish the two varieties studied. Individuals from the two different parent populations also differed consistently, over the four year period, with respect to flowering frequency and onset of flowering providing further indication that these characteristics were genetically determined.

Studies conducted on woody perennial (Clausen, Keck and Hiesey 1940, 1948) and grass (Rice and Mack 1991) populations from altitudinally distinct sites, planted into reciprocal field gardens, indicate that individuals from high altitude parent populations possess a slower growth rate with an associated increase in the time required to commence flowering in comparison to individuals from low altitude parental sites, as reported for *T. triandra* in this Chapter. These differences in growth rate and time to flowering between altitudinally distinct populations reflect

adaptation to parental climate where plants from high altitude sites experience more severe climatic conditions. This trend was also evident when allocation to vegetative and reproductive output is compared between sites for either *Themeda* ecotype; at increased altitude tiller allocation to vegetative growth increased and reproductive output decreased. Similarly, in excess of 93% of Zululand ecotype seedlings flowered at all sites while maximum flowering of Drakensberg ecotype seedling was recorded in the Zululand field garden (62%) with only 14% of seedling transplants flowering in the Drakensberg field garden. These figures are somewhat misleading, however, because the Drakensberg and Zululand ecotypes of *T. triandra* possess different strategies for perpetuation of the species in the natural environment. The Zululand ecotype, adapted to growth under sub-tropical conditions, displays a rapid growth rate and high reproductive allocation and, as documented for sub-tropical east African savanna grasslands perpetuates from seed (O'Conner 1994). In contrast, the Drakensberg ecotype of *Themeda triandra* perpetuates vegetatively (Everson 1994) and allocation to reproduction is consequently low. In addition, it is well documented that *T. triandra* in the Drakensberg flowers most abundantly in the second season following fire (Tainton 1984; Everson 1994) on account of the resource allocation needed to regenerate tillers removed in burning. Similarly, only limited tiller allocation to reproduction takes place in the season of establishment.

This field study was also designed to test the survival of *Themeda triandra* ecotypes sampled from high and low altitude populations when grown in reciprocal climates. As awareness of the need to use indigenous plants in re-vegetation of disturbed areas grows, greater use will be made of native grass seed. As described by Sindel *et.al.* (1993), however, seed of indigenous grasses such as *T. triandra* is in short supply and may always be so given the limited number of seeds produced per plant and the difficulty experienced in harvesting seed of indigenous plants. It is likely therefore that whenever seed of indigenous grasses is required, and it can be sourced, the seed will be used irrespective of the geographic origin of such seed. The use of seedlings derived from field harvested seed was

therefore necessary to test plant performance and survival in reciprocal climates and the results which have been presented in this Chapter support the decision made. It is of particular importance to note that *T. triandra* individuals from both populations were able to survive and reproduce under climatic conditions in reciprocal habitats. This point is of great importance from the perspective of using *T. triandra* for rehabilitation of disturbed areas because the data indicate that, although adaptive selection for environmental variables present at parent sites has taken place, ecotypes of this grass have a wide environmental tolerance. Although these data indicate that individuals from altitudinally distinct parent populations are able to survive and reproduce in reciprocal climates, this research has not addressed whether field sown seed harvested in reciprocal environments will germinate under reciprocal field conditions. As discussed in Chapter 5, the germination response of *T. triandra* seed from Zululand and Drakensberg populations differs significantly at seed shed. A high proportion of Zululand seed is able to germinate at seed shed, prior to becoming dormant after approximately 4 weeks whereafter a period of after-ripening is required to enable germination. In contrast, Drakensberg seed is largely dormant when shed, and possesses an after-ripening requirement. Given the similar range of temperatures over which seed of both ecotypes germinates it is probable that field germination of *T. triandra* seed harvested in the Drakensberg and Zululand would occur in reciprocal environments, but this needs to be confirmed. In addition, the field germination of seed produced under reciprocal climates warrants investigation because the successful use of any climax or sub-climax species in re-vegetation is determined not only by initial establishment, but also by perenniation of the species. Furthermore, having established that altitudinally extreme ecotypes of *T. triandra* can survive and reproduce in reciprocal climates, and knowing that hybridisation between ecotypic populations of the species is possible (Liebenberg 1986), the dangers, if any, of creating new hybrid strains of *T. triandra* by sowing seed from one *Themeda triandra* ecotype into an area dominated by another should be researched as a matter of urgency. At present *T. triandra* seed and seed of a number of other indigenous grass species, harvested in the Potchefstroom region,

is available commercially and is being sown in rehabilitation projects in Gauteng, Northern KwaZulu Natal (pers. comm. Van Wyk, S) and Mpumalanga (Baxter, B. personal observation), with moderate success.

CHAPTER 11: GENERAL DISCUSSION AND CONCLUSIONS

11.1 An overview of the problem addressed by this study

The dominant position of the fire-climax grass *T. triandra* in montane and high altitude grasslands is maintained by vegetative means and not through regular seedling regeneration (Everson 1985; Everson 1994). Seed production of the species is accordingly low (McDougall 1989; Everson 1994). The availability of seed is further limited by morphological constraints (Sindel *et.al.* 1993) with few spikelets bearing filled seeds on each reproductive tiller (Gibbs-Russel *et.al.* 1990). With over or under utilisation this palatable grass is displaced as a dominant species in grassland and savanna communities. Although sought after as a grazing grass, the results of early experiments which involved the clearing of grassland vegetation from trial blocks indicated that *T. triandra* would not re-colonise bared ground leading to the widely advanced belief (Tainton 1984) that *T. triandra* will not re-establish from seed. Increasingly, however, it has been shown that *T. triandra* can be re-established from sown seed (Rommel *et.al.* 1988; McDougall 1989; Sindel and Groves 1990; Sindel *et.al.* 1993) suggesting that low seed availability and poor seed dispersal are responsible for the failure of *T. triandra* to re-establish in bared ground. This suggestion is supported by Everson (1994), who reports that seed of *T. triandra* is not dispersed further than 2.5 meters from the parent plant.

The research reported in this Thesis was initiated to further the level of understanding of aspects relating to *T. triandra* seed germination, to enable the germination and establishment of sown *T. triandra* seed to be optimised.

11.2 The relevant seed biology of *Themeda triandra*

Themeda triandra seed ripens and is shed during mid to late summer over a period of approximately three to five weeks (McDougall 1989). At seed shed *T. triandra*

seed is dormant. The depth of dormancy varies between populations (Groves *et.al.* 1982; Chapter 3). Although Groves *et.al.* (1982), in a study of geographically separate *T. triandra* populations, did not link depth of dormancy to any particular climatic variable, the performance of *T. triandra* seed from the altitudinally extreme populations reported in this Thesis suggests that the level of primary seed dormancy in *T. triandra* reflects differences in the severity of the winter period. The *Themeda triandra* populations which experience a severe winter period (Drakensberg) produced deeply dormant seed while seed from the subtropical Zululand population was less dormant at seed shed. Irrespective of the level of seed dormancy at seed shed, during dry after-ripening dormancy is lost. Under controlled conditions (25°C±3°C) the loss of seed dormancy in both study populations coincided with the onset of spring. Results obtained from the field burial experiment conducted (Chapter 6) indicate that the rate of dormancy loss may be accelerated in buried seed. It is probable that under field conditions buried *T. triandra* seed moves from a condition of dormancy to quiescence prior to the onset of winter, but that environmental conditions preclude germination until spring. In the high altitude Drakensberg grasslands few *T. triandra* seedlings emerge immediately following seed shed. Seedling mortality during winter is high and is associated with severe seedling root pruning caused by frost upheaval of the surface soil layer (Everson 1994). Natural selection thus favours seedlings which emerge from dormant seed.

11.3 Understanding and optimising the germination of *Themeda triandra* seed

The mechanisms governing seed dormancy and the germination of *T. triandra* seed appear to be universal amongst ecotypes, although the magnitude of the *T. triandra* seed germination response to environmental and applied variables varies between ecotypes. With increased altitude, and hence an increase in the severity of the environment, the level of *T. triandra* seed dormancy increases. Seed germination in *T. triandra* is restricted, in part, by the physical restraint imposed by the robust

silicious glumes and dormancy of the embryo is enforced at seed shed by endogenous compounds which are inhibitory to germination. These compounds are similar in action to abscisic acid. With increased dry after-ripening the proportion of germinable *T. triandra* seed increases. The germination process appears to be governed by endogenous gibberellins, while plant-derived smoke and possibly kinetin counteract the effect of endogenous inhibitors, thus playing a permissive role in facilitating *T. triandra* seed germination. In contradiction to previous studies (Cresswell and Nelson 1971, 1972 and 1973) boron is without effect in promoting *T. triandra* seed germination.

The greater understanding of germination of *T. triandra* seed gained from the data reported in Chapters 3 through 9 provide the means to maximise germination of available *T. triandra* seed. At seed shed the majority of seed is dormant and must undergo a period of dry after-ripening during which time seed dormancy is progressively lost. Associated with the loss of primary seed dormancy, *T. triandra* seed germination is enabled across an increased range of temperatures and at lower water potentials. Field investigation (see Chapter 6) of these trends obtained under controlled conditions confirmed that the same processes take place in buried *T. triandra* seed, although at a faster rate. After-ripened (non-dormant) *T. triandra* seed is thus better equipped to survive fluctuations in soil moisture status during the germination process.

The rapid loss of viability in seed of *T. triandra* during uncontrolled seed storage (Everson 1994) is unlikely to significantly effect the level of germinable seed in the first spring season, but carryover of viable seed into a second season following planting will be limited. On account of the increased rate of seed deterioration during uncontrolled storage (Everson 1994) and the high level of seed predation in the field (O'Conner 1994), germination of available seed for use in re-vegetation will be maximised if harvested seed is maintained in dry-storage through the first winter and after-ripened (non-dormant) seed planted early in the following spring.

Early planting and associated germination affords a longer period for the emergent seedling to become established and well rooted. This is of particular important in high altitude grasslands which experience severe winter conditions with heavy frost because Everson (1994) reports high *T. triandra* seedling mortality in Drakensberg grasslands associated with root pruning caused by frost-upheaval of the soil surface.

The successful accelerated after-ripening and loss of dormancy in *T. triandra* seed, by storage at elevated temperatures, or short-duration exposure of dry seed to high temperatures, has been demonstrated in this Thesis. These treatments afford a convenient and cost effective means of rendering large volumes of dormant *T. triandra* seed germinable. The use of these technologies, however, is not recommended to obtain non-dormant seed for planting in the summer following seed shed because seedlings are unlikely to be sufficiently established to survive the first winter. The natural process of after-ripening which takes place at room temperature and renders seed germinable by the onset of the spring period should rather be allowed to take place in harvested seed kept in dry storage.

The exception, however, is when *T. triandra* is to be re-established from planted seedlings (see Section 11.5). To enable field planting of seedlings, approximately 6 weeks in age when planted, during spring or early summer, germination of *T. triandra* seed under controlled conditions prior to completion of the after-ripening process may be necessary in which instance *T. triandra* seed germination could be maximised by acceleration of seed after-ripening, pre-treatment of seed or the priming of seed prior to germination.

11.4 What is rehabilitation?

In the event of significant disturbance, such as is associated with mining, vegetation must re-establish on bared ground. The re-establishment of vegetation on severely disturbed sites follows three possible paths, namely natural

recolonisation, restoration or rehabilitation (Bradshaw 1987). Natural recolonisation of extensive areas of disturbance in grassland and savanna communities is unlikely to succeed due to the poorly developed seed dispersal mechanisms of climax species such as *T. triandra* which are adapted to perpetuate vegetatively (Everson 1994) and the failure of seed of perennial grasses to persist in the soil seed bank (Everson 1994). Restoration, which involves the recreation of an entire vegetation community, is not feasible in grassland and savanna areas as too little is known about the requirements of individual species and interspecific interactions to achieve success. The process of rehabilitation, better termed revegetation, involves establishment of a plant cover to meet specific objectives. In the South African coal mining industry the primary objective of land rehabilitation, as set be the Chamber of Mines of South Africa is to reduce soil movement (erosion) by the rapid establishment and subsequent maintenance of a vegetation cover (Anon 1981). The nature of the vegetation cover is not specified, although pasture grasses are recommended. Increasingly, however, the focus of land rehabilitation is the re-establishment of indigenous species which were present in the vegetation community prior to disturbance (Klco 1988; Kendle 1994; Anon 1995). Ideally, the revegetated landscape should contain indigenous species in the same proportions as present in the pre-disturbance vegetation community.

11.5 Use of *Themeda triandra* in revegetation

Within the eastern highveld of the Transvaal (now Mpumalanga, including portions of Gauteng) 2.7 million hectares of agricultural land is underlain by coal, of which 409 500 hectares may be influenced by high-recovery mining methods. Currently, 40 percent of high-recovery coal mining in Mpumalanga is achieved by opencast methods (Anon 1990) which, if extrapolated to the high recovery minable reserve, equates to a total of 163 800 hectares of land which may be mined and rehabilitated. In the Mpumalanga Province of South Africa, more than 150 hectares of agricultural land is currently opencast mined each month

(de Vlieg, J. pers.comm.). Although the majority of opencast mined land reverts to agricultural use post-mining, concern has been raised regarding the reduced agricultural potential of rehabilitated coal-mined land in South Africa on account of water pollution, soil compaction and uncertainty over the sustainability of rehabilitated pasture (Anon 1990) due to the limited diversity of species present (Breytenbach 1996).

The objectives of mine-site revegetation are to provide a short-term plant cover which establishes rapidly to minimise erosion and a long term stable cover which is self perpetuating and able to sustain economically viable agricultural practices (Anon 1981). Species currently used extensively in revegetation include the pasture grasses *Eragrostis teff* (short-term annual), *Chloris gayana, Digitaria eriantha* and *Cenchrus ciliaris* (perennial species), while legume crops, including *Medicago sativa* (lucerne) and various clovers, have also been used in revegetation programmes. Although livestock production figures for stock grazed on rehabilitated pasture equal or exceed those obtained from adjacent unmined land (Dyson, Elliot, Reynolds, Dight and Hill 1987) the use of improved pasture species such as *Cenchrus ciliaris* has been questioned because of the ability of such species to dominate the post mining landscape and become naturalised in adjacent undisturbed areas (Fox and Tacey 1994). Moreover, the establishment of large tracts of land to a limited number of introduced pasture species may lead to unbalanced plant communities (Fox and Tacey 1994) which are not representative of the original pre-mining floral community (van Leeuwen 1994). The goals for revegetating land in Australia (Taylor, Luscombe and Hill 1994; Anon 1995), the United Kingdom (Kendle 1994) and the United States of America (Klco 1988) now focus increasingly on re-establishing indigenous plant species which were present in the pre-mining vegetation community and on the recreation of physically and biologically diverse habitats (Robinson 1994). The stability (Tacey, Treloar and Gordine 1993) and resilience (MacMahon 1992) of re-established plant communities is a function of species diversity. Increased use of a diversity of

indigenous species, such as *Themeda triandra*, in the rehabilitation of disturbed land in South Africa must therefore be encouraged.

As documented in Section 11.1 the widely held dogma that *T. triandra* does not re-establish from seed has been challenged by reports of successful establishment of *T. triandra* from sown seed (Rommel *et.al.* 1988; McDougall 1989; Sindel and Groves 1990; Sindel *et.al.* 1993). Within this Thesis techniques which can be used to reduce the level of dormancy in *T. triandra* seed have been reported. Similarly, the pre-treatment of *T. triandra* seed to improve the level of seed germination on planting has been discussed (Chapter 9). It is questionable whether the application of techniques for alleviating dormancy, or pre-treating, *T. triandra* seed are warranted when seed is to be applied on an extensive scale. Primary seed dormancy in *T. triandra* is lost during dry-after ripening and the loss of dormancy in stored seed coincides with the onset of spring. Consequently, to maximise the establishment of *T. triandra* from sown seed, in extensive revegetation applications, seed should be sown and incorporated in early spring of the season following harvest. As discussed in Chapter 5, non-dormant *T. triandra* seed is able to germinate at a lower range of temperature, which coincide with early spring temperatures, following after-ripening. Early spring planting affords the emergent *T. triandra* seedling the maximum growth period prior to the onset of winter, which is of particular importance in high altitude grasslands which experience severe winter frost.

A notable success of the transplant experiments reported in this Thesis was the high survival of *T. triandra* seedlings planted into field gardens. Notwithstanding the fact that these experiments were designed to minimise competition, at all sites seedling survival exceeded 90 percent. This technique of "growing-up" veld grass seedlings in seedling trays until the seedling root system is well developed has application in rehabilitation of sensitive sites such as within conservation areas, where the use of exotic pasture species is deemed undesirable. The technique may

also find application in providing a means of reintroducing nucleus populations of indigenous species into extensive areas of rehabilitated pasture where the potential for spread of native grass seed is currently negligible. Intensive seed manipulation to maximise *T. triandra* seed germination may be warranted for such specialised revegetation applications. Osmotic pre-treatment of *T. triandra* seed, to maximise germination on sowing of seed into seedling trays, is advocated.

The technique of planting *T. triandra* seedlings has been developed further by B. Baxter and J.E. Granger in a series of studies which are outside of the scope of this Thesis. *Themeda triandra* seedlings have been successfully planted into areas cleared of exotic gum, pine and wattle trees in the Kamberg Nature Reserve (Natal Parks, Game and Fish Preservation Board). In this subsequent work post-winter field survival of *T. triandra* seedlings exceeded 85 percent with seedling mortality attributed primarily to grazing damage rather than climatic factors (Granger, J.E. pers. comm.).

The tolerance of both planted seedlings and mature *T. triandra* individuals to environmental conditions present in altitudinally extreme sites augers well for successful re-establishment of the grass and indicates the tolerance of the species to a broad range of environmental conditions. As suggested by Hacker (1988) for the *Digitaria milanjiana* complex, however, when selecting indigenous species for pasture (or rehabilitation) use, ecotypes should only be considered if appropriate mechanisms exist to enable the grass to perpetuate through seedling regeneration, under the climatic conditions of the rehabilitation site. Within *Themeda triandra* ecotypes the seed dormancy mechanisms appear to be universal. Ecotypic differences in the level of seed dormancy do occur, however, and reflect adaptive genetic variation in response to parental climate, particularly with respect to the response of *T. triandra* seed to stratification. It is therefore recommended that seed of *T. triandra* which is to be used in rehabilitation is collected in the same geographic region (provenance) as the disturbed site. Within a provenance, species

have undergone natural selection in isolation to individuals from other regions (Coates and Sokolowski 1989) and are thus best suited to local climatic and edaphic conditions and ecological processes (van Leeuwen 1994). The transfer of *T. triandra* seed from one geographic region to another, for use in revegetation of disturbed land, is consequently not recommended.

11.6 Future research

Numerous opportunities for further research have been identified and discussed throughout this Thesis. In concluding, three key areas for further research warrant mention:

Firstly, the mechanism/s by which plant-derived smoke stimulates seed germination should be elucidated as should the active component/s of plant-derived smoke be identified. The intellectual and economic potential of such research is vast and the results may be of significance for conservation of rare and endangered plant species, and for management of fire-dependant plant communities.

Secondly, the germination potential and performance of seed of the late successional fire-climax grass *T. triandra* has been demonstrated in this Thesis. The germination and seedling performance of other indigenous grass species, particularly early and mid successional species, warrants urgent research attention to facilitate the informed use of indigenous grasses in landscape revegetation.

Thirdly, considerable variability in seed form exists between different native grass species, making the use of conventional agricultural equipment impractical when sowing a diverse mixture of indigenous seeds. Techniques to enable seed of indigenous grasses to be successfully planted on a large scale need to be developed. Similarly, seed harvesting techniques need to be optimised to increase the volume of native grass seed available for use in revegetation.

These three research areas have been highlighted because there is a growing awareness of the need to re-establish diverse plant communities following disturbance in order to achieve a stable and self perpetuating vegetation community which requires reduced maintenance.

Appendix 1: Use of the dwarf-rice bioassay for gibberellin-like activity to investigate whether plant-derived smoke mimics, or facilitates synthesis of, gibberellins in *T. triandra* seed.

Introduction

Both smoke and GA_3 significantly promote germination of dormant *Themeda triandra* seed. When applied in combination these compounds act synergistically to further significantly promote seed germination. The possibility that smoke mimics gibberellin action directly , or that smoke facilitates endogenous synthesis of gibberellin-like compounds warranted further investigation. As smoke extracts contain a large number of unidentified compounds, isolation of the bioactive functions is a complex procedure involving numerous, different chromatographic isolation phases (van Staden, Drewes and Jäger 1994). Consequently, the dwarf rice bioassay for gibberellin-like activity was adopted as a comparatively simple and proven method to screen for gibberellins. The methods used were those of Murakami (1970), as adapted by Drewes (1989). A dilution series of aqueous smoke extract was assayed directly for gibberellin-like activity. Secondly, extracts were prepared from seeds of *T. triandra* which were imbibed in either water or smoke (aq) and tested for endogenous gibberellin-like activity. Both bioassays were repeated at least twice.

A dilution sieries of smoke contained no GA-like activity (Table Ap1). Similarly, imbibition of seed of *T. triandra* in smoke solutions did not affect the level of endogenous gibberellin-like activity in comparison to the level of GA-like activity recorded in seed imbibed in water (Table Ap2). The length of the second leaf sheath in response to a dilution series of GA_3 in the concentration range 0 to 1000 ng GA_3 is provided for reference (Table Ap3).

Table Ap1: The level of GA-like activity present in a dilution series of an aqueous smoke solution prepared from burning grass material. Within a column data followed by the same letter are not significantly different ($P \leq 0.05$).

Dilution of stock extract	Leaf sheath length (mm)	
10	13.77 (0.43)	a
1.0	13.88 (0.52)	a
0.1	13.75 (0.60)	a
0.01	13.76 (0.40)	a
0.001	14.45 (0.35)	a
0.0001	13.80 (0.40)	a

Table Ap2: The level of GA-like activity present in extracts prepared from *T. triandra* seeds imbibed in smoke (aq) or water for increasing time periods. Data are presented as ng GA_3 equivalents. An * in the ANOVA column reflects a significant difference in the livel of GA-like activity between imbibition treatments; **ns** = not significant ($P \leq 0.05$).

Imbibition time	Water	Smoke	ANOVA
control	2.80	2.80	ns
3	0.80	1.00	ns
6	2.95	2.80	ns
12	3.00	2.70	ns
24	68.10	62.00	ns
48	62.00	60.00	ns

Table A3: The length (mm) of the second leaf sheath of dwarf-rice seedlings in response to a dilution series of GA_3.

GA_3 (ng)	Leaf sheath length (mm)	
0	13.87 (0.4)	a
0.1	16.04 (1.86)	ab
1.0	20.91 (0.58)	b
10	30.55 (1.38)	c
100	42.73 (2.44)	d
1000	46.58 (1.64)	d

APPENDIX 2: Schematic layout of field transplant gardens established at Cathedral Peak, Pietermaritzburg and Eteza to assess the performance and survival of *T. triandra* seedling and tiller transplants under reciprocal climatic conditions.

At all sites a total of 100 seedlings and 60 tiller transplants were planted per population. The timing of planting and of data collection is tabulated (Table Ap4).

Table Ap4: Record of field planting times and dates on which data collection was undertaken. Field work was undertaken on consecutive days from the date tabulated, commencing with the Eteza site, followed by the intermediate Ukulinga (PMB) site and ending at the high altitude Drakensberg site.

Date	Seedling age	Activity
07-08-1992	6	Seedlings and tillers planted into field
12-11-1992	12	Data collection
30-12-1992	18	Data collection
05-02-1993	24	Data collection
22-03-1993	30	Data collection
03-05-1993	36	Final data collection
06-09-1993	-	First post-winter evaluation
17-10-1993	-	Second post-winter evaluation

A schematic layout of each field garden is provided (Figures Ap1 to Ap3).

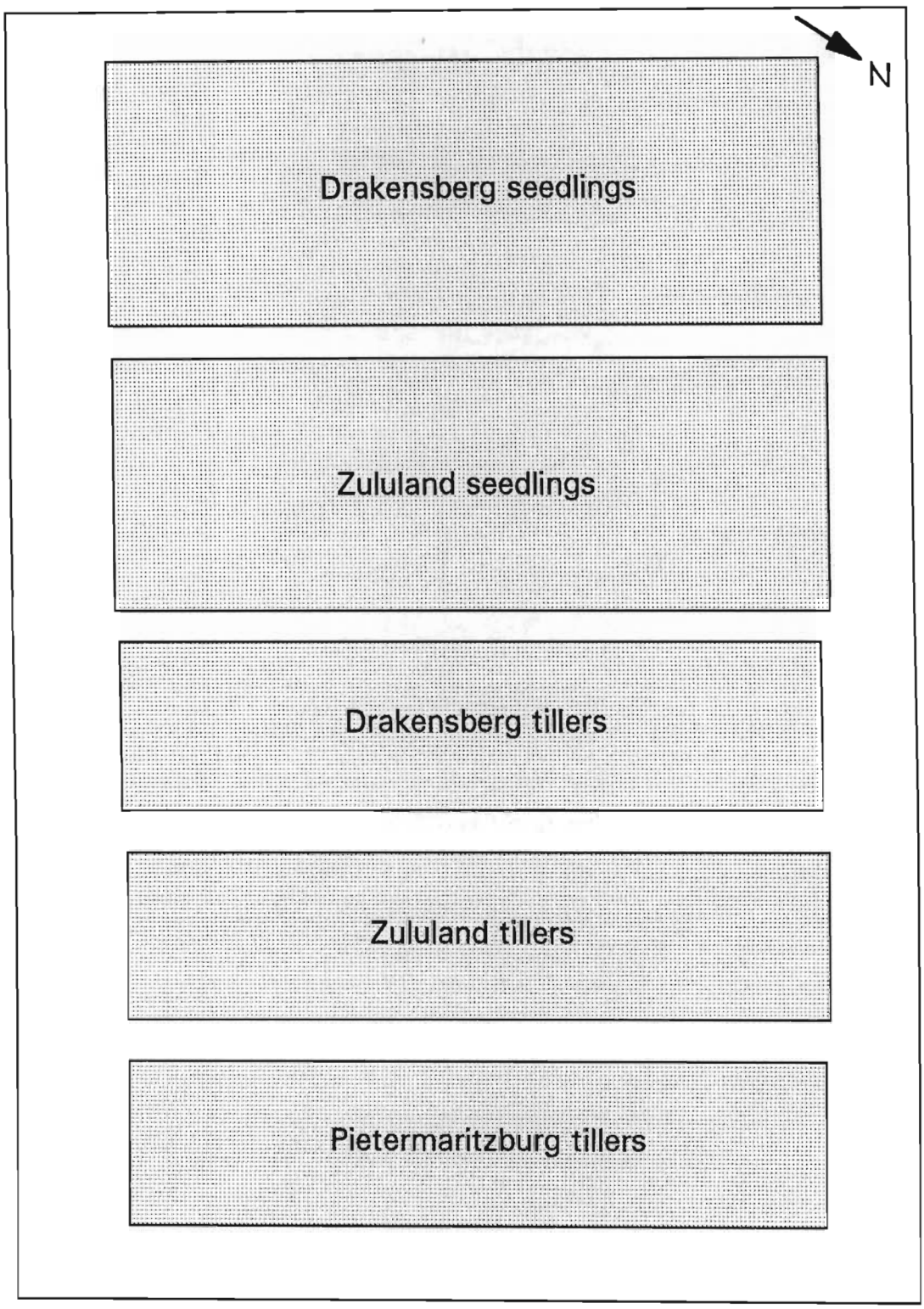


Figure Ap1: Schematic layout of the low altitude field garden established at Eteza, Zululand.

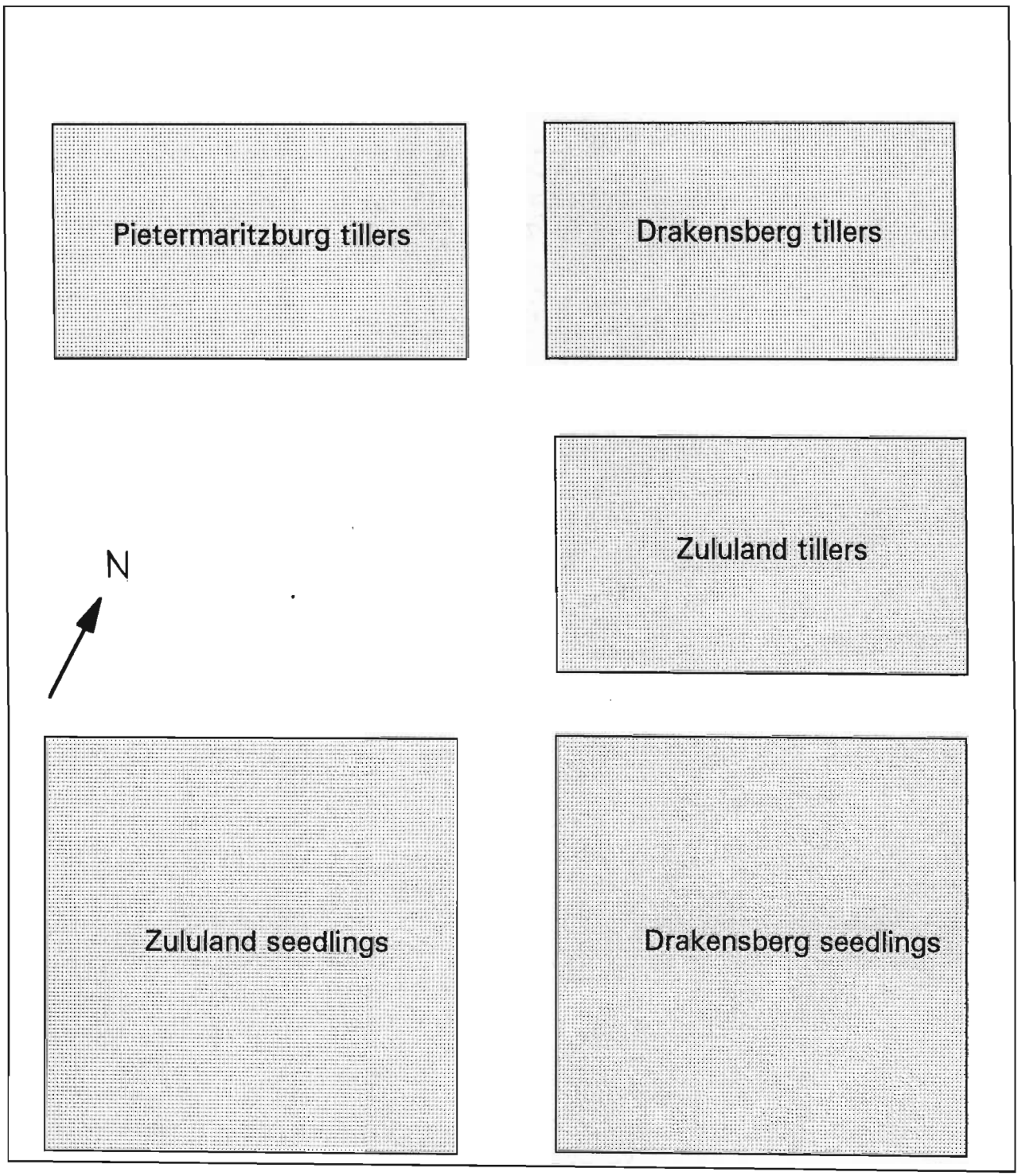


Figure Ap2: Schematic layout of the field garden established at an intermediate altitude at the University of Natal, Pietermaritzburg.

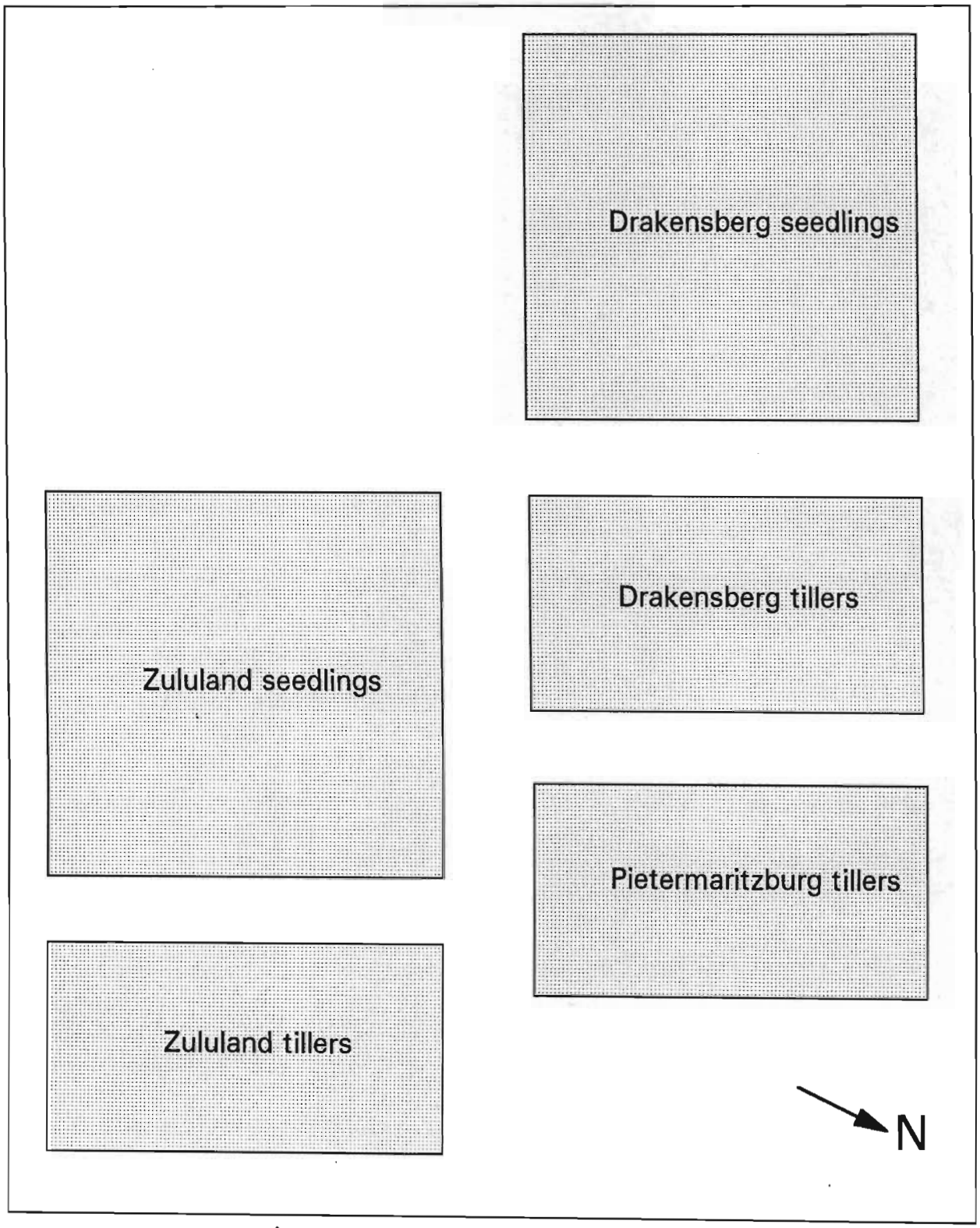


Figure Ap3: Schematic layout of the high altitude field garden established at Cathedral Peak.

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