**Aspects of the ecology of grass seedlings used for revegetation of degraded land**

By

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# Abstract

As restoration ecology has matured as a science there has been increased interest in the relationship between species diversity and landscape health. Degraded landscapes tend to be resource poor, which limits species diversity as only species which are capable of growing and reproducing in these resource limiting environments can inhabit the area. Additionally, the established species are strong competitors for resources and will exclude, by way of inter-specific competition, weaker competitor species attempting to invade the degraded area. Several studies have demonstrated that with increased species diversity the overall productivity and functionality of the grassland increases.

Seedling development and competitive interactions between grass seedlings has a significant impact on the final community structure and species diversity. It is for this reason that aspects of the ecology of grass seedlings were investigated.

The growth and competitiveness of *Chloris gayana, Cynodon dactylon, Digitaria eriantha, Eragrostis curvula* and *E. tef* seedlings were determined under three environmental stimuli, namely nitrogen availability, light availability and exposure to plant-derived smoke (in the form of smoke-infused water).

The primary conclusion from the competition experiments was that the species can be split into superior and inferior competitors at the seedling stage. *Chloris gayana, E. curvula* and *E. tef* were the most competitive seedlings as they had the largest negative effect on the growth of other species (high nitrogen Relative Interactive Index (RII) = -0.449, -0.203 and -0.379 respectively) and they were least affected by competition (high nitrogen RII = -0.251, -0.168 and -0.248 respectively). The calculated RII indicates the strength of the competitive interactions, the more negative the RII the stronger the competitive interaction. Nutrient availability had limited effect on the competitive hierarchy of the tested species. *Chloris gayana* seedlings, however, increased in competitiveness with an increase in available nutrients. In other words, there was a decreased negative response to competition in a high nutrient environment (high nitrogen RII -0.251, no nitrogen RII -0.605). When *D. eriantha* was grown under varying shade, nutrient and competition levels it was evident that the primary stress factor was light deficiency ($p<0.001$), and nutrient availability had no affect on seedling growth

(p=0.069). Smoke-infused water had no consistent affect on the germination success or the seedling's root and shoot vigour for the five grasses.

These results indicate that the introduction of a "2-phase" or "multi-phase" restoration plan may be beneficial for the development of species diverse rehabilitated grasslands. Manipulating the time and space that the different species are planted, or the distribution of nutrient concentration over the area, may increase the survivorship of all the species that are introduced to a restoration site.

## Preface

The work presented in this dissertation is the original work of the author unless stated otherwise in the text. This work has not been submitted in part or entirety for any degree or diploma at any other university or academic institution.

The experimental work was carried out at the School of Biological and Conservation Sciences, University of KwaZulu-Natal, from January 2006 to December 2008 under the supervision of Professor K. P. Kirkman and Mr. C. D. Morris.

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## **Acknowledgements**

# Chapter 1: Introduction

The grassland biome is the second largest biome in South Africa and covers 28% of the country. The largest biome, the savanna biome, which covers 32.5% of South Africa is also rich is grass species (Figure 1.1) (DEAT 2007). The grassland biome occurs over the majority of eastern South Africa and extends along the eastern and south eastern seaboard up into the northern inland provinces (Figure 1.1). It is rich in biodiversity and has a high level of endemic species of both flora and fauna. The conservation and maintenance of these grasslands is, therefore, of major importance for biodiversity. Only 1.9% of South African grasslands are formally conserved which, according to the South African National Biodiversity Institute (SANBI 2007), is not sufficient to maintain the diversity patterns and ecological processes which are required to maintain the integrity of the grassland system. In order for South African grasslands to be effectively conserved at least 36.7% of the grassland biome, which is divided into 15 priority conservation areas (green areas in Figure 1.2), is required (SANBI 2007).
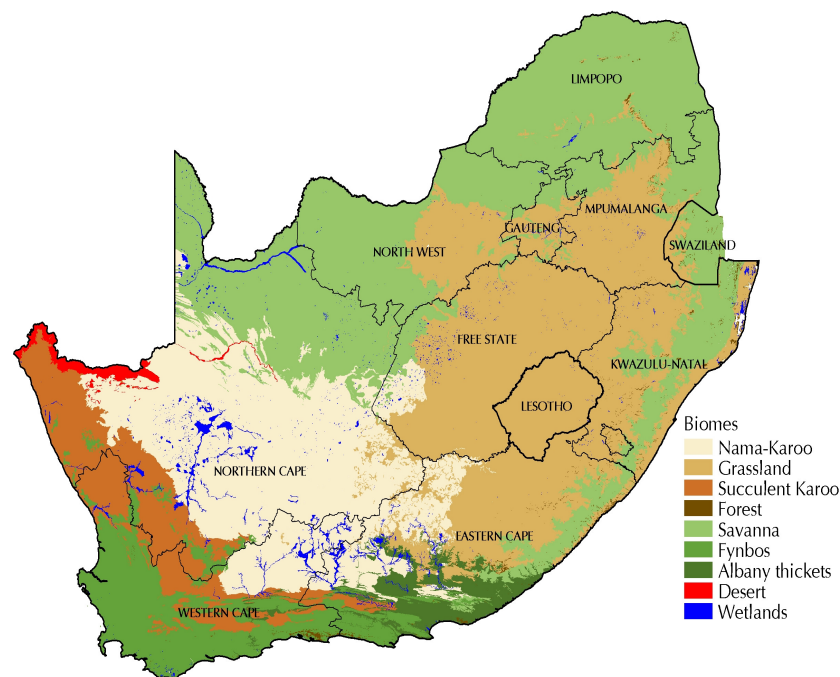


**Figure 1.1:** Distribution of the vegetative biomes of South Africa (DEAT 2007)

This dissertation is in the format of a paper intended for submission to the African Journal of Range and Forage Science. There will be some information overlap within the dissertation.
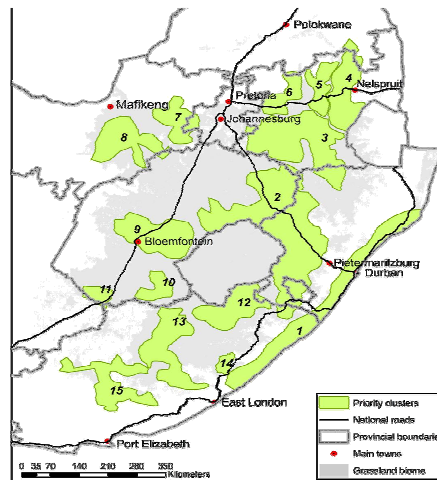
**Figure 1.2:** Distribution of the grassland biome in South Africa. The green areas represent priority conservation clusters of the biome. Map from the South African National Biodiversity Institute (SANBI 2007).


Biodiversity refers to the variety of species that occupy a given area and can be subdivided into three classifications: alpha, beta and gamma diversity. Grasslands have a high alpha diversity which is a measure of the number of different kinds of species in a given area (species richness), a high beta diversity which is the measure of diversity between habitat patches and a moderate gamma diversity which is a measure of how many species in an area are also present in another area (a measure of endemism) (SANBI 2007).

Unfortunately, the climatic conditions which promote the development of grasslands are also important for anthropogenic development such as cultivation, forestry and crop production. Additionally, South African grasslands contain great mineral wealth such as coal and gold, resulting in large areas of these grasslands being designated to mining practices. Rapid urbanization is also a real threat to the preservation of South Africa's grasslands. The problem with such agricultural developments and grassland uses is that the physical properties and vegetative structure of the landscape are severely altered. For example, the chemical and physical properties of the soil are altered through the addition of fertilizers, tilling and drainage lines. The vegetation structure is altered through artificially increasing productivity or a complete removal of the vegetative cover (Walker *et al.* 2004). It has been shown that the repeated addition of nitrogen rich fertilizers to a grassland, with the intention to increase the grasslands productivity, results in a

decrease in the species richness of the grassland sward due to the dominance of a few highly competitive species (Walker *et al.* 2004). Cramer *et al.* (2008) describes the adverse affects of anthropogenic alteration on the landscape it in this way:

> "All cultivation leaves a legacy. Biomass alteration, tillage, fertilization and changed hydrology alter ecosystem processes such that the legacy of cultivation can be seen in vegetation composition and structure hundreds, if not thousands, of years later".

South Africa has one of the most widespread cases of physical soil degradation in the world. Soil degradation processes range from soil crusting (a thick crust which forms over the soil surface preventing water infiltration and inhibiting root growth) to soil compaction (DEAT 2007). Additionally, soil acidification, caused by chemical use, mining and coal burning industries is common to South African soils (DEAT 2007). In total, approximately 30% of South African grasslands have been transformed through anthropogenic developments (SANBI 2007), most of which is irreversible without intervention through rehabilitation practices.

It is likely that grasslands which have been transformed through a degradation process will possess unique degradation attributes and rehabilitation challenges. These attributes are determined, in part, by the previous land use and the time since the disturbance has occurred. For example, areas that were previously mined on the Eastern Highveld of South Africa will have severely degraded soil structure, limiting nutrients and highly acidic soils (Mentis 2006). In contrast, areas that were previously under pasture (cultivation) could have high nutrient levels. Farming areas where the soil was historically tilled will have substantially lowered soil carbon levels (Reeder *et al.* 1998). As expected the restoration approach to these three scenarios would be very different.

Several factors need to be considered when deciding on a restoration approach for degraded grassland. A restoration ecologist needs to consider what the primary degradation factors are and what ecological components have been transformed (King and Hobbs 2006). Once the underlying degradation processes are understood the restoration of the degraded abiotic and biotic components can begin. Restoration can be a passive process where the recovery of the grassland is consistent with natural succession once the degradation process is stopped. Alternatively, restoration needs to be an active process of monitoring the restoration progression and adapting management practices according to the

response of the landscape to management and rehabilitation processes (Lake 2001).

Rehabilitation goals have shifted significantly over the past 10 years with the improvement of legislation regarding the protecting of South Africa's natural resources. Consequently, there has been an increase in the ecological understanding of community organisation and development.

Historically, mining companies would rehabilitate post-mining areas with commercially available species which have high requirements for water and nutrients. Over-fertilization of the post-mining areas would occur in order to establish a healthy grass sward. Once the grassland is "rehabilitated", according to the legislative requirements, resource addition into the system ceases and the grass cover deteriorates, decreasing the potential socio-economic use of the area. Consequently, the development of a mono-specific grass sward was promoted (Limpitlaw *et al.* 2005). Mono-specific grasslands are not capable of supporting grazing throughout the year, especially if the grass which is planted requires, but is not receiving, high resource inputs. Increasing the species diversity on the restored post-mining area would promote a more sustainable, year round, grazing system (Limpitlaw *et al.* 2005).

Originally the goals for rehabilitation were to achieve a level of vegetative cover that would decrease soil erosion potential and increase water retention (King and Hobbs 2006). There was limited focus on the development of a species diverse area as it was assumed that natural succession would promote a stable, species diverse grassland community. The increase in species, however, takes longer than originally proposed (sometimes over 70 years (Cramer *et al.* 2008)) and, as a result, many of the rehabilitated grasslands became dominated by a low number of highly competitive grasses. Consequently, it takes longer for the rehabilitated area to stabilise and maintain effective ecological processes, such as nutrient cycling. Today ecologists are more concerned with the ecological state and functioning of the rehabilitated area and aim to increase species diversity, as well as other ecological processes, allowing for the development of a self-sustaining ecosystem.

The increasing importance of the ecological state of a rehabilitated area is reflected in the integration of these concerns into the South African National Environmental Management Act (Act No. 107, 1998) where it was stated that:

> "Environmental management must be integrated, acknowledging that all elements of the environment are linked and interrelated, and it must take into account the effects of decisions on all aspects of the environment and all people in the environment by pursuing the selection of the best practicable environmental option."
> (Section 2.4b)

and that

> "The costs of remedying pollution, environmental degradation and consequent adverse health effects and of preventing, controlling or minimising further pollution, environmental damage or adverse health effects must be paid for by those responsible for harming the environment."
> (Section 2.4p)

One of the primary decisions that a restoration ecologist makes is what suite of species should be used to re-vegetate the area. The outcome of complete dominance of a single species, or the stable co-existence of a large suite of species in a rehabilitated area, is dependent on the species which the ecologist selects and how the system is managed. Fynn *et al.* (2009) demonstrated that there is a correlation of the species' invasiveness and invasibility and some of the species' growth characteristics, particularly grass height, growth rate, leaf mass, number of tillers and specific leaf area. They concluded that, in order for rehabilitated grasslands to be more species diverse, none of the species in the propagules mix should be tall or fast growing, nor should they have high total leaf mass, high tiller number or a low specific leaf area. In addition to determining the rehabilitation species growth characteristics the climatic conditions, the species present historically in the landscape and availability of seed needs to be considered when selecting rehabilitation species. Several abiotic factors, such as nutrients, light, moisture and the species' competitive ability (which is linked to a species' invasiveness (Fynn *et al.* 2009)), will contribute to the species' survival success.

The ecology of the humid grasslands of South Africa is well researched in terms of the sward structure, competition and nutrient availability (Wiltshire 1973, O'Connor 1994, Fynn and O'Connor 2000, and Tomlinson *et al.* 2007). Much work has also been carried out on the effects of grazing intensity and fire regime on the structure of the grassland (O'Connor 1994). There is, however, a large gap in research concerning how grasslands function with respect to grass seedling interactions and seedling ecology. The competition which is experienced by

seedlings could have a significant effect on the seedlings' establishment success and growth rate and, therefore, the structure of the community (Pywell *et al.* 2007). Consequently, environmental factors which influence the competition dynamics and growth rate of a grass seedling need to be determined. It is also important to understand the effect of available resources, and their relative availability, on mediating the competitive interactions among seedlings.

When the characteristics of a seedling are defined, in terms of the seedling's ability to grow under certain environmental conditions, species which are able to co-exist at the seedling stage can be identified. For a particular rehabilitation project the selection of the correct suite of species, based on the seedlings' growth and competitive characteristics, could lead to a decrease in seedling mortality due to the decreased competitive pressures. Consequently, there will be an overall increase in the initial species diversity of the grassland sward (Cramer *et al.* 2008).

There is no precise definition of what a seedling is, or more specifically, when a seedling has made the transition to an adult plant. It has been shown, however that there are three stages to a seedling's development, these being the heterophilic stage (relies on the cotyledonous reserves for growth), transition stage and autotrophic stage (Whalley *et al.* 1966). Fenner (1987) and Ries and Svejcar (1991) explain that a plant is still considered a seedling when it continues to use cotyledonous reserves, irrespective of its need to do so, that is, even if it is photosynthetically capable of producing sufficient resources for survival. This definition poses a problem in "field studies" as it is difficult to determine when the seedling is autotrophic or not. Even though it is not clearly defined it is generally accepted that when a study refers to a seedling and seedling growth it is referring to a young plant that has one or two leaves in addition to cotyledons (Fenner 1987). Crested wheatgrass (*Agropyron desertorum*) and blue grama (*Bouteloua gracilis*) seedlings were considered established after 21 days of growth as they were independent of cotyledonous reserves, had 4-6 leaves, 2 adventitious roots and 1-2 tillers per plant. Additionally, most of the seedlings from these two species survived the winter period after the first growing season (Ries and Svejcar 1991). It could be argued, therefore, that once a seedling is established it is no longer considered a seedling but an adult plant.

There are several environmental factors which influence the growth of adult grasses that also influence seedling growth, such as temperature (Black 1955,

Williams 1983), light wavelength and availability (Williams 1983), water (O'Connor 1994, 1996) and nutrients (Roberts 1960, Campbell 1968, McWilliam *et al.* 1970, Bisigato and Bertiller 1999, Monaco *et al.* 2003). In addition to these, factors such as the microsite where germination occurs (Silvertown 1980, Williams 1983), disturbance intensity prior to germination (Jutila and Grace 2002), litter cover (Fowler 1986, Jutila and Grace 2002), pathogenic fungi (Kirkpatrick and Bazzaz 1979), time to germination (Ross and Harper 1972, Fowler 1986, Klink 1996, O'Connor 1997), competition with neighbouring vegetation (O'Connor 1996) and exposure to plant-derived smoke compounds (Van Staden *et al.* 2004) have been shown to affect the establishment and development of seedlings.

## 1.1 Research objectives

One of the primary objectives of the South African National Biodiversity Institute's grasslands program (SANBI 2007) is to incorporate the preservation of grassland biodiversity into management plans and policies to contribute to, and maintain, the grasslands' vital ecosystem functions (i.e. water and soil retention, nutrient cycling and reproductive potential). In order to achieve a goal of a species diverse rehabilitated land the way in which grass seedlings interact with one another under different environmental conditions, and how competition affects seedling vigour, needs to be understood (Carr *et al.* 2007).

The aim of this dissertation is to determine the response of grass seedlings to different environmental stimuli and determine how these environmental conditions affect the seedlings' competitive ability. To achieve this several aspects of the ecology of South African grass seedlings are investigated. This dissertation focuses on three environmental stimuli that may be important for the growth and competitive ability of grass seedlings. Two of the stimuli investigated (nitrogen and light availability) are natural resources essential for plant growth. The third stimulus, smoke-infused water, is not naturally occurring in all environments but is a resource which can be artificially manipulated and which may prove advantageous to rehabilitation by increasing germination success and seedling growth rates. Three experiments were conducted to investigate the response of grass seedlings to these stimuli:

1. To determine the effect of nutrient availability on the competitive ability (response to competition and competitive effect) of *Chloris gayana, Cynodon dactylon, Digitaria eriantha, Eragrostis curvula* and *Eragrostis tef.*

   - It is predicted that these five species will not have the same competitive ability, and they will be affected differently by the availability of nutrients.

2. To determine what affects shading, nutrient availability and seedling density has on the growth of *D. eriantha* seedlings.

   - Shading, low nutrient availability and competitive pressure can all be negative influences on the growth of *D. eriantha* seedlings. It is therefore predicted that these environmental factors will have a compounding negative effect on the seedlings growth.

3. To investigate if the "smoke affect" is evident in the South African grasses *C. gayana, C. dactylon, D. eriantha, E. curvula* and *E. tef.*

   - Smoke-infused water reportedly increases germination percentage and seedling vigor in a variety of plant species (Van Staden *et al.* 2006, Kulkarni *et al.* 2007). It is unclear, however, what the affect of smoke-infused water is on the germination success and seedling growth rate (of the root and shoot) of South African grasses.

*Chloris gayana, C. dactylon, D. eriantha, E. curvula* and *E. tef,* five common South Africa grasses, were selected because of the variety of morphological properties (height, leaf size and growth structure) and high germination rates of over 80% (refer to Chapter 3).

*1.2 Limitations of study*

The experiments were carried out in environmental conditions where water, nutrients, seedling densities and competition from test and non-test species were controlled. In a natural situation these environmental conditions are not regulated and can have a significant effect on the growth of the seedlings. Further tests, in a natural environment, will need to be carried out in order to confirm any conclusions drawn from these experiments. This phase of developing new ideas towards a restoration plan which incorporates biodiversity is crucial for the understanding of the interactions that will take place in field trials.

Ideally local veld grasses are used in restoration projects as they have adapted to the local environment and disturbance regime of the area. In some grassland

communities the species from these experiments are not the dominant species and may not be suitable for that specific rehabilitation project. On the other hand, grass seeds are limiting resources in a natural environment (Martin and Wilsey 2006) and the use of commercially available species may be preferred as seed collection from neighbouring grasslands is time consuming and the germinability of the collected seed is not assured.

## Chapter 2: Literature Review

The restoration of previously disturbed grasslands has been a focus of ecological disciplines for a long period of time but restoration ecology has only been recognised as a discipline in its own rights in the last 15 years (Young *et al.* 2005). Early in development of restoration ecology, however, it became evident that there are concepts within the discipline which could be used to improve our understanding, and testing, of general ecological theories (Bradshaw 1987, Jordan *et al.* 1987). During this time two questions have emerged (Young *et al.* 2005), firstly, what set of ecological principles and concepts serve as an essential basis for effective restoration? Secondly, are there conceptual areas of ecology that are unique to restoration and rehabilitation practices?

The principles and ecological theory of rehabilitation and degradation are reviewed in section 2.1. The subsequent section (section 2.2) is a review of the models, methods and evaluation techniques used in restoration.

### 2.1 Introduction to restoration ecology

Many of the landscapes that require intensive restoration are degraded due to the economic activities of man (Jackson *et al.* 1995). The South African National Biodiversity Institute (SANBI 2007) estimates that 36.7% of the grassland biome is a priority for conservation and 30% of South African grasslands are irreversibly transformed through actions such as mining (0.3%), urban settlements (1.9%), forestry (3.6%) and cultivation (22.4%). An additional 6.4% of the grassland biome has been severely degraded through bad management and overgrazing. It may seem that mining and urban settlements have the smallest impact on the grasslands because of the relatively small areas which are affected but a "relative ecological impact" study indicated that these land uses had the highest negative impact on the grasslands. In total, only 64.5% of the grasslands are still considered "natural" and even these areas are under threat (SANBI 2007).

### 2.1.1 Recognising degradation

Timely recognition of the presence of degradation processes in a landscape will promote the development of a management plan to impede degradation progression. If the process of degradation is not recognised, or if action against

degradation is not taken, permanent changes in the landscape are inevitable and restoring a landscape to its original state may not be possible.

There are four system components that can be examined to asses the extent of degradation and the effect of restoration on the ecological system. These are the structural and functional components of the system as well as the biotic and abiotic factors (King and Hobbs 2006). As an example, the distinction between structural and functional components of an abiotic factor is when soil erosion is considered. The structural component to soil erosion is a change in the landscape due to the formation of rills and gullies. On a functional level soil erosion is a sign of degraded soil-water dynamics across the landscape. An example of a biotic relationship is if there are alien invasive species moving into the landscape. Structurally the outcome of this invasion is an undesirable species composition. Functionally, it is an indication of the superior competitiveness of the invading species compared to the desirable species (King and Hobbs 2006) or an indication that the overall ecological system is out of balance due to a disturbance or change of management regime. These four system components are not mutually exclusive and have to be considered in combination to attain a complete overview of the state of the ecological degradation of the system (King and Hobbs 2006). An understanding of how these four components interact with each other in a particular landscape will guide the development of an optimal restoration strategy.

Changes in the landscape caused by degradation processes occur in a stepwise feedback manner between two or more ecological factors. Whisenant (1999, 2002) developed a model where each step within the stepwise degradation cycle must be viewed in terms of having a structural or functional basis as well as being biotic or abiotic. During the degradation process the four components mentioned above work on each other creating a downward, self-regulating spiral into a more degraded state (Figure 2.1a). The changes which are taking place affect all four components of the feedback system because of the connectivity between them. Autogenic recovery (Whisenant *et al.* 1995, Whisenant 1999) takes place when there are restorative processes acting on the components of the system. This is also a self-regulating system but it flows in the opposite direction to the degradation process (Figure 2.1b).

In general, with a decrease in biotic and abiotic components present in the ecological system there will be an increase in the severity of degradation and the

rate in which degradation occurs (Schlesinger *et al.* 1990, Kassas 1995, Le Houerou 1996). For example, with a decrease in grass basal cover the rate at which water run-off and soil erosion occurs will increase. An increased erosion rate decreases the chance of seedling establishment and, therefore, a decrease in potential for vegetative cover to increase (e.g. Figure 2.1a).

A restoration map (King and Hobbs 2006) is a useful tool to investigate interactive processes and to determine how the system components are linked. The map is the outcome of the investigations regarding which components of the system are affecting the degradation state and, therefore, which of the four components should be targeted for restoration. In order for restoration to take place at least one component of the system needs to be identified which will reverse the degradation spiral and allow for improvement of the ecosystem components.

There are three phases of degradation which have been identified. They were first identified by Milton *et al.* (1994) and were later reviewed by Whisenant (1999, 2002), Hobbs and Harris (2001) and King and Hobbs (2006).

1. The biotic function of the system is degraded but autogenic recovery is still possible if the factor causing the degradation is removed. If the degradation process persists then the first threshold of the ecosystem is crossed. The threshold is identified by severe degradation to biotic components in the system.

2. Manipulation of biotic components in the system is required in addition to removing the identified component causing degradation. Direct manipulation of abiotic components may not be necessary even though they have been degraded through the loss of other components. The second threshold is crossed at this point if the factor causing the degradation is not removed.

3. The biotic and abiotic components are severely degraded and the abiotic components also require manipulation.

a)

Loss of plant cover

Increased erosion                    Reduced soil
                                     water

Deteriorating                                 Decreased
soil structure                                plant
                                              production

Reduced soil                          Greater soil
fertility                             temperatures

b)      Increased water infiltration

Increased water
availability to
plants
                                           Increased
                                           plant
                                           production

Increased vegetative
Cover

              Increased soil
              fertility
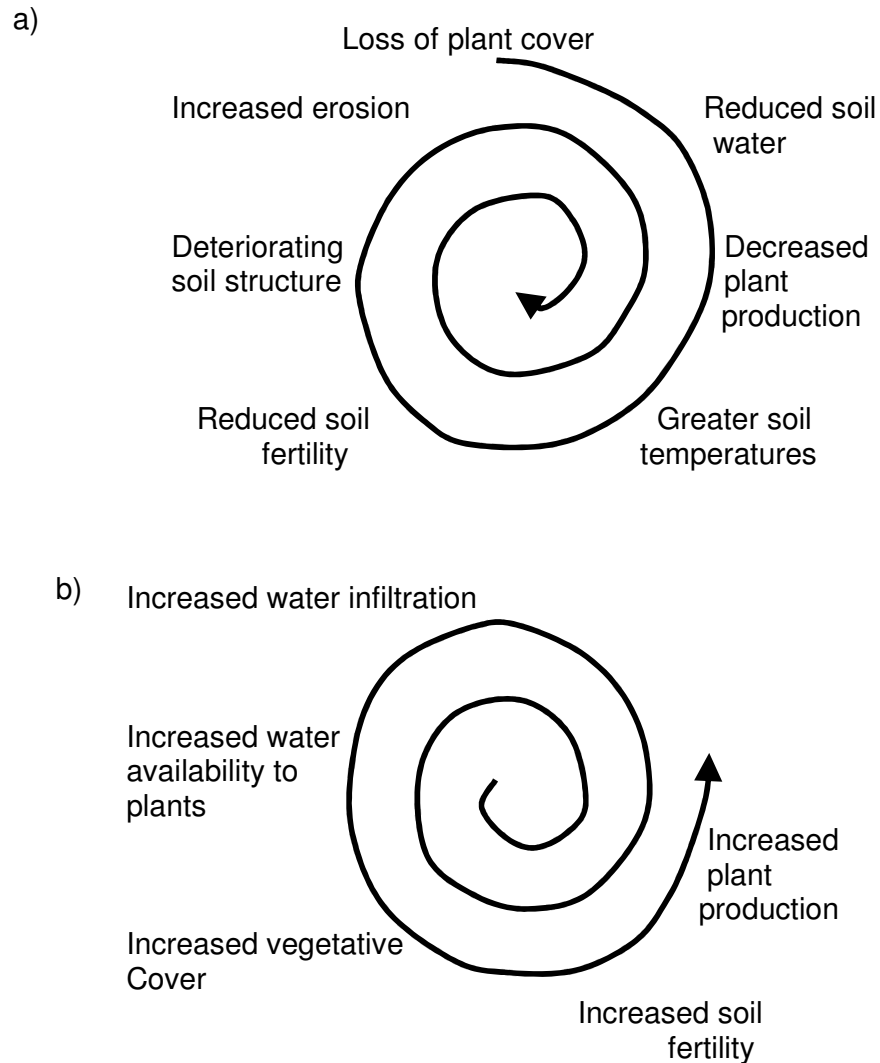
**Figure 2.1:** Simplified examples of degradation (a) and restoration (b) spirals occurring in stepwise feedback mechanisms as depicted in Whisenant (1999, 2002)

Abiotic factors in the ecological system are referred to as "primary drivers" of restoration even though the processes of degradation, and the initiation of autogenic recovery, is driven by the feedback processes of a combination of the biotic and abiotic factors (Whisenant 1999). They are the "primary drivers" because there is some level of abiotic structure and functionality required before biotic components can successfully establish (King and Hobbs 2006). For example: grass cover will not increase on a degraded area if there is poor soil

structure and excess erosion. It is more effective to concentrate on the recovery of the soil than trying to get grasses to establish.

In general, it can be expected that grasslands that have higher levels of resources (e.g. water and nutrients) are in a healthy state and will be regulated by biotic interactions (e.g. input of organic materials into the soil). These areas will also be relatively stable and resistant to change with a change in environmental conditions and disturbance. Grasslands with low resource levels are influenced on a more abiotic level (e.g. water infiltration and soil temperatures) and are closer to a degradation threshold than resource rich environments (Tongway and Ludwig 1996, Whisenant 1999).

### 2.1.2 A resistant and resilient landscape

The resistance and resilience of a landscape is measured by the extent to which a disturbance affects the ecosystem, on a structural and functional level, and the rate at which the system recovers from a disturbance. A landscape is considered to be resistant to degradation if the original species composition, and its structural and functional properties, are maintained after a disturbance event (Stringham *et al.* 2001, Pellant *et al.* 2005). If the landscape is not considered to be resistant to degradation but possesses resilience then the recovery of the landscape after a degradation event will be rapid (Stringham *et al.* 2001, Pellant *et al.* 2005).

A fully functional landscape is both resistant and resilient to change and a stable ecological state will be maintained during moderate disturbance (Stringham *et al.* 2001). The resistance of the landscape to disturbance may promote a fluctuation of plant species over time (Stringham *et al.* 2001) but these fluctuations are not transitions between ecological states, rather phase shifts within an ecological state that the system can recover from.

A disturbed landscape, which possesses limited resistance and resilience properties, will show a deterioration of ecological processes. When ecological processes deteriorate a change in species composition and functional processes of the system is inevitable (Pellant *et al.* 2005), an ecological threshold could be crossed and an alternative stable state could be formed (Briske *et al.* 2008).

*2.1.3 Restoration versus rehabilitation*

There is a fine line distinguishing restoration from rehabilitation and, because the two concepts are so closely related, distinguishing characteristics need clarification. Restoration is generally referred to as the changing of the degraded landscape to its original structure and function (King and Hobbs 2006). McKay *et. al.* (2005) defines restoration as the "augmentation of re-establishment of an extinct population or community". The National Research Council (NRC 1995) defines it as "returning a system to a close approximation of its condition prior to disturbance, with both the structure and function of the system recreated."

Restoration, however, can promote the introduction of new genes or genotypes into a population when the vegetative material used is not of the same origin as the historically present vegetation. The restored vegetation is, therefore, not necessarily adapted to the local environmental conditions present (McKay *et al.* 2005). Arguably, the change in the genetic makeup of a population is rehabilitation (reclamation) and not restoration. Rehabilitation is the effort to restore functionality (King and Hobbs 2006), specific services and some vegetation (Bautista *et al.* 2004) to the system which is severely degraded. Typically an ecological threshold has been crossed decreasing the likelihood that the restored vegetative type is identical to the original (King and Hobbs 2006).

Two approaches to rehabilitation are possible:

1. A structural approach leads to a landscape that resembles the previous state. This could be regarded as a "quick fix" because there is little guarantee that the rehabilitated state will persist over time (King and Hobbs 2006) if the factor causing degradation is not identified and corrected.

2. A functional approach looks at the recovery of the ecological processes that have been or are being degraded. There is a greater chance that the changes made to the system during the rehabilitation process will persist if the factor causing the degradation is removed. This process is much slower than the structural approach (King and Hobbs 2006) and the final structure of the vegetation in the landscape (e.g. species present) may differ significantly from the previous state.

The ability of a landscape to respond and adjust appropriately to disturbance needs to be taken into account when developing a restoration plan. When the ecological factors that are driving degradation process are understood

management practices could be implemented which could decrease the rate of degradation and promote recovery. Currently there are several models that are used for developing rehabilitation practices and several techniques that are used to assess the rehabilitation success. These models and techniques are discussed in section 2.2.1 and 2.2.3 respectively.

## 2.2 Restoration models, methods and evaluation

To develop concepts which will contribute to the science of restoration ecology there must be an understanding of how vegetative communities are constructed, how they develop and how they react to disturbances (Young *et al.* 2005). It is equally important that new knowledge and ecological understanding is continually incorporated into ecological theories and methods for evaluating ecosystem health. It is becoming increasingly apparent, however, that this is not being done and the models used for restoration of degraded landscapes, and the theories on which they are based, are not in line with current ecological thought (Friedel 1991).

It is important, therefore, to have a sound understanding of the current models, and rehabilitation and evaluation methods, so that new insight of ecological processes can be incorporated into the rehabilitation of degraded grasslands.

## 2.2.1 Ecosystem models used in restoration

Clementsian concepts such as "there is a single stable climatic species composition for a given area" and "continuous successional pathways" are no longer as dominant in ecological theories as when they were first proposed. Current theories suggest that there can be several ecological states that a community can tend towards and the final community composition and structure depends on the environmental conditions present and how the area is managed. There is also a general agreement that the successional pathway evident in a community is not continuous and, in many cases, is an irreversible transition between ecological states (Westoby *et al.* 1989).

### 2.2.1.1 Succession theory

Clements was first to indicate the importance of managing grasslands in accordance to the principles outlined in the range successional model (Westoby *et al.* 1989). This model is based on the argument that each grassland's vegetative

community will progress towards a single climax species composition (Westoby *et al.* 1989) which is governed, primarily, by climatic conditions. Secondary climax community determinants are variations in soil structure and nutrient availability. Subsequent determinants may be the management and utilization of the area. Areas which are over-utilized and badly managed will have a different climax species composition to those which are utilized sufficiently and have been managed optimally (Westoby *et al.* 1989).

Two factors, given as examples by Westoby *et al.* (1989), which affect the direction of the successional pathway are stocking rate and rainfall (Figure 2.2). Above average rainfall will promote community development along the successional pathway. If there is below average rainfall the progression of the vegetative community along the successional pathway will cease and may, under extreme circumstances (e.g. in a prolonged drought), cause the vegetative structure to deteriorate back to a previous successional state. Increased grazing pressure will decrease the rate at which natural succession progresses. According to the successional model, if the stimulus hampering the vegetative succession is removed the vegetation would naturally continue, linearly, along the previous successional path towards a pre-established climax community (Westoby *et al.* 1989).

In order to rehabilitate a degraded area the correct stimuli need to be present in, or added to, the system to drive the natural successional pathway. Promoting a rehabilitative stepwise feedback mechanism in the area would benefit the autogenic recovery and develop the successional tendency of the system.
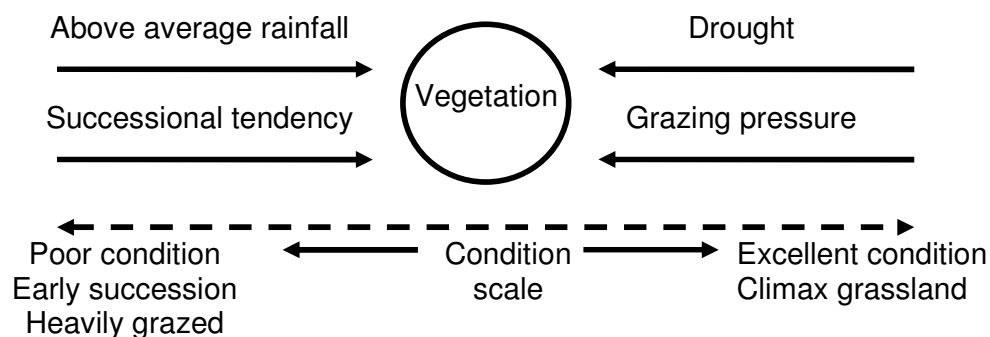


**Figure 2.2:** Schematic representation of the range succession model (Westoby *et al.* 1989). Grazing pressure and rainfall are presented as drivers of the succession process.

The theory that there is a single successional pathway that will lead to a single climax plant community is inadequate to describe complex successional pathways evident in nature (Stringham *et al.* 2001). In many instances there are a variety of successional pathways which occur in a landscape after a disturbance, and the incorporation of these pathways into the range succession model is not possible. For this reason some ecologists and rangeland managers no longer rely on the successional model for predicting rehabilitation outcomes (Stringham *et al.* 2001).

*2.2.1.2 State and transition models*

A state and transition model is based on the premise that within an ecological state, which is defined by a set of ecological boundaries, a healthy vegetative community will fluctuate in species composition and structure in accordance with the climatic conditions and the natural disturbance regime. If, however, there are significant changes in climatic conditions or if a large disturbance occurs the vegetative community could shift to another ecological state.

The ecological state, in the state and transition model, is one or more biological communities in an area which are distinguishable by relatively large differences in the plant functional groups, soil properties, ecosystem processes (Herrick *et al.* 2005b, Pellant *et al.* 2005) and their response to disturbances (Pellant *et al.* 2005). Fluctuations of the dominant vegetative composition and community structure can occur within a state (transient transition) and may be caused by changes in season (there may be a fluctuation in dominant plant group from a warm season plant (C4) in the summer to a cool season plant (C3) in the winter), pulses of influencing factors (e.g. episodic rainfall events) and a change in management practices. It may, therefore, be difficult to determine where the ecological boundaries lie for a particular community and if the changes which occur in the vegetation are merely phase shifts within the state, or transitions between states (Pellant *et al.* 2005). Factors influencing the shift within a state should be considered when defining the state and ecological boundaries of an area.

Transitions can either be transient (temporary) or persistent (Westoby *et al.* 1989). A transient transition is reversible, can be passively restored (Stringham *et al.* 2001) and can only occur within a state. A persisting transition occurs when the vegetative structure or ecological processes of an area cross the ecological

boundaries of two or more states. A persisting transition is irreversible without mechanical altering.

State and transition models can help managers to decide where monitoring should take place as ecological boundaries and thresholds can be identified (Herrick *et al.* 2005b). It is important to accurately identify the factors that are changing so that there are no shifts in the ecological state which could be avoided by minor alterations to management practices. Figure 2.3 is a diagrammatic representation of a state and transition model that can be used to assess the changes that are occurring within and between the states in an ecosystem. In this diagram a reference state is used to judge the potential risk of crossing a transitional threshold. The importance of a reference state to evaluate the condition of the area is highlighted in section 2.2.3.1.
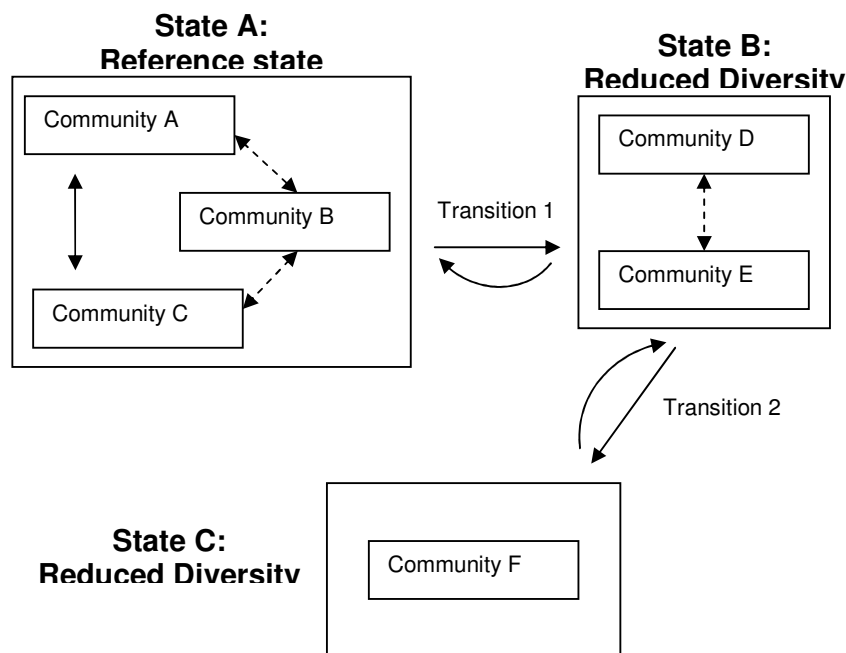


**Figure 2.3**: Generic state and transition model (adjusted from Stringham *et al.* 2001, Tongway and Hindley 2004, Pellant *et al.* 2005). Within a state: dashed lines represent reversible transitions, solid lines are community pathways. Solid lines between states are persisting transitions which are only reversible with mechanical altering (curved arrows). The boxes encompassing the states are the thresholds.

Briske *et al.* (2008) re-looked at the state and transition model to determine a method to assess restoration. They incorporated several ecological factors into the model and were particularly interested in the importance of ecosystem resilience in determining the ecological boundaries of a biological system. The incorporation of ecosystem resilience into the state and transition model clearly showed that systems which lack resilience are more susceptible to changes in state. In general, there are no obvious signs of declining vegetative structure and ecosystem processes when there is a gradual decline in resilience. The degradation of the system and the crossing of transitional boundaries are, therefore, difficult to assess.

Ecosystem processes can be governed by a number of feedback systems that enhance ecosystem resilience or decrease it. Enhancing feedback systems maintain the vegetative structure and ecosystem processes away from the threshold boundaries. In a system where an ecological threshold has been crossed an enhancing feedback system forces the vegetative structure and ecological processes away from the previous state (enhancing ecosystems transition across the threshold) increasing the rate of degradation (Figure 2.4). A decreasing feedback system will always drive the vegetative structure and ecological processes towards a threshold boundary (Figure 2.4). In a healthy system a decreasing feedback system is the result of degradation processes but in a system that has crossed a threshold a decreasing feedback system is beneficial and aids in rehabilitation (decreases the resilience of the degraded system promoting rehabilitation). The response of the alternative state to restoration efforts depends on how long the enhancing feed back system has been acting on the state and the extent to which the biotic and abiotic systems have been modified.

In order for restoration to be successful the decreasing feedback mechanisms need to be introduced into the alternative state. When the vegetative community and ecosystem functions resemble those immediately after the change in state it may be possible to restore the vegetation and ecosystem function to that of the previous state (i.e. from "State 2" to "State 1" in Figure 2.4).
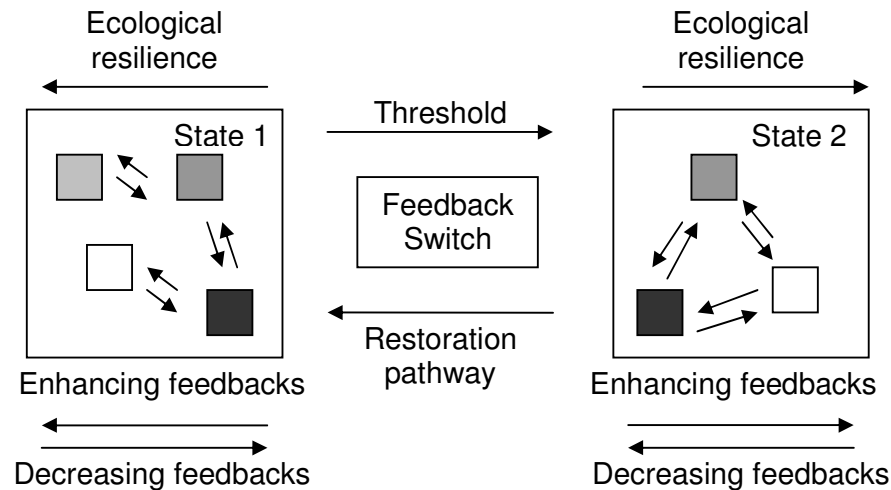
**Figure 2.4:** Revised state and transition model incorporating the resilience based concept as discussed by Briske *et al.* (2008).

### 2.2.1.3 Community Assembly Theory

The community assembly theory associates the assemblage of a plant community with a sequential series of species invasion and extinction events. The invasiveness of a species and the ability of the invaders to displace the established species are determined by the interactions which are taking place between the invader and the target community, assuming that competition is a major deterministic process governing the structure of the community. A specie's competitive ability and survival can, in turn, be influenced by climatic conditions and resource availability (Moyle and Light 1996).

Determining the community assembly sequence of areas surrounding a rehabilitation site will increase the predictability of restoration results as the next "step" in the vegetative community development could be calculated (Moyle and Light 1996). When dealing with community development there are several factors which need to be considered, for example:

1. The order in which species are naturally introduced into a community is important (Palmer *et al.* 1997) as it will influence the approach that restoration managers employ.
2. The extent of the degradation at the rehabilitation site, and the distance of the site to the nearest regional species pool will determine if species need to be manually introduced into the rehabilitation site or if they will naturally

disperse into the area (Cairns 1993, cited by Palmer *et al.* 1997). The species dispersal mechanisms and general establishment success need to be carefully considered e.g. grasses disperse slowly, especially if they reproduce vegetatively.

3. The time and spatial scale of anthropogenic intervention alters the course of community development (Palmer *et al.* 1997).

At a mine reclamation project in Wyoming (USA) Parmenter and MacMahon (1983) found that planting vegetation in different spatial patterns led to different vegetation spatial structures, age-structures, biota and soil dynamics. The implications of this finding is important for restoration ecology as it suggests that every step of the restoration process needs to be viewed as part of the whole process of community development. For example, adding seeds at different time intervals could change the direction that the community interactions drive the rehabilitation succession (Palmer *et al.* 1997).

Through the development of the community assembly rules and testing of associated theories, a number of generalizations have emerged (Case 1991):

1. Invasion success decreases with an increase in species richness and with the time that the community has been collecting species (age of the stable community).

2. Invasion, even by a superior competitor, is difficult when there is a low number of invading species, or individuals per species.

3. The community's resistance to invasion increases with an increase in the number of species interacting with one another.

4. The final species composition of a community is dependent on the order of invasion. Multiple stable states are possible from one species pool invading at different time intervals and at different spatial scales (contrary to the successional theory).

5. Communities can shift between alternative states as invasions and extinctions proceed.

6. With an increase in the species pool there is a decrease in the stability of each species but there is an overall increase in community stability. There are strengths and weaknesses that are played off between the species and some species will compensate functionally for others (Tilman *et al.* 1994).

**2.2.2 Effective rehabilitation methods for South African grasslands**

The severity of the landscape degradation and the size of the degraded area will affect which rehabilitation and revegetation techniques will be implemented. When limited vegetation is present in the landscape the primary goal would be to increase soil stability and introduce pioneer species to the system. If, however, there is vegetative cover (even at the earliest successional level) then different rehabilitation methods can be employed.

Three of the most effective revegetation methods used in rehabilitation are grass plugs, direct seeding and using facilitator plants. These methods are discussed in detail in section 2.2.2.2, 2.2.2.3 and 2.2.2.4 respectively. The first two revegetation methods discussed, grass plugs and direct seeding, can be used for either rehabilitation scenarios mentioned above. The third method, using nurse or facilitator plants, can only be implemented when there is established vegetation present.

*2.2.2.1 Importance of donor plant populations relative to restoration site*

Determining the correct source population for revegetating the restoration site is crucial as there are several genetic and environmental factors which can influence the success of the vegetation establishment. The donor plants suitability for the rehabilitation sites environmental conditions, geographic isolation of the donor population from the restoration site and the founder effect (brought about by genetic drift) are factors which need to be evaluated when selecting a donor plant population.

Local adaptation is described by Bischoff *et al.* (2006) as a significant difference between populations of the same species in contrasting habitats and is predominantly expressed through phenotypic characteristics (outward expression of genetic information). For example, *Themeda triandra* plants can differ significantly in culm height (Rattray 1960) depending on the environmental and climatic conditions in which the population is established.

Contrasting habitats where local adaptation takes place can occur at a small scale (patches within a landscape) or at a large scale of different landscapes or continents (Bischoff *et al.* 2006). If populations are geographically similar (within the same landscape) but their environmental and climatic conditions are different (due to soil nutrients, soil type, topography, etc) there is a strong possibility that

local adaptation has occurred between those populations. If there are two populations that are geographically separated on a large scale but which have similar environmental conditions, then the vegetation could be phenotypically similar. The species within the environmentally similar populations will probably have similar growth and competitive traits (Bischoff *et al.* 2006).

The founder effect occurs when a few individuals establish a community and the genetic diversity between the individuals is not representative of the original population (Mander 1997). Decreased genetic diversity will decrease the community's probability of surviving a catastrophic event or a shift in climatic conditions. Small populations are more susceptible to the effects of translocated genes which are not adapted to the environmental conditions. The gene pool is small and the effects of the maladapted genes will be more influential in the local re-established population (Hufford and Mazer 2003, Rice and Emery 2003).

In unpredictable environments it is important to re-vegetate an area from donor communities with similar genotypic variations to the historical source population. Using phenotypes which do not readily adapt to significant environmental fluctuations will cause the newly established vegetation to die out (Lande and Shannon 1996). Likewise, highly variable but predictable environments require higher genetically variable founder populations allowing the population to adapt to the varying environmental pressures (Lande and Shannon 1996). Conversely, if the environmental conditions in an area are stable there is little need for the vegetation to continuously adapt to new influencing factors. Transplanting phenotypes which differ significantly from the historic phenotype could lead to local extinction of that species.

Therefore, gene flow can either prevent or facilitate rehabilitation. The challenge is allowing enough genetic flow to prevent local extinction but not so much that the founder population can not adapt quickly to their new environment.

### 2.2.2.2 Grass plugs

Seedling trays which can hold seedlings for long periods of time, without decreasing their growth rates or survival probability, but which also allow for easy seedling removal are important for seedling propagation. Commercially available trays, such as Speedling[©] and Viro-cell[©], as well as tube stock systems can be used (Cole *et al.* 2000). Growing media or "plug media" is made from disease and

seed free materials and is available commercially but can be made privately (Cole *et al.* 2000). Seedling propagation is most successful in a greenhouse or a warm, controlled environment with a timed irrigation system (such as a misting device).

Pre-planting preparations and post planting management is essential for good survival of the grass transplants. Correct site preparation may entail several weed removal sessions (depending on the seed bank), soil preparation (such as tilling, removing rocks or stumps) and fertilization (Cole *et al.* 2000). The climatic conditions before and after planting are also important. Two to three days of moist soil is important for optimal germination which means that a planting should take place after a good rainfall event followed by cool, overcast climatic conditions (Cole *et al.* 2000). Additionally, post-planting rains will increase the probability of successful establishment of the grass plugs (Du Toit 2009).

Grass plugs can either be hand planted or machines can be used. When hand planting grass plugs a hole is made in the soil the same size as the seedling and the seedling is placed deeply in the hole. It is important that the base of the grass plug makes contact with the base of the hole and that some of the seedling leaf is visible above ground. A slow-release fertilizer can be added in liquid or solid form (Cole *et al.* 2000). There are commercially available 'hole making' tools but any make shift tool (e.g. a broom handle) is sufficient.

When using a mechanical seedling planter an injection irrigation system that can deliver 125 – 250ml of water automatically with each seedling is ideal. Up to 1000 plants an hour can be planted using machinery such as the Potiputki planter (Cole *et al.* 2000).

### 2.2.2.3 Direct seeding

Direct seeding costs less than planting with pre-propagated seedlings but is less effective as more seeds, which are limited resources, are required (Anon. 1998). Understanding species preferences in terms of sowing time and depth, and their specific germination requirements can significantly increase the reliability of direct seeding techniques (Carr *et al.* 2007).

In accordance to the guidelines set out by FloraBank (Anon. 1998) seeds need to be collected from between 20 and 100 plants in order for genetic diversity to be accounted for. Collections should be taken from one large population but if there are only small populations available then the seeds must be pooled together

before planting. Collection sites must be matched with the re-seeding site in terms of soil texture and origin, altitude, aspect, slope position and latitudes. Generally, a sowing density of 100 to 200 germinable seeds/m$^2$ is recommended but stipulated seed density is highly variable depending on the germination percentage of the species used, expected precipitation, and expected level of weed competition (Cole *et al.* 2000).

Growing a nursery crop of grasses from the veld collected seeds and harvesting the rehabilitation seed stock from the nursery will significantly decrease the number of seeds needed from the veld. A secondary advantage is that the rehabilitation seed stock harvested from the nursery plants will be weed-free (Gibson-Roy 2008).

There are several methods that can be used for seeding: broadcasting by hand, using seed planting machinery or brush and hydro mulching (Anon. 1998). Broadcasting by hand is the most suitable technique when working with large seeds, those which have awns or where good surface mulch is present to protect the seeds. Seeds sown in this manner should be lightly raked or harrowed to bury them slightly and improve seed-soil contact (Cole *et al.* 2000). There are several seed sowing machines which can be used to plant grass seeds, but there are none that are capable of planting seeds at the right depth and sowing rate in their chaffy form (with bracts still attached) (Cole *et al.* 2000).  Some mechanical planters are designed to be multi-functional and incorporate weed control, ground preparation, seed application and sometimes some form of seed treatment (water, fertilizer etc) (Anon. 1998).

Machines which have proven successful at planting grass seeds are:

1. The "light mesh hopper" which bounces seeds in a mesh drum and seeds fall through in a random order and pattern.
2. "Crocodile planter" which is a drum seeder that disturbs the soil surface as it passes and makes water collecting pits in which the seeds are placed.
3. The "germinator" which is an air-drill seed planter that reduces the disturbance to the soil and, therefore, weed competition (Cole *et al.* 2000).

All of the currently used seed planters have positive and negative attributes and it is up to the manager to decide which mechanism is best for their specific needs. The species being sown, soil characteristics and availability of manual labour should be considered when making this decision.

Brush Mulching is when branches and culms (with seeds still attached) are spread over the re-seeding site. As the culms and seeds dry out the seeds will fall and the vegetative layer will provide a protected area for germination (Anon. 1998). This is a popular technique for areas that are severely eroded or degraded as the branches form a protective layer over the soil thereby decreasing soil erosion, water loss and soil temperature (Anon. 1998).

Hydro mulching is very different to brush mulching. With hydro mulching the seeds are mixed into slurry consisting of water, fertilizer and mulch material and this is sprayed onto the bare ground using high pressure hoses. This is a common technique on inaccessible sites and steep slopes (Anon. 1998).

The optimal time at which seeds are sown will differ between species and environmental conditions. There should be sufficient time after planting for the seedling to grow to maturity, and flower, to increase the probability of survival over the dormancy period (Cole *et al.* 2000). Allowing for seed set in the first year of planting is important in seedbank development and thickening of the sward. In the second year it may be more important to extend vegetative growth period (delay / inhibit reproduction) allowing for the development of rhizomes, stolons or above ground vegetative structure in order to increase the probability of prolonged survival (Cole *et al.* 2000)

### 2.2.2.4 Facilitator / nurse plants:

Seedling growth and establishment can be severely hampered by high soil temperatures, low soil moisture, rain drop action (eroding the soil at the base of the young plant) and herbivory. If a seedling establishes close to a mature plant it would be protected from a range of these environmental factors and its chance of survival would be significantly increased. The established plant next to which the seedling is growing is referred to as a facilitator or nurse plant.

Shade, cast by nurse plants, aids the growth and establishment of seedlings by decreasing the soil temperature and increasing the available soil moisture. Nurse plants also prevent direct rain drop impact on the surrounding soil and increases soil stability. The roots of the nurse plant stabilize the soil structure and prevent soil erosion around the seedling. Soil, water and nutrients are trapped against the nurse plants base improving the microhabitat in which the seedling establishes (King and Hobbs 2006). Nurse plants are also referred to as facilitator plants as

they enhance the natural regeneration progression (such as nutrient cycling, litter generation and water retention) within the landscape (Parrotta *et al.* 1977, Callaway 1995, Callaway and Walker 1997, Lamb *et al.* 1997, Bruno *et al.* 2003, Gomez-Aparicio *et al.* 2004, and Young *et al.* 2005).

An experiment conducted by Fowler (1986) on three grasses from Edwards Plateau, Texas showed that nurse plants may be detrimental to the survival of seedlings which germinate in close proximity (<2cm) to its base. Although it was noticeably wetter at the base of the nurse plant the growth rate and survival percentage of the seedlings grown 0cm and 2cm from the base was less than seedlings grown 4cm to 6cm away. Fowler (1986) concluded that the intensity of competition experienced by the seedling is dependent on the size of the nurse plant, proximity of the seedling to the nurse plant and on the size and location of nearby adult plants.

### 2.2.3 Assessment of restoration success

Frequently monitoring post-rehabilitation changes in vegetative cover, biomass production and species composition is crucial for evaluating the recovery of the rehabilitation area (Herrick *et al.* 2005a). Constant evaluation of the rehabilitated site will allow managers to anticipate divergences in the recovery plan and adjust their restoration approach accordingly. Several short-term monitoring sites are required within the restored area. These sites should be selected because of their unique landscape attributes such as topography, elevation, degree of degradation and sensitivity to management practices (Herrick *et al.* 2005a).

An intensive long term monitoring regime is beneficial to the recovery process of the area. Plant basal cover, soil structure and water infiltration rates of the long term monitoring sites will give additional information of the state of the recovering system as a whole. Generally, some of the short term monitoring sites are doubled as long term monitoring sites as the "short term data" will assist in explaining the changes observed in the long term recovery trends (Herrick *et al.* 2005a).

As the degraded area recovers the ecological stability and the structural and functional processes, which govern the ecological system, increases. At this stage in the rehabilitation process it may be possible to increase the length of time between monitoring events and possibly even monitor the recovery progression every five to ten years.

The Society for Ecological Restoration Science and Policy Working Group (SER) (2002) monitor restoration success using a three step system. These steps are:

1. to directly compare the restoration site to the reference site,
2. to analyse the quantitative and qualitative attributes of the site and
3. to determine the state of the structure and functional processes of the restored area.

Several landscape evaluation and monitoring methods have proven successful in determining the success of the rehabilitation efforts. These methods are discussed below in relation to the three monitoring steps adopted by the SER.

*2.2.3.1 Reference sites as a tool for determining restoration progress*

A reference site is an area in good ecological health and which is geographically and topographically similar to the rehabilitation site. The recovery trajectory of the restoration site is compared to the ecological processes in the reference site as a means of monitoring the outcome of the rehabilitation efforts (Friedel 1991). Well chosen reference sites are essential to the assesment of the rehabilitation success. Without having a model to judge the development of the vegetation structure, hydraulic functioning and species competition there can be no defined "end point" where the restored system is viewed as fully functional or fully recovered.

The spatial position of the reference site to the rehabilitation site is important. The reference site must not be situated on an area that is atypically favoured with respect to soil nutrients and rainfall, or which has an unknown or a significantly different disturbance history (e.g. historical fire occurrence should be similar across both sites) (Friedel 1991).

Ecologically it is not possible to determine which successional pathway the rehabilitation process will follow. The final vegetative structure could be very different to that of the reference site due to the disturbance that the area is recovering from. Some have argued, therefore, that the use of a reference site to determine the "end point" of the rehabilitation progress is "old ecology" which implies that the rehabilitated area should have the same vegetative structure as the chosen reference site (Brewer and Menzel 2009). To overcome the issues of the position of the reference and determining the correct successional pathway it

has been suggested that multiple reference sites are selected (Wilson 1984, Bosch *et al.* 1987, cited in Friedel 1991, Brewer and Menzel 2009). Having multiple reference sites will give an indication of the natural variability of vegetation structure across the landscape under different disturbance regimes and climatic conditions.

Finding a suitable reference site may not always be possible. In severely degraded landscapes there may not be an area which is considered to be in good ecological health. Similarly, when rehabilitating endangered vegetation units (e.g. the *Aristida junciformis* dominated grasslands classified to as 'Ngongoni veld (Mucina and Rutherford 2006)) there may not be another area of grassland geographically or topographically similar to the restoration site. Brewer and Menzel (2009) were confronted with such a problem and devised a method to construct a "virtual reference site" from surrounding, closely related vegetative groups. Brewer and Menzel (2009) undertook a restoration project of oak woodlands for which there are no local reference sites available. There were, however, similar vegetation types which had different environmental conditions and associated soil types. In order to develop an ecological trajectory of the possible climax community of the oak woodlands a species list was compiled from the rejected reference sites and appropriate species (those that would naturally occur in the region concerned) were noted. Using this technique there was at least a guideline for the required vegetation structure and species composition which would indicate the success of the restoration efforts.

*2.2.3.2 Analysis of qualitative and quantitative attributes of restoration sites*

What to measure, how to measure it, and how those measurements are interpreted in terms of evaluating the rehabilitation processes are still debated (Friedel 1991, Palmer *et al.* 1997). It is generally accepted, however, that the evaluation of each rehabilitation project should be determined independently.

The objectives of rehabilitation projects differ significantly between rehabilitation sites with different types of disturbance and historical land use. For example: if there were dongas forming in an area due to increased erosion rate the rehabilitation goals would be to decrease donga size and increase vegetative cover to stabilize the soil. These goals, and methods for evaluating rehabilitation

success, will be very different than if trying to re-vegetate an area that has been over utilized through bad management practices.

In order to evaluate the extent of the landscape degradation and the rehabilitation success several landscape attributes need evaluation. The majority of the measurable landscape attributes can be described as being qualitative or quantitative indicators. Qualitative indicators are the basis for the indicators of landscape health. They are generally based on physical attributes of a site and are often difficult to measure. Quantitative indicators are used when a direct comparison is required between two locations or where the data is intended to be used to determine trends in the landscape (Pellant *et al.* 2005). In some cases the qualitative indicators are correlated to quantitative indicators. Inter-site comparisons of the qualitative indicators can therefore take place using the quantitative data (Pellant *et al.* 2005). Some of the more widely used methods for measuring the success of restoration, such as Rangeland Health Indicators (section 2.2.3.2.1) (Pyke *et al.* 2002) and Vital Landscape Attributes (section 2.2.3.2.2) (Aronson and Le Floch 1996), measure qualitative and quantitative indicators. These methods are designed to measure more than a single quantitative or qualitative attribute in the landscape in order to increase the accuracy of the assessment and to include functional and structural system components.

### 2.2.3.2.1 Rangeland Health Indicators

All ecological processes functioning in a landscape can be grouped into three ecological components: soil/site stability, hydraulic function and biotic integrity (Pellant *et al.* 2005). These components are called "attributes of rangeland health" as they are the primary components for monitoring rangeland health and will indicate if there are degradation or recovery tendencies. Monitoring such a broad system of components, however, is virtually impossible, especially if the intention is to monitor changes in the attributes on an annual basis as suggested by Herrick *et al.* (2005a).

There are biological systems which are present in a landscape which contribute to the health of each of the three rangeland health attributes. Monitoring these biological systems will indicate if, and where, there is degradation or recovery in the landscapes. These systems are referred to as rangeland health indicators.

There is a suite of 17 indicators which range from identifying water flow patterns to percentage bare ground and the size of gaps between vegetation. These indicators are easily measured and each indicator is used to determine the health of one or more of the rangeland health attributes. For example: bare ground and soil stability are quantitative measurements of the "soil/site stability" attribute, but bare ground can also be an indicator for "hydraulic function" as it affects water flow pattern and flow rate (Pellant *et al.* 2005).

The rangeland health indicators must be used in conjunction with each other in order to accurately assess the rangelands health (Pellant *et al.* 2005). No single indicator is sufficient to represent the complexity that exists in the rangeland ecology (Pellant *et al.* 2005).

Refer to the "technical reference for the interpretation of rangeland health indicators" by Pellant *et al.* (2005) (page 13) for a detailed description of the identified qualitative and quantitative indicator characteristics and how they relate to each of the three rangeland health attributes.

### 2.2.3.2.2 Vital Landscape Attributes (VLA)

The effect of a disturbance on the vegetation, soil properties or functional processes can be observed in the immediate area of the disturbance, within neighbouring vegetation unit, throughout the ecosystems or across the landscape. It is therefore imperative that the effect of the disturbance, and the recovery from the disturbance, is evaluated across the whole landscape. Consequently, VLAs have been developed to measure the functioning and the health of multiple ecosystems within a landscape and the ecotones between the affected landscapes (Aronson and Le Floc'h 1996). Because of the VLAs' sensitivity to environmental changes, and their adaptability to a range of landscape types, they can be used to monitor the success of rehabilitation projects and compare rehabilitation techniques used on different landscapes (Aronson and Le Floc'h 1996).

It is important to look at the change in system function at the "inter-ecosystem" scale as it is more likely that the sources and sinks of resource flow are identified and corrected (Aronson and Le Floc'h 1996). Additionally, at a landscape level several degradation causing anthropogenic factors, which are the predominant cause for landscape degradation and fragmentation, will be identified. These

degradation promoters (e.g. human populations, industries and economic potential) could have been overlooked if degradation assessment was isolated to a vegetation unit or ecosystem level (Aronson and Le Floc'h 1996).

Aronson and Le Floc'h (1996) describe the 16 VLAs that have been reduced and adapted from a list of 23 VEAs. These 16 attributes can be classified into 3 major components (1) structural and biotic composition (2) functional interactions among ecosystems (3) degree, type and cause of fragmentation and degradation. Refer to Aronson and LeFloc'h (1996) for a detailed description of these attributes.

*2.2.3.3 Evaluating the structure and function of the restored area*

Changes in the ecological processes in a landscape manifest themselves as changes in structural and functional components of the system. These changes in the system components only appear once the causing factor has been acting on the system for a long time. It may, therefore, be too late to act on the changes in ecological processes once physical changes are evident as the damage to the system function may already be severe. It is important, therefore, to constantly assess the tangible components in the area for the slightest change; changes in several components over a short period of time could act as a warning for a more serious transformation incidence (Pellant *et al.* 2005).

Two adjoining landscapes can have an affect on each other. The interactions within or between the landscapes are referred to as direct and indirect inter-rangeland relationships. Direct inter-rangeland relationships are when changes in one landscape directly impact on the landscape down slope (Pellant *et al.* 2005). An example of this is when there is a decrease in water run-off or soil erosion from an upslope landscape. The landscape at the base of the slope will be at a disadvantage as it would have normally benefited from the extra water and soil nutrient deposition. Indirect inter-rangeland relationships are when a biotic component is introduced to a landscape and it is the affects of that component on another that impact on the landscape down slope (Pellant *et al.* 2005). For example, if the stocking rate of an area is decreased there is an increase in the vegetative cover. Increased biomass utilizes more available soil moisture thereby decreasing the water runoff to the lower landscape. Likewise increased degradation of the upslope, due to overgrazing, will increase erosion and thereby increase soil deposition on the lower slopes (Fynn and O'Connor 2000).

The two primary methods currently used to evaluate the structural and functional progression of restoration projects are the Landscape Function Analysis (LFA) and Ecosystem Function Analysis (EFA). These two techniques are discussed in detail below (Section 2.2.3.3.1 and section 2.2.3.3.2 respectively).

*2.2.3.3.1 Landscape Function Analysis* (LFA)

Ludwig and Tongway (1997) developed the trigger-transfer-reserve-pulse (TTRP) model (Figure 2.5) as a conceptual model to better understand processes which influence landscape dynamics. An amalgamation of this model, other similar conceptual models and landscape and soil condition evaluation procedures led to the development of a nested hierarchical system (Tongway 2005). This system was the foundation for the development of the Landscape Function Analysis (LFA).

The LFA is a relatively simple landscape monitoring procedure using indicators similar to ones used in a VLA (section 2.2.3.2.2). The LFA monitoring procedure determines how well the ecosystem is functioning, how the landscape utilizes its resources, recycles its nutrients and retains its abiotic components. It is a rapid, simple, repeatable and adaptive management tool which managers are able to use to compare the relative impacts of different management strategies on the functioning of the system across different land types (Tongway and Hindley 2004, Ampt 2007).

The diagram shows boxes labeled: TRIGGER (driver), LOSSES, TRANSFER (process), RESERVE (patch), PULSE (response), connected by numbered arrows 1 through 6.

1. Run-on
   Storage capture
   Deposition
   Saltation capture
2. Germination & growth
   Uptake processes
   Nutrient mineralization
3. Run-off
   Sheet erosion
   Rill flow and erosion
   Wind erosion
4. Harvesting
   Fire
   Deep drainage
5. Seed pool
   replenishment
   Organic matter cycling
   Harvest by micro-fauna
6. Physical obstruction
   Absorption process

**Figure 2.5:** The TTRP (trigger-transfer-reserve-pulse) system framework developed by Ludwig and Tongway (1997). It represents the sequences of ecosystem processes and feedback loops. The corresponding descriptions are some of the processes which exist at locations in the framework (Tongway and Hindley 2004, Tongway 2005, Ampt 2007).

Determining the landscape organisation is the first step of the LFA and a map of the patches and inter-patches of the assessment area is produced (Tongway and Hindley 2004). For example, a patch can be defined as the position of a tuft of grass or the canopy cover of a tree, the inter-patch is the bare ground between the two. The second step is assessing 11 soil surface indicators for each patch and inter-patch (Tongway and Hindley 2004, Ampt 2007). The soil surface indicators can be extrapolated to determine the (i) stability or resistance of the area to erosion (ii) infiltration and water holding capacity and (iii) the level of nutrient cycling (refer to Table 2.1). These indicators were initially developed to quantify processes such as surface hydrology, erosion, crust formation and litter decomposition (Tongway and Hindley 2004).

**Table 2.1**: Soil surface indicators used in the landscape function analysis and the indices that can be derived from their assessment (Tongway and Hindley 2004, Ampt 2007)

|    | **Indicator** | **Derived indices** |
|----|---------------|---------------------|
| 1  | Soil cover | Stability |
| 2  | Basal cover or perennial grass & tree/shrub canopy cover | Infiltration & nutrient cycling |
| 3a | Litter cover | Stability |
| 3b | Litter cover, origin and degree of composition | Infiltration |
| 4  | Cryptogam cover (fungi, lichen and algae) | Stability & nutrient cycling |
| 5  | Crust brokenness | Stability |
| 6  | Erosion type and severity | Stability |
| 7  | Deposited material | Stability |
| 8  | Microtopography | Infiltration & nutrient cycling |
| 9  | Surface resistance to disturbance | Stability & infiltration |
| 10 | Slake test | Stability & infiltration |
| 11 | Soil texture | Infiltration |

The LFA data need to be collected at the extremes of the landscape health scale (degraded and pristine) as well as several sites which are considered to be of intermediate health. Landscape function analyses on the extreme end of the functional scale are required to produce a sigmoidal response curve in which the slope can be calculated. The slope of the sigmoidal curve will depict the robustness or fragility of the system; the landscapes ability to absorb stress and disturbances present in the system. A fragile landscape (Figure 2.6a) shows a rapid decrease in landscape function with a relatively small increase in stress or disturbance. A robust landscape (Figure 2.6b), however, does not reach a critical threshold (where the landscapes function begins to rapidly decline) until a substantial amount of stress or disturbance is present in the system.
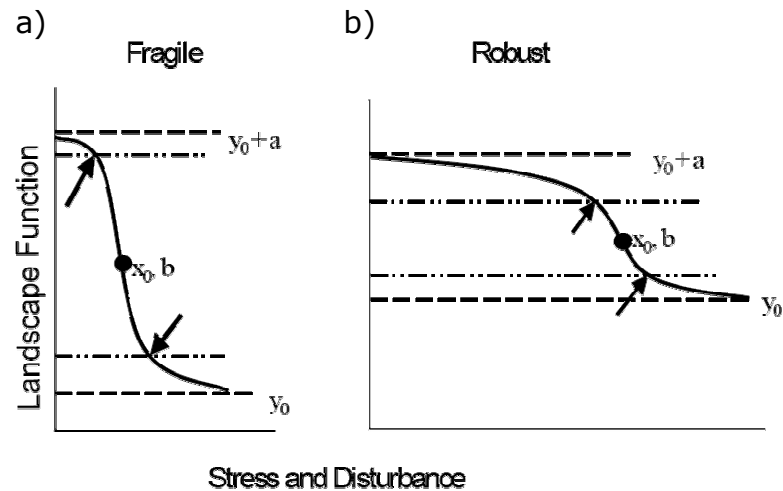
a) Fragile
b) Robust

**Figure 2.6:** Proposed response curves of fragile (a) and robust (b) landscapes to stress or disturbance. Critical thresholds of the landscape are identified by arrows. The slope 'b' is a sensitivity indicator of fragility or robustness. $Y_0+a$ represents the biological potential of the site and $Y_0$ is the limit of the system function under the existing stresses and disturbances. $X_0$ represents the time or space value reflecting 'self-sustainability' (Palmer *et al.* 2001, Tongway and Hindley 2004, Ampt 2007).

Equation 2.1 provides for the practical values that reflect the nature of the landscape. The slope (b) is a sensitivity indicator of fragility or robustness of the ecosystem, ($Y_0+a$) represents the biological potential of the site and $X_0$ represents the time or space value reflecting 'self-sustainability'.

$$y = (y_0+a)/1+e^{-(x-xo)/b}$$  **(Equation 2.1)**

Landscape function analyses have been used successfully on mine reclamation sites where the degraded area would represent the lowest asymptote of the response curve. The upper asymptote is the reference site for the rehabilitation project and acts as the 'target value' for the rehabilitation success (Tongway and Hindley 2004). If the LFA procedures are used over a period of time the extent to which the landscape recovers from the disturbance can be verified as the landscape function score will shift along the gradient of the slope towards the upper asymptotes (Ampt *et al.* 2003).

*2.2.3.3.2 Ecosystem Function Analysis (EFA)*

The ecosystem function analysis (EFA) has three inter-relating evaluation components: landscape function analysis (Section 2.2.3.3.1), vegetation and structure composition, and habitat complexity (Randall 2004). The strength of the EFA model is that it incorporates the principles of the widely accepted LFA with the assessment of the structural and biotic components of the landscape, thereby incorporating all the ecosystem components into one analysis. By doing so an ecologically sensitive evaluation technique has been developed, one which is known to present signs of rehabilitation failure before other evaluation methods (Randall 2004).

The vegetation and structural composition of an ecosystem indicates the health of several components of the system. The species present in the landscape and their relative growth rates can indicate which nutrients are present in the soil and possibly their abundance ratios. Vegetation structure can also suggest the presence of shelter and food for fauna (Randall 2004). For example: the presence of trees in the landscape indicates a possibility that roosting birds inhabit the area. Ecosystem function analyses demonstrate the potential for changes in the vegetative structure, species composition and the functional role of vegetation in the system (Tongway and Hindley 2004).

The third evaluation component, habitat complexity, uses biological indicators to evaluate the extent to which fauna can inhabit the landscape. As vegetation increases in complexity (size, composition and diversity) the number of sites and their suitability for inhabitation also increases (Tongway and Hindley 2004). It follows, therefore, that when fauna inhabit an area, and the number of faunal species increase, it is an indication of an increase of vegetation establishment and complexity. Habitat complexity indicators commonly used are: ants, reptiles and soil litter biota (Randall 2004).

### 2.3 Germination and seedling establishment

Seeds can succumb to one of four fates: they can either be preyed upon, die before germination, emerge, followed by death or emerge and persist to successfully establish immediately after abscission from the parent plant or after a period of dormancy (Kalisz 1991, O'Connor 1997). The germination of seeds, and the seedlings successful establishment, is influenced by several physical and environmental factors (Williams 1983).

Biological aspects of the seed which affect the germination time and emergence success are hard seed coats, after ripening and the need for aerobic environments (facilitated by the seed coat's physical properties) (Rose 1915). Environmental conditions such as frost, pathogens (Rose 1915) and light availability (Williams 1983) or physical conditions such as pathogens (Rose 1915), water availability (Oomes and Elberse 1976), soil temperature and nutrient levels (Williams 1983, Monaco *et al.* 2003) could also affect germination success.

Any change in these factors, even at a small scale, can influence the growth characteristics of the plant. Microvariations in the physical environment surrounding the seed (e.g. light availability) could influence changes in the plants leaf blade thickness, root structure and even flowering time. A change in any one of these morphological characteristics could influence the individual's growth and reproductive success (sexual and vegetative) (Silvertown 1980).

Studies have shown that a slight change in the light quality and/or temperature can affect the germination rate (Thompson *et al.* 1977), emergence success and seedling weight (Jutila and Grace 2002). Often these changes in environmental stimuli are brought about through a disturbance of the vegetative layer in the vicinity of the seed. When the vegetative layer above the seed is disturbed the light quality and soil surface temperature, and possibly a number of other factors, is altered, promoting germination. Disturbances on a landscape scale through fire, herbicide treatments or cultivation can induce a flush of seedlings (Wiseman *et al.* 2002) whilst small scale disturbances (e.g. selective grazing) can induce localised seedling establishment.

Jutila and Grace (2002) and Wiseman *et al.* (2002) studied the affect of different naturally occurring disturbances on the germination of monocotyledonous and dicotyledonous plants. Their results showed that more seedlings were present in areas where the vegetation was cut and removed (to simulate grazing) than the

areas where the vegetation was cut and not removed. Areas where vegetation was removed with the least disturbance to the soil (spraying vegetation with glysophate herbicide) resulted in the greatest number of seedlings and the seedlings with the greatest individual biomass. They concluded that vegetation covering the seed and the degree of disturbance when the vegetation is removed is a major factor in germination success and seedling establishment.

There have been several studies into the importance of the time at which germination occurs. Germination early in the growing season is thought to give a seedling a competitive advantage since it experiences an extended growing period which results in a larger seedling (Fowler 1986), higher survival probability and a higher reproductive success (Ross and Harper 1972). Fowler (1986) demonstrated that the adult plants of early germinating *Themeda triandra* seeds were larger than late germinating *T. triandra* plants after the first season's growth. The increased growth was attributed to an extended growing season. Conversely, O'Connor (1997) and Fowler (1986) suggest that seedlings which emerged early may be susceptible to high mortality rates as the "optimal germination cues" received by the seed may have occurred during a "warm spell" in the dormancy season. Early germination may also expose seedlings to increased grazing pressure through selective grazing (Klink 1996) as seedlings are more palatable than mature plants. If, however, the seeds germinate at approximately the same time then all the seedlings would be exposed to relatively equal grazing pressure.

Williams (1983) indicated that the species which are least sensitive to environmental stimuli such as light and temperatures, were least likely to form a persistent seed bank compared to species that have a narrow range of germination stimuli. If the specific germination requirements are not met in a particular season the seeds are incorporated into the seed bank and lay in a state of dormancy until the required environmental stimuli are present.

There is a range of physiological and environmental factors which can impose dormancy onto a seed. Dormancy types, and methods used to break dormancy in seeds, are discussed below (section 2.3.1). Thereafter physical and environmental factors which affect germination and seedling establishment are discussed (section 2.3.2 and 2.3.3 respectively).

**2.3.1 Dormancy**

Seed dormancy is present in most plant groups, including grasses, and has been described by Bradford and Nonogaki (2007) as a physiological state in which metabolically active seeds do not progress to germination and growth, even after imbibition. Complicated interactions and feedback loops of hormones (auxins, gibberellins, cytokinins, etc.), genes, enzymes and proteins contribute to the initiation and breaking of dormancy (Bradford and Nonogaki 2007).

*2.3.1.1 Types of dormancy*

There are several opinions surrounding the classification of dormancy type, many of which contradict each other. For example, Harper (1959) argued that seeds experience "enforced dormancy" when they are prevented from germinating due to environmental conditions (low moisture levels, available light intensity and wavelength etc). Baskin and Baskin (1998) argued that this is not a state of dormancy but "non-dormancy". They argued that delayed germination due to adverse environmental conditions is not physiologically the same as being in a state of dormancy.

The most widely accepted definitions of dormancy are that of Nikolaeva (1977) where dormancy is split into two primary categories: endogenous and exogenous dormancy. These deal with properties controlled by the embryo (endogenous dormancy) and those which result from properties of the endosperm and tissues of the seed or fruit (exogenous dormancy). Silvertown (1999) describes a range of dormancy types which are associated with endogenous and exogenous dormancy. Endogenous dormancy can be:

1. physiological dormancy, for example the inability of the embryo to break through the protective seed envelope,
2. morphological dormancy which is due to an undeveloped embryo that will mature once the seed is abscised from the parent plant or
3. a combination of physiological and morphological dormancy known as morphophysiological dormancy.

Exogenous dormancy is either, or a combination of:

1. physical dormancy  primarily influenced from an impermeable seed coat,
2. chemical dormancy induced by chemical germination inhibitors or

3. mechanical dormancy which is brought about by factors that decrease the potential for the seeds and embryo growth e.g. hard, woody exterior to the seed.

In some cases there are subdivisions of each of these dormancy types. Nikolaeva (1977) breaks physiological dormancy into deep, intermediate and non-deep categories in accordance to what is required to break the dormancy. Simpson (1990) summarized the above classifications into three types: Endo-dormancy which is regulated by physiological factors inside the seed, para-dormancy is regulated by physiological factors outside the seed and eco-dormancy is regulated by environmental factors.

### 2.3.1.2 Breaking seed dormancy

There are several chemical and mechanical mechanisms that can be employed to break seed dormancy. The majority of these techniques are used to break the hard seed coat so that oxygen and water can come into contact with the embryo, thereby promoting germination. Dormancy induced by other factors such as chemical and physiological factors (e.g. an underdeveloped embryo) is not as readily broken. Often a period of storage, commonly referred to as after-ripening, is required before the dormancy can be broken.

Seeds which imbibe the same volume of water with and without the seed coat do not have a physiologically hard seed coat, even if the outer coat of the seed seems physically hard (Rose 1915). Seed hard-coatedness can be broken by mechanical and chemical treatments. Mechanically breaking the seed coat requires the application of the right amount of pressure to the seed to ensure that the seed coat is damaged (cracked) without damaging the embryo or cracking the seed (Rose 1915). The most common methods for damaging the seed coat are scrubbing, cutting off the tip of the seed or using high pressured air or sand to scarify the seed coat surface. Alternatively, seeds can be soaked in chemicals which will soften the seed coat without causing mechanical damage. Chemicals which have been used include sulphuric acid, ether, chloroform, sodium and potassium hydroxide (Rose 1915). The disadvantage of this method is the chemicals could kill the embryo if they seep through weakened areas of the seed coat and into the embryo.

Seeds which are not physiologically ready for germination after abscission from the parent plant need to undergo a period of after-ripening. During this period of dormancy physiological and chemical changes occur within the seed, for example the lengthening of the seeds hypocotyl may take place (Eckerson 1913, Adkins *et al.* 2002). During post-abscission hypocotyl lengthening the seed will remain dormant, even if water is imbibed, and will only germinate once the hypocotyl has increased to a predetermined length (Eckerson 1913, Adkins *et al.* 2002). Silvertown (1999) suggests that there is a need for the embryo to "strengthen" so that the young hypocotyl can break through the seed coat.

In grasses the scenario is similar. Many grasses fail to germinate at first wetting or they germinate slowly, depicting a liner increase in germination percentage over time (Gordon 1973, Allen *et al.* 1994). After-ripening dormancy can often be broken by hydration after a period of dry storage (Allen *et al.* 1995), which may be a short event (for example *Bromus tectorum* required 1 weeks storage (Thill *et al.* 1980)) or could be for an extended period of time (longer than 5 months (Milby and Johnson 1987)).

## 2.3.2 Physical factors affecting germination and seedling establishment

Structural components of the ecological system which influence the germination rate and seedling growth are known as physical factors. It is these structures that shape the micro-environment in which the seed germinates and which determines the seedling's survival probability. The physical factors act on the immediate microenvironment that the seed is exposed to. For example, soil structure affects the water holding capacity of the soil, which in turn affects germination.

The physical factors which can alter the time to germination are: the microsite in which the seeds germinate (Harper *et al.* 1961), depth of sowing (Cole *et al.* 2000) and litter cover (Jutila and Grace 2002). Several of these factors have correlating environmental stimuli which are discussed in section 2.3.3.

### 2.3.2.1 Microsites

Microsites are the immediate area in which the seed germinates. The quality of the microsite, measured as germination potential, depends on a variety of environmental and physical factors such as microtopography (McWilliam *et al.*

1970, Fowler 1986), aerial cover from surrounding vegetation, litter cover (Fowler 1986, Jutila and Grace 2002) and water content. The age of the biological soil crusts has also been shown to affect the emergence and establishment success of annual and perennial species (Langhans *et al.* 2008).

O'Connor (1997) found that the microsite influences the seed bank dynamics of *Themeda triandra* suggesting, in support of Harper's (1977) findings, that seedling success depends on the microsite in which it emerges and grows. These "safe germination sites" are species specific (Harper *et al.* 1961) and in a given area there may be an assortment of microsites suitable for the germination and seedling establishment of a variety of species. Fowler (1986) concluded, through experimentation, that the specific requirements of a species for successful germination in the microsite may be responsible for some of the plant species diversity present in natural communities. Further discussion of the specific requirements of seeds from the microsite (light, water, nutrients, oxygen and temperature) is presented in section 2.3.3.

### 2.3.2.2 Sowing depth

Black (1955) found that the only effect that depth of sowing had on the emergence of *Trifolium subterraneum* seeds (weighing approximately 7.91mg) was time of emergence and the pre-emergence weight. Seeds sown at 1.3cm from the soil surface emerged first, followed by those sown at 2.5cm, followed by seeds sown at increasing depths. The primary difference observed in seedling structure between the treatments was an increase in the hypocotyl length (and a corresponding pre-emergence weight) with an increase in sowing depth.

The preferred depth of sowing is highly dependent on the species that are present and the site condition (Cole *et al.* 2000). Seeds of most species have a preferred sowing depth of 5 – 10mm but larger seeds may tolerate sowing depths up to 25mm (Cole *et al.* 2000) due to a higher cotyledonous nutrient reserve.

Miss-timing the emergence event with the environmental conditions could be more detrimental to seeds sown deeper in the soil. Seedlings germinating near the soil surface have a greater cotyledonous resource reserve to assist in the seedling establishment if it emerges in unfavourable conditions. The seed deeper in the soil would use more of the cotyledonous reserves growing to the surface and may

have insufficient reserves to establish if they germinated in unfavourable conditions, decreasing the probability of survival.

*2.3.2.3 Litter cover*

Litter cover, which can consist of leaves, branches or other organic materials resting on the soil surface can change the environmental conditions at the microsite and, therefore, germination success (Jutila and Grace 2002). Julia and Grace (2002) demonstrated that, even though litter cover on the soil surface had little affect on the light penetration into the soil, there was a significant decrease in the average number of germinated seeds with an increase in litter cover. Further experimentation showed, however, that there was a decrease in seedling mortality with increased litter cover (Fowler 1986). The decreased germination rate with increasing litter cover could be attributed to a decrease in light quality, not quantity and decreased diurnal soil temperature fluctuation. Decreased seedling mortality could be influenced by increased soil moisture content (less soil water evaporation) and decreased seedling competition. The above mentioned attributes (i.e. water availability, diurnal temperature fluctuations and light quality) and their effects on germination and seedling establishment are discussed in sections 2.3.3.1, 2.3.3.3 and 2.3.3.4 respectively.

## 2.3.3 Environmental factors affecting germination and seedling establishment

Environmental factors which influence the germination of seeds and seedling establishment are a combination of the climatic conditions in the landscape and the resources which are available to the seed. Subsequently, the impact of these environmental factors on the seed and seedling can occur at a micro-scale (within the microsite) or at a landscape or ecosystem scale (e.g. seasonal fluctuations).

The primary environmental factors which influence germination and seedling establishment are water (McWilliam *et al.* 1970, Oomes and Elberse 1976), nutrient availability (Roberts 1960, Campbell 1968, McWilliam *et al.* 1970, Bisigato and Bertiller 1999), temperature (Morinaga 1926, Williams 1983, Probert *et al.* 1986, Silvertown 1999), light availability and quality (Morinaga 1926, Silvertown 1980), exposure to the butenolide compound present in plant derived smoke (Baxter and Van Staden 1994, Dixon *et al.* 1995, Drewes *et al.* 1995, Sparg *et al.*

2005, Van Staden *et al.* 2006) and the presence of pathogenic fungi in or on the seed (Kirkpatrick and Bazzaz 1979).

### 2.3.3.1 Water availability

The volume of water available to the seed for imbibition is dependent on the matric potential of the soil. The lower the matric potential the more energy is required to move the water particles from the soil particles into the seed (Oomes and Elberse 1976) decreasing the germination rate and percentage (McWilliam *et al.* 1970). The rate of water imbibition is also influenced by the surface area of the seed in contact with water particles. Large seeds sown in a sandy soil (large soil particles) will have a small surface area in contact with the soil, and therefore less soil water will be imbibed than if sown in finer clay soil where the soil particles are closely packed around the seed (Oomes and Elberse 1976).

Oomes and Elberse (1976) found that higher germination success occurs in seeds sown close to the soil surface compared to those sown in protected sites (e.g. sites which are covered with organic litter). At the soil surface there is an increased rate of water loss and a greater fluctuation in water availability which affects the consistency of the water imbibition rate. Irregular water loss and absorption alters the seeds rate of shrinking and swelling (Oomes and Elberse 1976) which may cause sufficient weakening of the seed coat for physical changes to occur (e.g. cracking). Germination success increases as weakened seed coats allow oxygen, which is required for germination to take place, to enter the embryo (Morinaga 1926, Raven *et al.* 1976).

In general, it is essential that a seed is exposed to water for a minimum of two days before germination can occur. There should be sufficient soil moisture to promote seedling growth, without threat of desiccation, until the rootlet is capable of taking up water. Too much soil water, however, will decrease germination success as the rate of gaseous exchange is hampered.

### 2.3.3.2 Nutrient availability

In general, nutrient and mineral resources from the seed's immediate environment have no affect on germination rate or germination success (Bisigato and Bertiller 1999, Monaco *et al.* 2003). It is only once the seed has germinated and the seedling has depleted the resources available from the seed's endosperm

(carbohydrates, protein and minerals) that the external resources become essential for the seedling's growth and establishment (Bisigato and Bertiller 1999). Should the seed germinate in sub-optimal conditions the seedling would rely more heavily on the endosperm's resources for its initial establishment.

Seedlings are able to utilize resources from the micro-environment within days of germination and the availability of nitrogen (Roberts 1960, Campbell 1968), phosphorus and mineral nutrients such as calcium, potassium and magnesium are essential for seedling development (McWilliam *et al.* 1970). Seedlings from surface germinating seeds will have increased initial growth rates as the seedling can extract resources from the environment and the endosperm concurrently. Seedlings which require the majority of the endosperm reserves to grow to the soil surface will initially have a decreased growth rate after emergence. The seedling will rely on the photosynthetic capabilities of the newly emerged leaves to enable the development of a primary root system which is required before the secondary above-ground vegetative development occurs.

Nutrient levels and soil organic matter content is related to the amount of aerial vegetative cover (Bisigato and Bertiller 1999) because with aerial cover there is an increased rate of organic deposition. A varying plant cover in an area could contribute to the development of soil microhabitats and uneven distribution of nutrients. In arid environments there are patches where there is permanent vegetation and inter-patches that only have vegetative cover after a rainfall event. These inter-patches promote alternative habitats for those species which are poor competitors for soil nutrients. These species have adapted to areas of low resources (Bisigato and Bertiller 1999) and once a resource pulse has occurred they germinate, grow and mature at a rapid rate in order to reproduce in a limited time frame.

In summary, nutrients are essential for the growth and development of seedlings but the response to changes is resource availability is species specific. For example, annuals are sensitive to low levels of nutrients as the growth rates are faster and require more nutrients per unit time. Perennials, on the other hand, grow slowly and conserve the available resources more effectively.

*2.3.3.3 Temperature*

There is a variable, species specific response of seeds to temperature as a germination cue; consequently there are several definitions for "the optimal germination temperature". In 1932 Edwards reviewed several definitions of optimal germination temperature, many of which are still regarded as the most accurate, most widely applicable definitions. These are:

1. The upper limit of a range of temperatures with the same final percentage germination than when tests are continued for extended periods of time.
2. The temperature yielding the highest germination after a set time period.
3. The temperature at which the least time is required before the first or the last seedling appears.
4. The temperature at which the greatest seedling height (or seedling vigour) is achieved after a set period for seed germination and subsequent seedling growth.
5. The temperature with the least average incubation time. This method takes the whole seed sample into account.
6. The temperature at which the mean rate of seedling growth and final germination percentage is highest.

The most popular definition of optimal temperature today is a combination of definition 2 and 5 i.e. the temperature that gives the highest germination percentage in the least amount of time. One situation that is not discussed in these definitions is when alternation of temperatures is required to achieve greatest germination success. Alternation of temperature occurs with the diurnal fluctuations corresponding to the day and night temperature regime that the majority of plants have adapted to.

Several studies have demonstrates that alternation of temperatures is a primary influencing factor in germination (Morinaga 1926, Williams 1983, Probert *et al.* 1986, Silvertown 1999) but it is a predominantly species specific response which is dependent on the species life history. For example: the optimal temperatures for British grass seeds is between $9^oC$ and $12^oC$ alternating with a 12 hour exposure to $20^oC$ (Williams 1983). African species such as *Cynodon dactylon* germinate best under alternating temperatures of $10^oC$ and $38^oC$, $10^oC$ and $32^oC$ or $15^oC$ and $38^oC$ (>80% germination). *Poa compressa*, on the other

hand, showed little variation in response to the alternation of temperature treatments (Morinaga 1926).

In general, the minimum time of the seeds exposure to either the high or low temperature is between 4.5 and 8 hours. There is little change in the germination success if the high or low temperatures exposure was between 6 and 18 hours (Morinaga 1926). Probert *et al.* (1986) found that the 85 species they studied showed a marked increase in germination when the temperature cycles comprised of a 16 hour cool phase and an 8 hour warm phase. They also noticed that the optimum amplitude of the temperature (change between the low and high temperatures) was narrow, with different optimums expressed between species. For example, when the diurnal temperature changes were greater than $10^{o}$C the rate and percentage of germination was less than when there was an amplitude change of less than $10^{o}$C.

The ecological significance of alternation of temperatures is three-fold: Firstly, it aids in the incorporation of the seed into the seed bank (Williams 1983). If the diurnal temperature fluctuations are not optimal for germination then dormancy will be enforced and the seed will be incorporated into the seed bank during the following growing season. Secondly, a requirement of diurnal temperature fluctuations for germination may be a 'gap-detecting mechanism' (Williams 1983). There will be a greater diurnal temperature fluctuation when there are gaps formed in the canopy vegetation layer than when there is continuous aerial cover. An increase in the amplitude of the alternating temperatures will, therefore, indicate the formation of a gap or a disturbance in the vegetative layer above the seed. Thirdly, seasonal temperature changes can be detected as the time of exposure to the hot and cold cycles (Van Assche and Van Nerum 1997) and the change in the heating and cooling time will change with season. For example, in the summer months there is a faster heating and slower cooling rate and there is an increased time in the hot cycle as there are more daylight hours.

Morinaga (1926) demonstrated that, in some instances, the need for alternation of temperature is removed if the seed coat is broken. The alternation of temperatures may, therefore, promote seed coat weakening making it more susceptible to cracking when the embryo swells or water is imbibed.

Totterdell and Roberts (1980) identified nine attributes of diurnal temperature cycles which could influence germination. These are:

1. The number of cycles of alternation of temperatures (number of hot-cold cycles).

2. The amplitude (range) of the fluctuations of temperature.

3/4. Value of the upper and lower temperature (actual temperature not the range of temperatures).

5/6. The time spent at each temperature within each cycle.

7/8. The time taken for the seed to cool or to warm with each temperature cycle.

9. The time of the cycles from the start of the seeds imbibition.

### 2.3.3.4 Light availability and quality

Light is a primary germination cue for a majority of plant species (Morinaga 1926, Silvertown 1980) but the quality (wavelength and intensity) and the quantity of light which is required for germination is species specific (Silvertown 1980, Williams 1983). For example, some species may require exposure to light for a few seconds (Wesson and Wareing 1969) whilst others require a prolonged or specific photoperiod (Black and Wareing 1954, Cumming 1963).

Leafy vegetation absorbs light wavelengths required for photosynthetic processes but reflects far-red light and is, therefore, the only light wavelength available to seeds covered by dense vegetation. Far-red light is a germination inhibitor (Williams 1983) and imposes leaf-canopy induced dormancy (Silvertown 1980) onto grass seeds. Leaf-canopy induced dormancy is relatively common in plants and was shown to affect six of seven annual species and 11 of 20 perennials when tested by Silvertown (1980). Five of the nine perennials that did not show leaf-canopy induced dormancy were the five heaviest seeded perennial species. All but two of the small seeded perennials showed leaf-canopy induced dormancy demonstrating that smaller seeds are more sensitive than large seeds to shading.

There is some evidence that the colour of the seed coat has an influence on the sensitivity of the seed to changes in light wavelengths (Silvertown 1980). Of the smaller seeds studied by Silvertown (1980) the two species which did not show leaf-canopy induced dormancy had a black seed coat, which may be impermeable to far-red light.

A gap in the vegetative layer (indicated by and increase in the light quality and quantity available to the seed) breaks the leaf-canopy induced dormancy if there are no additional germination inhibitors acting on the seed. For example: The differences in light quality experienced by seeds buried in soil or covered by vegetation could be viewed as complimentary dormancy inducers (Silvertown 1980) and are mutually exclusive. A seed on the soil surface that is experiencing leaf-canopy induced dormancy will not germinate if it is incorporated into the soil (where light wavelength and intensity changes) and if there is no evidence that there is a gap in the vegetative canopy (Silvertown 1980). In other words; dormancy will not be broken, even though the dormancy stimulus has changed, unless there is a positive environmental stimulus showing that the environmental conditions are optimal for seedling establishment.

*2.3.3.5 Plant-derived smoke as a germination stimulus*

Fire has been the historical driving force behind the development of the natural biodiversity of South African grasslands. There has been an evolutionary relationship between fire, the effects of fires on the landscape and common grasslands species. It was often noted that after a fire there is a flush of seedlings of grasses (Blank and Young 1998) and *Acacia* species (Blank and Young 1998) as well as a general increase in the landscape health and functionality. It was thought, and correctly so, that the seedling flush was brought about by an increase in light availability, a nutrient pulse and heat shock. Subsequently it was determined that the seedling vigour of post-fire germinated seeds was greater than that of opportunistic seedlings (when the seeds germinated without a fire event). Additionally, the post-fire germinating seedlings showed signs of de-etiolation, which is a reduced rate of shoot elongation, expansion of true leaves and the development of mature chloroplasts (Symons and Reid 2003). These observations encouraged the investigation into additional germination inducers which would act on a seed during, or after, a fire.

3-methyl-2H-furo[2,3-*c*]pyran-2-one is a butenolide compound which has been isolated from plant-derived smoke (Van Staden *et al.* 2004). It promotes germination by breaking hard seed coat dormancy, stimulates preliminary physical germination processes (Harker 1959, cited in Kulkarni *et al.* 2007), and increases seedling vigour (Blank and Young 1998, Sparg *et al.* 2005, Van Staden *et al.* 2006,

Kulkarni *et al.* 2007). Plant-derived smoke (both in an aerosol and aqueous form) is now widely recognised as a germination cue for both fire-dependent and fire independent species (Baxter and Van Staden 1994, Dixon *et al.* 1995, Drewes *et al.* 1995, Sparg *et al.* 2005, Van Staden *et al.* 2006).

Thomas *et al.* (2007) discussed the correlation that is evident between heat shock (when a seed is exposed to intense heat from fire) and plant-derived smoke and how the interaction of these factors promote germination. They examined the response of 16 species from the Ericaceae and Myrtaceae families which are common in fire-prone regions of Australia. In some species the smoke/heat interactions are independent of each other but in other instances both smoke and heat are needed to promote germination. In general, there is no significant response of seeds to heat in the absence of smoke but there are some instances where smoke is not required (Blank and Young 1998). In some instances the combination of smoke and heat were inhibitory, decreasing germination levels below that of the control (Thomas *et al.* 2007).

Depending on the characteristics of the fire, seeds will be exposed to different temperatures for different durations. Hot, slow fires will heat the soil much more than a fire that moves quickly through the vegetation with limited effect on the soil temperature. The seed's burial depth also influences the extent of the heat shock (Thomas *et al.* 2007). If seeds are buried above the optimal depth they are exposed to high temperatures which could be lethal. Seeds which are buried too deep may not experience heat shock at all (Thomas *et al.* 2007).

Jager *et al.* (1996) showed that the active butenolide components of smoke are volatilized (as smoke) between $160^{o}C$ to $200^{o}C$, and these compounds are captured when the smoke is incorporated into water. Smoke-infused water can be stored in an aqueous solution for long periods of time (>12 months) without losing its efficacy (Drewes *et al.* 1995, Van Staden *et al.* 2000)). The compound is naturally stored in soil (Roche *et al.* 1995) as it may bind to soil particles (Keeley and Fotheringham 1998) and can be absorbed into charred wood from which it can be released at a later stage (Keeley *et al.* 1985).

The butenolide compound is highly active and is effective at very small concentrations (Van Staden *et al.* 2004). Dilutions between 1:100 and 1:1000 of a particular batch of smoke-infused water have had significant stimulatory effects on a range of species (Drewes *et al.* 1995, Light *et al.* 2002, Light and Van Staden

2003, Van Staden *et al.* 2004, Kulkarni *et al.* 2007). There is a minimum threshold level of butenolide that the seed must imbibe in order for the optimal germination response (Light *et al.* 2002). Tests on Grand Rapids lettuce seeds, on which a substantial amount of work on the properties of plant-derived smoke has been carried out, showed that highly diluted smoke extract with a concentration of 1:1000 could be used when imbibing the seeds for periods up to 6 hours. The smoke-infused water concentration could be increased to 1:100 and the imbibition time decreased to about 1 hour for the same response (Light *et al.* 2002). Constant exposure of the seed to the extract at high concentrations could lead to some germination inhibition which could be negated by rinsing the seeds with distilled water, even after 24 hours of imbibition (Light *et al.* 2002). The stimulatory effect that the butenolide compound has on seeds is permanent (Baldwin *et al.* 1994, cited by Blank and Young 1998), that is, it is not possible to remove the germination cue from the seed.

Jager *et al.* (1996) showed that batches of smoke-infused water, which were produced from a variety of species, using plant tissue and leaf material, either stimulated and/or inhibited the germination of the light-sensitive Grand Rapids lettuce seeds. Even smoke from carbon based tissue paper was shown to promote germination suggesting that the active butenolide compound is found throughout nature (Jager *et al.* 1996) and the origin of the smoke may not matter. However, Baxter *et al.* (1995) showed that *Themeda triandra* did not show the same response when exposed to smoke derived from 27 different species. This implies that not all species produce the smoke compound with the same potential to stimulate germination.

Kulkarni *et al.* (2007) showed that *Acacia* seeds treated with smoke-infused water produced seedlings with increased the shoot and root ratios, seedling vigour and seedling mass when grown in the dark. There was, however, limited success when the seeds were exposed to permanent light or alternating light and dark periods. Likewise, Van Staden *et al.* (2006) found that there was a significant increase in the growth of tomato, okra and bean seedlings when grown in complete darkness after treatment with smoke-infused water. But, seedlings that were exposed to a 16:8h light/dark cycle did not have a different germination rate and percentage than the control. Both authors concluded that there may be a negative interaction between butenolide and light; similar to the affect that light has

on the root stimulatory properties of auxin. The decrease in smoke affect under light conditions could pose a problem when working with pre-treated seeds as storage and germination would have to be in complete darkness, which is not always practical.

The smoke affect was tested on a variety of grasses of the sagebrush-steppe (USA) and there was some evidence that the height of grass seedlings was increased when treated with aerosol smoke and smoke-infused water (Blank and Young 1998). The only tests carried out on South African grasses was by Baxter *et al.* (1994) who looked at the effect of smoke treatments on *Themeda triandra.* An increase in germination was shown when treated with aqueous smoke but further tests showed that exposure to smoke from different species did not have a consistent affect on *T. triandra*'s germination percentage. *Themeda triandra* seeds treated with *Aristida junciformis* smoke showed the highest increase in germination percentage (a germination increase of 11%). There was no significant response of the *T. triandra* seeds to the smoke from 8 of the 27 species.

Van Staden *et al.* (2006) investigated the possibility that this butenolide compound could be used as a priming agent for maize. Maize showed to have a greater root mass when treated with smoke-infused water after eight days growth inferring an advantage onto the seedlings growth rate. Potentially, pre-treatment of grasses intended for rehabilitation with smoke compounds could be invaluable. The seeds will germinate more readily and the seedlings will be more vigorous and the combination of these factors could promote restoration. Several other aspects regarding the seedlings response to smoke, such as the optimal concentration of the smoke compound, if heat shock is required and the effect of increased vigour on the nutrient requirements of the seedling, need to be determined in pre-restoration trials.

### 2.3.3.6 Pathogenic Fungi

Pathogenic fungi are found both on the exterior and interior of a seed and significantly affect the seed germination rate and seedling growth when present in abundance. Some plants produce antifungal chemicals as a defence against the fungi, decreasing the pathogen abundance on the seed and decreasing the plants mortality (Kirkpatrick and Bazzaz 1979).

Seed fungi causes a variety of abnormalities in the seedling's growth if the seeds were exposed to certain fungi during the germination stage (Kirkpatrick and Bazzaz 1979). If the seedlings are infected with fungi after germination the growth abnormalities are limited, and the disturbance in the seedling growth process decreases with an increase in the seedlings age when infected (Kirkpatrick and Bazzaz 1979).

## 2.3.4 Summary of the response of seeds and seedlings to environmental and climatic conditions

Tables presenting the interactive effects of environmental and climatic factors on the germination success (Table 2.2), seedling survival probability and growth rates (Table 2.3) are presented below. These tables are summaries of the information available in the literature. They are a means to identify the stimuli which, according to the above literature, are most important for seedling survival and growth.

Table 2.2 indicates that the period of after ripening of the seed, light availability and wavelength, soil temperature and water availability play important roles in the germination of seeds as these factors have a positive relationship with a variety of other factors. Table 2.3 indicates that light, nutrient, water availability and ambient temperature are the most important factors in the seedling's survival and growth rates.

The effect of the butenolide compound, which is found in plant-derived smoke, is the only factor that does not have a clearly defined positive or negative influence on germination or seedling survival and growth. The ambiguity around the effect of the butenolide compound on seeds and seedlings could be an indication of a strong species specific response. Identifying the extent of the species specificity can only be achieved through further research.

It would make sense to study the affect of the most influential environmental factors on the growth and competitive ability of grass seedlings. Studying factors that have a limited affect on germination, survival and growth of the seedling will only tell us limited information about the seedlings ecological response to competition. Similarly, only factors which can be manipulated in experimental and natural conditions should be considered when investigating aspects of seedling ecology. Should the factor being investigated be difficult to control under

experimental conditions there is a possibility that the results could be inaccurate. Additionally, the duplication of the experimental conditions under natural conditions may be unrealistic. Therefore, the effects of nutrient and light availability will be studied in the following seedling competition experiments (Chapter 4 and 5). Additionally, the effect of plant-derived smoke on the germination rate and success and seedling growth rate of South African grasses will be investigated (Chapter 6).

**Table 2.2**: Interactive effects of factors shown to influence the germination of seeds for a variety of species. An increase in the factor can lead to an increase (+), decrease (-), variable response (V) or an ambiguous response (A) in germination success.

| | General response | Aerial cover | Aerobic conditions | After ripening | Alternation of temperature | Butenolide compound | Frost | Hard seed coat | Light availability | Light wavelength | Litter cover | Micro topography | Nutrient availability | Pathogens | Soil crust | Soil structure | Soil temperature | Sowing depth | Water availability |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Aerial cover | - | | | - | | | - | | - | - | - | | A | | | | - | - | - |
| Aerobic conditions | - | | | A | - | A | | - | | | - | | A | | | | | | - |
| After ripening | + | | | | V | A | | + | + | + | + | | + | | | | + | + | + |
| Alternation of temperature [1] | V | | | | | A | | + | + | + | | | + | | | + | V | | |
| Butenolide compound [2] | A | | | | | | A | A | - | - | A | | A | | | | A | A | A |
| Frost | - | | | | | | | | | | | | | | | | - | | |
| Hard seed coat | - | | | | | | | | | | | | | | | | + | | + |
| Light availability | + | | | | | | | | | + | | | + | | | + | + | | + |
| Light wavelength | + | | | | | | | | | | - | | + | | | | + | | + |
| Litter cover | - | | | | | | | | | | | | + | | | | | - | A |
| Micro topography [3] | A | | | | | | | | | | | | + | | - | + | | | + |
| Nutrient availability [4] | A | | | | | | | | | | | | | - | - | + | + | + | + |
| Pathogens | - | | | | | | | | | | | | | | | | | | |
| Soil crust | - | | | | | | | | | | | | | | | | - | - | - |
| Soil structure [3] | A | | | | | | | | | | | | | | | | + | + | + |
| Soil temperature [1] | V | | | | | | | | | | | | | | | | | | + |
| Sowing depth | - | | | | | | | | | | | | | | | | | | - |
| Water availability | + | | | | | | | | | | | | | | | | | | |

[1] variable response between species

[2] contradictory results presented by various authors

[3] dependant on degree of factor

[4] contradictory results presented by various authors, some variation may be due to species specific responses

**Table 2.3**: Interactive effects of factors shown to influence seedling survival and growth for a variety of species. An increase in the factor can lead to an increase (+), decrease (-), variable response (V) or an ambiguous response (A) in germination success.

| | General response | Aerial cover | Ambient temperature | Butenolide compound | Frost | Light availability | Litter cover | Nutrient availability | Pathogens | Sowing depth | Time to germination | Water availability |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Aerial cover | - | | + | A | - | | - | A | - | - | V | + |
| Ambient temperature [1] | + | | | + | | + | + | + | - | V | + | + |
| Butenolide compound [2] | A | | | | A | - | A | A | A | A | A | A |
| Frost | - | | | | | | + | + | - | + | + | - |
| Light availability | + | | | | | | + | + | | + | + | + |
| Litter cover | - | | | | | | | + | - | - | - | + |
| Nutrient availability [1] | + | | | | | | | | | + | + | + |
| Pathogens | - | | | | | | | | | - | - | - |
| Sowing depth | - | | | | | | | | | | - | + |
| Time to germination [3] | V | | | | | | | | | | | + |
| Water availability | + | | | | | | | | | | | |

[1] can vary considerably depending on species adaptions to the environment
[2] contradictory results presented by various authors, some variation may be
   due to species specific responses
[3] variable response between seasons and management practices

**Chapter 3: Germination of South African veld grass species: factors affecting species suitability for rehabilitation**

**3.1 Introduction**

There are several factors which influence the germination rate and germination percentage of seeds with the most obvious being light intensity and availability (Williams 1983), water availability (Oomes and Elberse 1976) and temperature (Williams 1983). There are several additional factors, such as fluctuation and range of temperatures experienced (Morinaga 1926, Williams 1983, Probert *et al.* 1986, Silvertown 1999), heat through fire (Williams 1983, Morrison *et al.* 1998), fungal activity (Rose 1915) and, in some instances, availability of nutrients. Dormancy (or a period of after ripening) is also important for a number of species as delayed germination may increase the seedling's survival probability and growth rate (Fowler 1986, Klink 1996, O'Connor 1997).

Understanding the environmental factors which affect germination will increase the ability to make accurate assumptions of seed germination success in a restoration area. Furthermore, understanding seed dynamics allows for careful selection of the abundance ratios seeds should be planted at in order to obtain the required seedling densities and final community structures. Such information will increase the efficiency and success of the rehabilitation process (Gibson-Roy *et al.* 2007). For example, if a hypothetical community composition is intended to have a 50/50 mix of two species, and one species exhibits a 50% lower germination rate than the other, that species should be sown at a higher density (a ratio of 2:1) in order for the final seedling ratio and community structure to be correct.

Low germination success is not uncommon in grass species. Gibson-Roy *et al.* (2007) found that two thirds of 64 grassland species tested showed less than 50% germination. Fowler (1986) on the other hand had more success when evaluating the germination of three grass species on a variety of soil micro-conditions. Two species had greater than 60% germination whilst one species had less than 10% germination after a 130 day trial. Six of ten grass species tested by Blank and Young (1998) demonstrate a germination rate of below 50%, and an additional two species had less than 60% germination. The germination percentage of *Themeda triandra* seeds from the montane grasslands of KwaZulu-Natal, South Africa is dependent on the age of the seed, as this affects the seed viability, and

the germination treatments (Everson *et al.* 2009). Germination was generally low in *T. triandra* seeds, the most favourable germination treatment being germination in an alternating light/dark environment (36%), followed by continuous darkness at 25$^o$C (28%) and burial for five months (20.8%). Treatments such as ambient light conditions, continuous light at 40$^o$C and continuous dark at 5$^o$C gave rise to very low germination percentages of between 0% and 2.2%.

This study aims to assess the percentage germination of grass species common to the Pietermaritzburg region of KwaZulu-Natal, South Africa under various germination conditions. Some of the species with high germination percentages will be used in seedling growth and competition trials to determine the species suitability for rehabilitation. The seedling growth trials will determine the affect of three environmental factors, namely nutrient availability, shading intensity and plant derived smoke-infused water on the seedling's growth and competitive ability.

## 3.2 Materials and methods

Germination tests were carried out on a wide variety of species, these being the tufted *Aristida junciformis* (Trin. & Rupr.)*, Digitaria eriantha* (Steudel)*, Eragrostis capensis* (Thunb.) Trin*, E. curvula* (Schrad.) Nees*, E. racemosa* (Thunb.) Steud.*, E. tef* (Trotter), *Hyparrhenia dregeana* (Nees.) Stapf.*, Melinis repens* (Willd.) Zizka.*, M. nerviglumis* (Franch.) Zizka.*, Panicum maximum* (Jacq.)*, Sporobolus pyramidalis* (P. Beauv.)*, S. africanus* (Poir.), *Themeda triandra* (Forsk.) and *Tristachya leucothrix* (Nees.). The stoloniferous grasses *Chloris gayana* (Kunth.), *Cynodon dactylon* (L.) Pers. and *Paspalum notatum* (Flugge.) were also included in the germination trials. These species were selected for germination trials as they are the dominant grass species in the local grasslands and harvesting of sufficient seeds for experimentation purposes was likely.

Most of the veld grass seeds were collected from the University of KwaZulu-Natal's (UKZN) grassland patch (4.06ha) behind the Life Science campus (Carbis Road), Pietermaritzburg. The dominant grass species present are *T. triandra, S. pyramidalis* and *S. africanus*. Other seed collection sites were a *T. triandra* dominated grassland plateau at Ukulinga, the University of KwaZulu-Natal's Research Farm (Murray Road, Pietermaritzburg) and at the Oribi Airport (Oribi Road, Pietermaritzburg), where there are large stands of *T. triandra* and *E. curvula*.

The veld type of these areas, according to Mucina and Rutherford (2006) is a transitional zone of 'Ngongoni Veld containing elements of KwaZulu-Natal Hinterland Thornveld with the distinguishing species of *Acacia sieberana*. The previous Acocks' (1988) classification for this area was Southern Tall Grassveld. Commercially available seeds of *Cynodon dactylon, Chloris gayana, Digitaria eriantha, Eragrostis curvula, E. tef* and *Panicum maximum* were purchased from McDonald Seeds, Pietermaritzburg.

Seeds from the Ukulinga Research farm and Oribi Airport were collected using a seed harvester during 2004 and 2005. Seeds collected from the UKZN grassland patch were collected in 2006 and were collected by hand. The procedure for seed collection was to collect seeds from a large number of individuals per species, to collect ripe seeds (although this was often difficult to determine), and to collect seeds for a variety of grassland species. Seeds collected from Ukulinga Research farm and Oribi Airport were stored in large woven bags and kept dry and cool. When required for germination trials the seeds were taken to a laboratory and, along with seeds collected from the UKZN grassland patch, were separated according to species. The seeds were then manually removed from the spikelet (by removing the glumes and separating the florets). The sorted seeds were placed in brown paper bags and labelled according to species, date of collection and collection site. These bags were stored in dark, cool and dry conditions.

The germination success (percentage germination) was assessed using two methods. The seed was considered to have germinated when the radical protruded from the seed coat.

1. Twenty five seeds per species were placed in a petri dish lined with Whatman's filter paper and placed in a germination chamber. The temperature in the germination chamber fluctuated between $25^{o}C$ and $30^{o}C$, and was set to constant light. The seeds were checked daily for germination and the number of seeds which had germinated per petri dish were recorded and then removed from the experiment. The germination trials continued for 20 days. It was expected that germination processes would be initiated at this time under the germination chamber conditions. Studies have shown that germination in grasses should occur within 10 days of wetting if the seeds are viable and are not in a state of dormancy (Fowler

1986, Klink 1996) . The seeds were watered every two to three days with approximately 5ml of distilled water. The experiment was replicated six times. Secondary germination trials were performed on four species namely, *C. gayana, P. notatum, S. africanus* and *S. pyramidalis* two months after the original testing to determine if there was a dormancy period. Four sets of trials were conducted, depending on seed availability, from May to November 2006.

2. One hundred seeds (4 replications of 25 seeds) of a number of species were germinated on a mist bed in the greenhouse at the University of KwaZulu-Natal's Neil Tainton Arboretum (Life Science Campus) from 18 December 2006 to 12 January 2007 (25 days). The position of the seed trays was randomized by species and replication number. The mist spray was set to irrigate the seeds for 20 seconds every 5 minutes to keep the seeds moist. The seeds were checked for germination daily and seeds which had germinated were counted and removed from the experiment. The species germinated on the mist bed were *T. triandra*, *T. leucothrix*, *E. racemosa* and *E. capensis* and the commercially acquired seeds *C. dactylon, C. gayana, D. eriantha,     E. curvula, E. tef* and *P. maximum.*

In the first germination chamber experiment it was noted that there was a large amount of fungal growth in the petri dishes. Additional germination trials were carried out to determine the affect of the fungal growth on germination. Pre-germination treatments were applied to *T. triandra*, *T. leucothrix*, *E. racemosa* and *E. capensis* seeds. Seeds were soaked in 1% Jik$^{©}$ solution (sodium hypochlorite) for 5 minutes to kill off the fungi present on the seeds and then rinsed thoroughly (Sauer and Burroughs 1986). Twenty seeds of each species were placed in the petri dishes and placed in the germination chamber. This was replicated three times. One hundred treated seeds (four replicates of 25 seeds) were placed on the mist bed with the same irrigation settings mentioned above.

Williams (1983) and Morinaga (1926) found that there is a significant increase in germination percentage when nitrogen was available, sometimes doubling the percentage germinated in a 0.002M nitrogen solution. For that reason a second pre-germination treatment was applied to the seeds of *T. triandra*, *T. leucothrix*, *E. racemosa* and *E. capensis*. The seeds were germinated in 80% Hoagland's solution (Hoagland and Arnon 1950) which is a nitrogen rich liquid fertilizer

comprising essential plant nutrients for vegetative growth. The major chemical compounds in the solution are calcium nitrate, potassium nitrate, monopotassium phosphate and magnesium sulphate. Twenty seeds of each species were placed in petri dishes and placed in the germination chamber, this was replicated three times. Seeds treated with Hoagland's solution were not germinated on the mist bed as the solution would have been severely diluted and could have leached out into neighbouring treatments. Twenty seeds per species, times three replications, were treated with a combination of the two pre-treatments (Jik[©] and Hoagland's solution) and germinated in the germination chamber.

## 3.3 Results

In the germination chamber, germination was highest for *S. africanus* (40% ±1.74) and increased to 88.67% ±0.65 after two months storage (Table 3.1). *Themeda triandra* and *T. leucothrix* seeds, which have been shown to have a dormancy period (O'Connor 1997, Tainton 1999), had less than 5% germination when the seeds were less than a year old or if they had been stored for a year and a half (Table 3.1). Other species which were expected to have a high germination rate, e.g. *A. junciformis*, because of its invasive characteristics (Tainton 1999), did not germinate well (8.67% ±0.48) (Table 3.1).

*Themeda triandra*, *T. leucothrix, E. racemosa* and *E. capensis* did not show a significant response to treatment with Jik[©] when germinated in the germination chamber (p>0.05). There was, however, a significant response when germinated on the mist bed. *Themeda triandra* (78% ±0.5 to 69% ±0.25) and *E. capensis* (33% ±0.63 to 40% ±1.15) decreased germination percentage whilst *T. leucothrix* (3% ±0.47 to 14% ±1.04) and *E. racemosa* (27% ±0.48 to 38% ±1.04) showed an increase in germination percentage (Table 3.2). When the seeds grown in the germination chamber were treated with Hoagland's solution, or a combination of Jik[©] and Hoagland's, the germination percentages were not significantly different (p>0.05) except when *E. racemosa* was treated with Hoagland's solution. *Eragrostis racemosa* doubled in germination percentage when treated with Jik[©] compared to the control (6.67% ±0.31 to 13.33% ±0.67), tripled when treated with Jik[©] and Hoagland's solution (18.33% ±1.33) and quadrupled when treated with just the Hoagland's solution (26.67% ±0.67) (Table 3.2).

The germination percentage of untreated seeds of *T. leucothrix* did not differ between the germination chamber and the mist bed (p>0.05). There was an increase in *E. racemosa* germination (6.67% ±0.33 to 27% ±0.48) and a decrease in *E. capensis* germination (66.67% ±1.45 to 33% ±0.63) on the mist bed compared to the germination chamber control treatments. The largest difference in the germination percentage between the germination chamber and mist bed treatments was with *T. triandra*. *Themeda triandra* increased from 0% germination in the germination chamber to 78% ±0.5 on the mist bed for the untreated seeds (Table 3.2).

The germination success of commercially available grass seeds, namely *C. gayana, C. dactylon, D. eriantha, E. curvula, E. tef* and *P. maximum* was tested on the mist bed. *Chloris gayana, C. dactylon, D. eriantha, E. curvula* and *E. tef* showed a high germination success of over 80%, whilst less than 9% ±1.03 of *P. maximum* seeds germinated (Table 3.3).

In summary, *S. africanus, T. triandra, E. capensis* and four of the five commercially available species *C. gayana, C. dactylon, D. eriantha, E. curvula* and *E. tef* demonstrated germination percentages of 50% and over. *Hyparrhenia dregeana* and *E. racemosa* had germination percentages between 30% and 40%. Seven of the 11 species germinated in the germination chamber had less than 10% germination (Table 3.1).

**Table 3.1:** Germination success of seeds germinated in a germination chamber at 30°C and under constant light (mean% ±standard error). Seeds were collected from the University of KwaZulu-Natal grassland, Ukulinga, and Oribi Airport.

| Collection place | Date of collection | Species | Germination % - Time 1 | Germination % - Time 2 |
|---|---|---|---|---|
| UKZN grassland | 16/04/2006 | *A. junciformis* | 8.67 ±0.48 | - |
| UKZN grassland | 16/04/2006 | *C. gayana* | 8.00 ±0.52 | 10 ±0.62 |
| UKZN grassland | 16/04/2006 | *H. dregeana* | 32.00 ±1.06 | - |
| UKZN grassland | 16/04/2006 | *M. nerviglumis* | 4.00 ±0.45 | - |
| UKZN grassland | 16/04/2006 | *M. repens* | 0 | - |
| UKZN grassland | 16/04/2006 | *P. notatum* | 0 | 0 |
| UKZN grassland | 16/04/2006 | *S. africanus* | 40.67 ±1.74 | 88.67 ±0.65 |
| UKZN grassland | 16/04/2006 | *S. pyramidalis* | 0.67 ±0.17 | 7.33 ±1.08 |
| Ukulinga | 09/11/2005 | *E. racemosa* | 2.00 ±0.22 | - |
| Ukulinga | 17/11/2005 | *E. racemosa* | 4.00 ±0.26 | - |
| Ukulinga | 09/11/2005 | *T. leucothrix* | 0 | - |
| Ukulinga | 13/10/2004 | *T. leucothrix* | 0 | - |
| Ukulinga | 17/11/2005 | *T. leucothrix* | 0 | - |
| Ukulinga | 27/10/2004 | *T. leucothrix* | 0 | - |
| Ukulinga | 09/11/2005 | *T. triandra* | 0.667 ±0.17 | - |
| Ukulinga | 13/10/2004 | *T. triandra* | 1.33 ±0.21 | - |
| Ukulinga | 17/11/2005 | *T. triandra* | 2.67 ±0.22 | - |
| Ukulinga | 27/10/2004 | *T. triandra* | 3.20 ±0.33 | - |
| Oribi Airport | 14/11/2005 | *E. racemosa* | 40.00 ±0.82 | - |
| Oribi Airport | 25/11/2004 | *E. racemosa* | 4.80 ±0.63 | - |
| Oribi Airport | 25/11/2004 | *T. leucothrix* | 0 | - |
| Oribi Airport | 14/11/2005 | *T. triandra* | 0 | - |
| Oribi Airport | 25/11/2004 | *T. triandra* | 0.67 ±0.17 | - |

**Table 3.2**: The effect of pre-germination treatment on the germination success (mean% ± standard error) of four grass species germinated in a greenhouse (mist bed) and germination chamber. Letters adjacent to the germination percentages show significant differences within species using the 5% least significant difference values. Treatments with letters in common (within species) are not significantly different.

| Species | Trial | Pre-germination treatment | % Germination | |
|---|---|---|---|---|
| *Themeda triandra* | Germination chamber | Control | 0 | a |
| | Germination chamber | Jik & Hoagland's | 1.67 ±0.33 | a |
| | Germination chamber | Jik | 0 | a |
| | Germination chamber | Hoagland's | 0 | a |
| | Mist bed | Control | 78 ±0.5 | b |
| | Mist bed | Jik | 69 ±0.25 | c |
| *Tristachya leucothrix* | Germination chamber | Control | 5.00 ±0.58 | a |
| | Germination chamber | Jik & Hoagland's | 1.67 ±0.33 | a |
| | Germination chamber | Jik | 0 | a |
| | Germination chamber | Hoagland's | 10.00 ±0 | ab |
| | Mist bed | Control | 3 ±0.47 | a |
| | Mist bed | Jik | 14 ± 1.04 | b |
| *Eragrostis racemosa* | Germination chamber | Control | 6.67 ±0.33 | a |
| | Germination chamber | Jik & Hoagland's | 18.33 ±1.33 | ab |
| | Germination chamber | Jik | 13.33 ±0.67 | a |
| | Germination chamber | Hoagland's | 26.67 ±0.67 | bc |
| | Mist bed | Control | 27 ±0.48 | b |
| | Mist bed | Jik | 38 ±1.04 | c |
| *Eragrostis capensis* | Germination chamber | Control | 66.67 ±1.45 | a |
| | Germination chamber | Jik & Hoagland's | 58.33 ±2.19 | ac |
| | Germination chamber | Jik | 71.67 ±2.19 | a |
| | Germination chamber | Hoagland's | 63.33 ±2.33 | a |
| | Mist bed | Control | 33 ±0.63 | b |
| | Mist bed | Jik | 40 ±1.15 | bc |

**Table 3.3:** Percentage germination (mean% ± standard error) of six species whose seeds were obtained from a local seed supplier. Seeds were germinated on a mist bed in a greenhouse.

| Species | % Germination |
|---------|---------------|
| *Chloris gayana* | 82 ±0.96 |
| *Cynodon dactylon* | 84 ±0.41 |
| *Digitaria eriantha* | 84 ±1.41 |
| *Eragrostis curvula* | 83 ±0.63 |
| *Eragrostis tef* | 84 ±1.08 |
| *Panicum maximum* | 9 ±1.03 |

## 3.4 Discussion

Several species which were tested in this germination trial had less than 10% germination but other species, such as *S. africanus* and *T. triandra* had over 70% germination. These results indicate that there is large variability in germination percentages between species. Similar variability in germination percentages of grassland species was demonstrated in germination trials carried out by Fowler (1986), Blank and Young (1998) and Gibson-Roy *et al.* (2007).

There was a significant increase in the germination percentage of *T. triandra* seeds when they were placed on the mist bed (78% ±0.5) compared to in the germination chamber (0%) without any pre-germination treatments. Cole and Lunt (2005) demonstrated that *T. triandra*'s optimal germination temperature is 30°C but Everson *et. al.* (2009) showed that *T. triandra* requires light/dark cycles for maximum germination. Light/dark cycles were not set up in the germination chamber which would explain the significant increase in *T. triandra*'s germination percentage on the mist bed where diurnal light fluctuations did occur. This argument could also hold true for *E. racemosa*'s germination requirements as it also showed a significant increase in germination percentage on the mist bed (6.67% ±0.33 vs. 27% ±0.48), although not to the same magnitude as *T. triandra*.

The germination conditions on the mist bed are not always the most appropriate. *Eragrostis capensis* had a higher germination percentage in the germination chamber (66.67% ±1.45 vs. 33% ±0.63) implying that this species germinates best under constant temperatures and does not require diurnal fluctuations in temperature or light. Conditions of relatively constant temperatures

and light availability can occur if the seeds are buried in the soil, especially if the soil has a light litter cover. These results suggest that *E. capensis* may require a specific burial depth prior to germination, a concept discussed by Cole *et al.* (2000).

The general sowing density guidelines for a variety of seeds is between 100 and 200 germinable seeds per square meter (Cole *et al.* 2000). This means that at 50% germination approximately 400 seeds/m$^2$ are required. The required seed density increases exponentially with a decrease in germination percentage. At 20% germination 1000 seeds/m$^2$ are required and at 10% germination 2000 seeds/m$^2$. Seeds are a limiting resource in grassland systems and the likelihood of harvesting enough seed from local grasslands for reseeding a neighbouring grassland decreases with a decrease in the species germinability. This is especially evident when calculating the number of seeds required in a hectare. For example, *S. africanus* has 88% germination which means that 227 seeds/m$^2$ (or 2.27 million seeds per hectare) are required in optimal germination conditions. Similarly, for a species like *S. pyramidalis*, which has a very low germination success of approximately 7%, would require 2857 seeds/m$^2$ or 28.57 million seeds per hectare for sufficient seedlings to establish in the grassland community. Species tested in these germination trials which have acceptable germination percentage for rehabilitation (>50% germination) were *S. africanus*, *T. triandra*, *E. capensis* and four of the five commercially available species *C. gayana, C. dactylon, D. eriantha, E. curvula* and *E. tef*.

The results from these germination trials emphasize how important it is to determine the germination percentage of the species intended for rehabilitation prior to sowing. Knowing the correct germination percentage will indicate which species will be best for rehabilitation as the number of seeds required can be calculated. Species with a low germination percentage, low seed set, or low abundance in the local grasslands are not appropriate for rehabilitation via direct seeding. Additionally, overestimating the percentage germination will significantly alter the required number of seeds per hectare resulting in a potentially undesirable change in the final community structure.

It may be beneficial to use species available from a local seed supplier for rehabilitation as the seeds are readily available to the restoration ecologist and will, generally, have a high germination percentage. Additionally, some of the more

dominant species in South African grasslands, namely *C. gayana, C. dactylon, D. eriantha, E. curvula* and *E. tef* (Zacharias 1990) are available commercially. There are, however, concerns around the adaptability of the seedlings and adult plants of commercially available species to the local environmental conditions. If the seeds are bought from a local seed supplier, however, there should be sufficient adaptation to local environmental conditions to ensure seedling, and adult plant survival.

# Chapter 4: Seedling growth and competition in five South African grasses: the nitrogen effect

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This chapter is in the format of a paper intended for submission to the African Journal of Range and Forage Science. There will be some information overlap within the dissertation.

# Chapter 4: Seedling growth and competition in five South African grasses: the nitrogen effect

## 4.1 Introduction

Nitrogen, phosphorus and other minerals such as calcium, potassium and magnesium are essential for seedling growth and development (Roberts 1960, Campbell 1968). A high nitrogen level in a seedling's microhabitat is, therefore, advantageous to that seedling's establishment success and survival probability (Bisigato and Bertiller 1999). Increased nitrogen availability will not only increase the seedling's growth rate; the time that the seedling is dependent on the cotyledonous reserves will decrease as the time till the seedling is photosynthetic, thereby producing its own energy, decreases. Low nitrogen levels at the time of seedling establishment may be detrimental to the seedlings survival. With a decrease in nitrogen availability more cotyledonous reserves will be used for developing a root system to maximise nutrient uptake (McWilliam *et al.* 1970). Consequently, there is delayed above-ground vegetation development and, therefore, a decreased above-ground competitive ability.

Two species growing in close proximity with each other and competing for the same resources can succumb to one of three scenarios: 1) neither species can survive, 2) one will dominate over the other or 3) both species will co-exist at equilibrium in a stable community (Tilman 1985). The competitive interactions between species for environmental resources and the competitive outcomes are outlined in Figure 4.1. Nitrogen availability can be regarded as "environmental resource 1" and therefore, according to Figure 4.1, species A will dominate over species B at high levels of nitrogen and low levels of "environmental resource 2". Species B, however, will dominate at low levels of nitrogen when "environmental resource 2" is high. What is important to note, and is illustrated in this diagram, is that there are tradeoffs in resource requirements. Species B is not a superior competitor for both, or all, environmental resources but has made a trade-off in its competitive ability requiring high levels of "environmental resource 2" for a sufficient supply of "environmental resource 1" even though it is not available at an optimum level.

The aim of this study was to examine the affect of nitrogen on grass seedling growth and competitive ability, with the specific objectives to determine:

1. How seedlings compete against, and respond to, competition from other seedlings.

2. Which seedlings are better competitors than others under varying nitrogen availability levels.

3. If the competitive ability of a species is consistent in both high and low nutrient conditions.
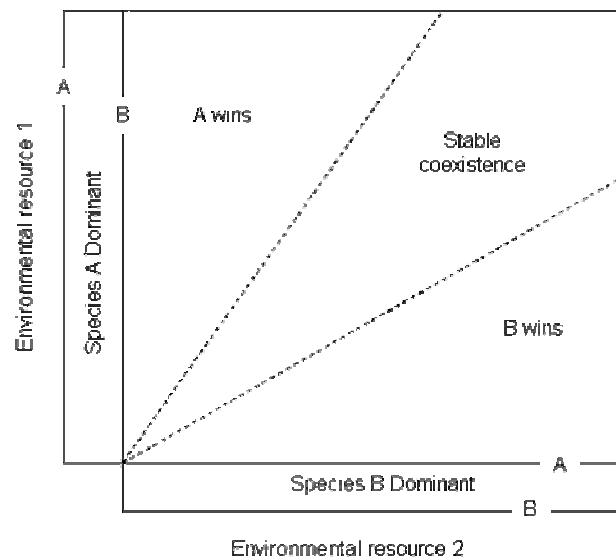


**Figure 4.1:** Growth isoclines as presented in Tilman (1985) represent the competitive outcome between two species competing for two environmental resources.

**4.2 Materials and Methods**

The growth and competition experiments were set up in a greenhouse at the Neil Tainton Arboretum, Life Science Campus, University of KwaZulu-Natal, Pietermaritzburg. The experimental trials ran from 13 August to 21 October 2007 and terminated after 8 weeks as there were some seedlings which had begun to tiller and produce inflorescences. The competitive effect of the flowering species on the neighbouring species would have been artificially decreased if the trial were continued past this point. The non-flowering species would have continued growing whilst the flowering species ceased their growth to use energy reserves to produce seeds. Subsequently, the non-flowering species may have had a greater

total seedling weight than if the flowering species continued to grow at pre-flowering rates.

Five common South Africa grasses were selected because of the variety of morphological properties (height, leaf size and growth structure) and high germination rates of over 80% (refer to Chapter 3). The species selected were *Chloris gayana*, *Cynodon dactylon*, *Digitaria eriantha*, *Eragrostis curvula* and *Eragrostis tef*. Physiological information about these species can be found in Chippendall (1959), Clayton *et al.* (2006 onwards), Gibbs Russell *et al.*(1991), Tainton *et al.* (1990), Tainton (1999) and van Oudtshoorn (2002).

*4.2.1 Experimental design*

The experiment was a fully factorial design of two nitrogen levels and three competition levels with six replications of each treatment. The high nitrogen treatments were given two nitrogen additions of 100ml of Hoagland's solution at 80% concentration per week (17.14mg of nitrogen per 100ml). The second nitrogen treatment (low nitrogen) did not have any Hoagland's solution added; however there may have been residual nitrogen in the growing medium and in the water. The term low nitrogen is used to indicate "no added nitrogen" treatments. All of the treatments were watered daily. Hoagland's solution is a nitrogen rich liquid fertilizer consisting of major elements of calcium nitrate, potassium nitrate, monopotassium phosphate and magnesium sulphate. The minor elements in the solution are boric acid, manganese chloride, zinc sulphate, copper sulphate, molybdic acid and FeEDTA (Hoagland and Arnon 1950).

The seedling competition combinations were: the growth of each species with another species (10 combinations) (Figure 4.2a), each species grown against itself (5 combinations) (Figure 4.2b) and each species grown with half the seedling density of the other two treatments to use as a control (5 combinations) (Figure 4.2c). The general design for the competition combinations was take from Buman *et al.* (1988). Each competition combination was placed in a separate tray and the treatments were completely randomised within replicates.

Within each of the competition combinations (Figure 4.2) two-to-three-day-old seedlings (30 for each treatment and 15 for the control) were planted in a lattice design of 5 seedlings by 6 seedlings and planted 2cm apart (approximate density of 2500 seedlings/$m^2$ for the non-control treatments) (Figure 4.2). In treatments

when two species were planted in a single tray the seedlings were planted alternately in the lattice design (Figure 4.2a). The seedlings in the outer border of the design (white circle in Figure 4.2) were a continuation of the treatments species composition, for example, if two species were grown with each other (Figure 4.2a) then the border species would also be alternating "species A" and "species B" in the same pattern as the internal lattice. Their growth characteristics were not measured as their purpose was to eliminate the edge effect of decreased competition as they were not surrounded by seedlings on all four sides. Effectively, the growth of six seedlings of each species present in the competition combination (Figure 4.2a) or 12 seedlings in the monospecific treatments (Figure 4.2b) were measured.

The density of 2500 seedlings/m$^2$ was selected based on the results of a study carried out by Cottam and Curtis (1956) which showed that even at a density of 2084 seedlings per m$^2$ there was still limited competition between seedlings. They also commented that most seedling experiments have artificially inflated densities and the results are not necessarily a true reflection of natural conditions. Because the overall aim of this study is to better understand seedling competitive interactions the seedling density needs to be higher than in naturally occurring populations.

Measurements of seedling height, number of tillers, number of live and dead leaves, number of inflorescences per seedling and mortality were recorded. The measurements were taken two weeks after planting and one week prior to harvesting. At the end of the experiment the above ground vegetative structures of all the competition combinations were harvested and separated according to species and treatment. The harvested material was dried at 60$^o$C for 3 days. The mass of each species per treatment (per replication) was recorded.
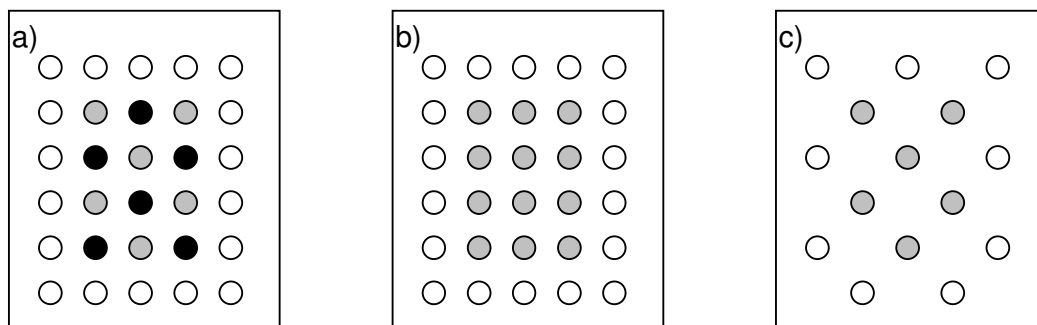
**Figure 4.2:** Layout of the seedling competition combination. ○ represents the border species that were not measured, ◯ represents species A and ● species B. Diagram a) is where one species was grown with another, b) when it was grown in a monoculture and c) when a species was grown with lowered competition. The border species (○) were a continuation of the species combinations for that treatment, in diagram a) they were an alternation of species A and B, and in diagram b) and c) they were also monospecific.

*4.2.2 Statistical analysis*

Two competition interactions could be determined from the data. For example, if species A was grown with species B the effect of A on B and B on A can be measured. The competition interactions are stated in terms of the effect that the neighbour species had on the growth of the target (competitive effect) and how the target species was affected by the presence of a neighbour (competitive response). The target is the species which is measured and the data of the target seedling is compared to when grown with different species (the neighbour).

Three statistical analyses were carried out on the seedling interaction data. Firstly, an analysis of variance was performed on all the data collected (seedling height, number of tillers, number of live and dead leaves, number of inflorescences per seedling, mortality and the dry weight of the harvested seedlings). The ANOVA identified where there were significant interactions taking place between the target and neighbour species. Secondly, a relative interactive index (RII) was calculated on the data set where significant interactions were indicated in the ANOVA. The RII shows where the interactions were taking place and the strength of the relationship. These RIIs were then analysed in an analysis of covariance to

determine which of the calculated interactions were significantly different from the controls and other treatments.

The relative interaction index (Armas *et al.* 2004) was computed (with 95% confidence intervals) to measure which species (target) had the greatest relative competitive effect on the growth of another species (the neighbour) and which target species was the most affected by competition (i.e. the competitive response). The RII represents the ratio of the net loss or gain of seedling mass through the competitive interaction and has defined limits of +1 and -1. A positive interaction represents a facilitative effect and a negative one a competitive effect and the more positive or negative the RII value the more intense the interaction. The relative interaction index's statistical properties are advantageous as the analysis is sensitive to small changes in the data and the RII values are symmetrical around zero (Armas *et al.* 2004). The RII is calculated as follows (equation 4.1):

$$RII = \frac{B_w - B_o}{B_w + B_o} \qquad \text{(equation 4.1)}$$

$B_o$ is the mass of the plant observed in the absence of inter- or intra-specific interactions and $B_w$ is the mass of the plant observed when growing with other plants.

The death of a single seedling could have influenced the growth of the surrounding seedlings. The competitive intensity experienced by a neighbouring species could have been further decreased if the seedling which died was one of the more competitive species. The number of live neighbour seedlings was therefore taken into consideration when determining a species competitive ability. The number of live border seedlings was also taken into consideration.

An analysis of covariance was performed on the calculated relative interaction indices. The covariate was the number of live neighbour seedlings in the tray and RII was the competitive response variable. The factors used to determine the competitive interactions were species (target and neighbour) and nutrient level. The least significant difference values were used to determine significant separation of the species means. All analyses were done using Genstat (v 9.1).

**4.3 Results**

The analysis of variance on all the collected data (seedling height, number of tillers, number of live and dead leaves, number of inflorescences per seedling, mortality and dry weight of the harvested seedlings) showed that the dry weight of the harvested seedlings was the only statistically significant measure of seedling competitiveness. It indicated there was a significant difference in the seedling weight between treatments ($p<0.05$). This indicates that the seedling competitive ability is not reflected in a single growth characteristic but rather a combination of several factors which is manifested in the seedling weight. Several of the factors measured during the competition trial (e.g. seedling height, number of leaves and number of tillers) would have contributed to the final seedling weight.

Two comparisons were made for each nutrient level to determine the competitive interactions which take place between the seedlings:
1. The competitive response of the target species (measured) to the presence of competition from the neighbour.
2. The competitive effect that the neighbour has on the growth of the target.

*4.3.1 Analysis of seedling dry weight*

ANOVAs on the square root transformed dry weight of the seedlings of the five grass species were performed to identify any significant relationships with respect to the seedling's response to competition. In the low nutrient treatments there was a significant difference in the response of the target species to competition from the neighbour and the competitive pressure imposed from the neighbour onto the target ($p<0.001$ and $p=0.016$ respectively). There was no significant interaction between the two factors ($p=0.107$) nor a significant covariate effect ($p=0.788$) (Table 4.1). The same trends are seen in the high nutrient treatment (Table 4.2). The response of the target species to competition from the neighbour and the competitive pressure imposed from the neighbour onto the target were highly significant ($p<0.001$), the interaction between the factors was not significant ($p=0.446$) and neither was the covariate ($p = 0.312$).

**Table 4.1:** Results of the Analysis of Covariance for the square root transformed dry weight of five grass species under low nutrient conditions adjusted for the covariance of total neighbour survival

| Source of variation | d.f.[1] | s.s.[2] | m.s.[3] | v.r.[4] | cov.ef.[5] | F pr.[6] |
|---|---|---|---|---|---|---|
| Target | 4 | 0.060 | 0.015 | 15.82 | 0.99 | <.001 |
| Neighbour | 5 | 0.014 | 0.003 | 2.88 | 0.58 | 0.016 |
| Target x Neighbour | 20 | 0.027 | 0.001 | 1.45 | 0.99 | 0.107 |
| Covariate | 1 | 0.000 | 0.000 | 0.07 | | 0.788 |
| Residual | 144 | 0.136 | 0.001 | | 0.99 | |
| Total | 179 | 0.323 | | | | |

[1] degrees of freedom  [2] sum of squares  [3] mean squares
[4] variance ratio  [5] covariance efficiency  [6] F probability

**Table 4.2:** Results of the Analysis of Covariance for the square root transformed dry weight of five grass species under high nutrient conditions adjusted for the covariance of total neighbour survival

| Source of variation | d.f.[1] | s.s.[2] | m.s.[3] | v.r.[4] | cov.ef.[5] | F pr.[6] |
|---|---|---|---|---|---|---|
| Target | 4 | 28.584 | 7.146 | 47.43 | 0.96 | <.001 |
| Neighbour | 5 | 12.876 | 2.575 | 17.09 | 0.99 | <.001 |
| Target x Neighbour | 20 | 3.014 | 0.151 | 1.00 | 0.99 | 0.466 |
| Covariate | 1 | 0.155 | 0.155 | 1.03 | | 0.312 |
| Residual | 142 | 21.393 | 0.151 | | 1.00 | |
| Total | 177 | 71.329 | | | | |

[1] degrees of freedom  [2] sum of squares  [3] mean squares
[4] variance ratio  [5] covariance efficiency  [6] F probability

The relative interactive indices (RII) were calculated to determine the species competitive interactions and between which species and treatments those interactions are taking place. Analysis of covariance was carried out to determine if there were significant interactions between the RII values. In the low nutrient (Table 4.3) and high nutrient (Table 4.4) treatments the responses of the target species to competition from the neighbour were significantly different (p<0.001 and p=0.051 respectively). The effect that the neighbour had on the growth of the target in the low nutrient treatments was not significantly different between species (p=0.107) but was significantly different in the high nutrient treatments (p<0.001). There was no significant interaction between the two factors in the low and high nutrient conditions (p=0.209 and p=0.566 respectively) nor did the covariate have a significant effect on the model (p=0.708 and p=0.268 respectively) (Table 4.3 and Table 4.4).

**Table 4.3:** Results of the Analysis of Covariance for the Relative Interaction
Indices for the dry weight of five grass species under low nutrient conditions
adjusted for the covariance of total neighbour survival

| Source of variation | d.f.[1] | s.s.[2] | m.s.[3] | v.r.[4] | cov.ef.[5] | F pr.[6] |
|---|---|---|---|---|---|---|
| Target | 4 | 1.661 | 0.415 | 5.78 | 0.99 | <.001 |
| Neighbour | 4 | 0.559 | 0.140 | 1.95 | 1.00 | 0.107 |
| Target x Neighbour | 16 | 1.493 | 0.093 | 1.30 | 0.99 | 0.209 |
| Covariate | 1 | 0.010 | 0.010 | 0.14 | | 0.708 |
| Residual | 119 | 8.554 | 0.072 | | 0.99 | |
| Total | 149 | 17.154 | | | | |

[1] degrees of freedom [2] sum of squares [3] mean squares
[4] variance ratio [5] covariance efficiency [6] F probability

**Table 4.4:** Results of the Analysis of Covariance for the Relative Interaction
Indices for dry weight of five grass species under high nutrient conditions adjusted
for the covariance of total neighbour survival

| Source of variation | d.f.[1] | s.s.[2] | m.s.[3] | v.r.[4] | cov.ef.[5] | F pr.[6] |
|---|---|---|---|---|---|---|
| Target | 4 | 0.825 | 0.206 | 2.43 | 1.00 | 0.051 |
| Neighbour | 4 | 3.583 | 0.896 | 10.58 | 0.94 | <.001 |
| Target x Neighbour | 16 | 1.225 | 0.077 | 0.90 | 1.00 | 0.566 |
| Covariate | 1 | 0.105 | 0.105 | 1.24 | | 0.268 |
| Residual | 117 | 9.906 | 0.085 | | 1.00 | |
| Total | 147 | 16.942 | | | | |

[1] degrees of freedom [2] sum of squares [3] mean squares
[4] variance ratio [5] covariance efficiency [6] F probability

*4.3.2 Competitive response*

In the low nitrogen treatment the growth of *C. gayana* and *D. eriantha* were
most affected by the presence of neighbours (more negative RII) while *E. curvula*
and *E. tef* were least affected (less negative RII) (Figure 4.3). *Digitaria eriantha*
was affected most by the presence of neighbours in the high nitrogen treatment
(more negative RII) and *C. gayana* and *E. curvula* were least affected (less
negative RII) (Figure 4.4). *Chloris gayana* became more resistant to competition
(less negative RII) when under high nutrient conditions than if it was under low
nutrient conditions.
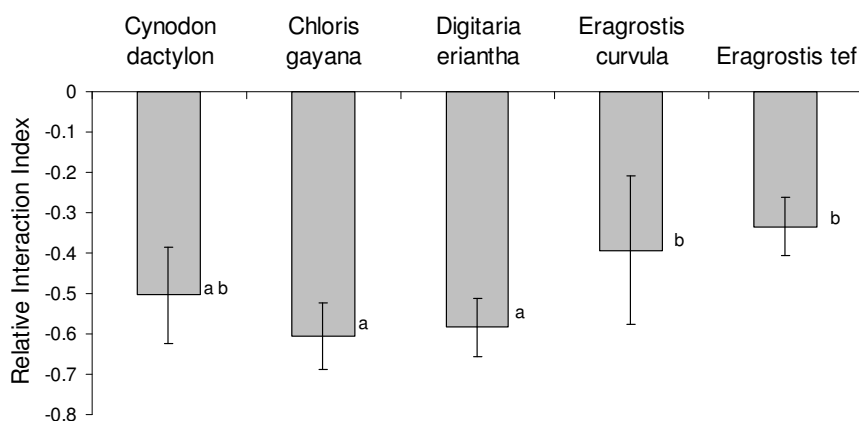
**Figure 4.3:** Mean competitive response (based on dry matter yield) of the target species to competition from all neighbours under low nutrient conditions. Bars with letters common are not significantly different (p>0.05) using the least significant difference value of 0.136. Vertical lines represent the 95% confidence intervals.
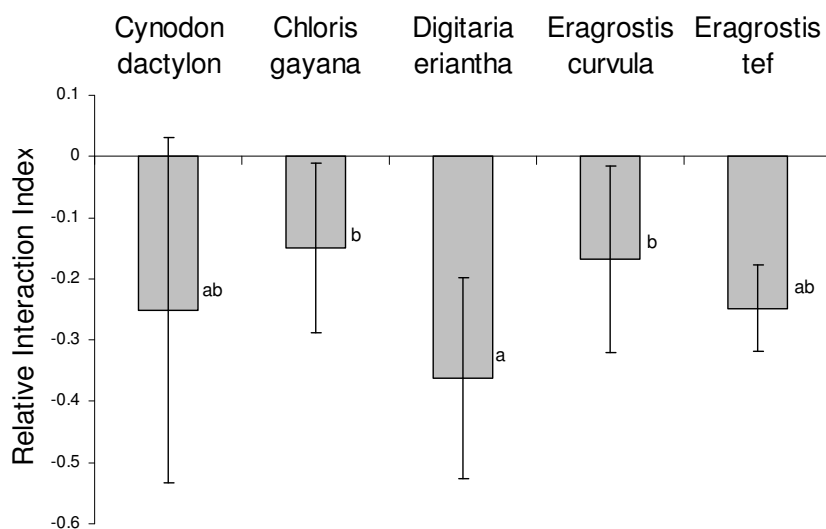


**Figure 4.4:** Competitive response (based on dry matter yield) of the target species to competition from the neighbour under high nutrient conditions. Bars with letters common are not significantly different (p>0.05) using the least significant difference value of 0.149. Vertical lines represent the 95% confidence

*4.3.3 Competitive effects on the target species*

In the low nitrogen treatment the different neighbour species had similar effects on the growth of the targets (p=0.107, Table 4.3). This could be due to the very large 95% confidence limits (most evident in *C. dactylon*) (Figure 4.5). In the high nitrogen treatment *C. gayana* had the largest effect (most negative RII) on the growth of the target species and *C. dactylon* and *D. eriantha* effected the target the least (least negative RII) (Figure 4.6).
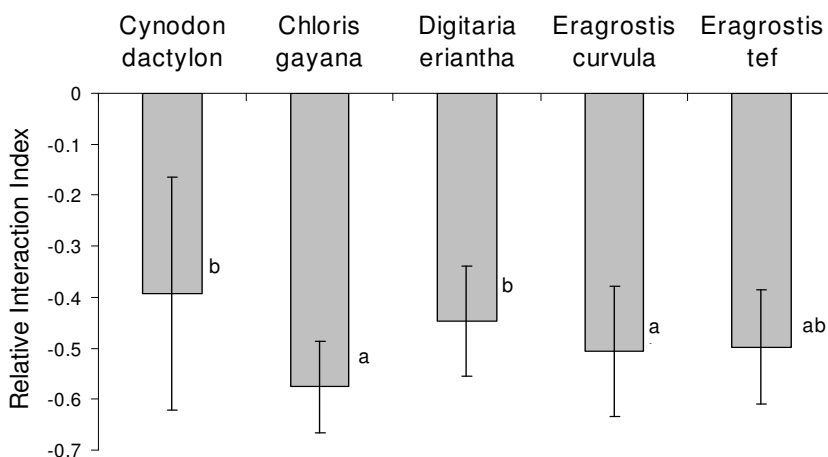


**Figure 4.5:** Competitive effect (based on dry matter yield) of neighbour species on target species under low nutrient conditions. Bars with letters common are not significantly different (p>0.05) using the least significant difference value of 0.136. Vertical lines represent the 95% confidence intervals.
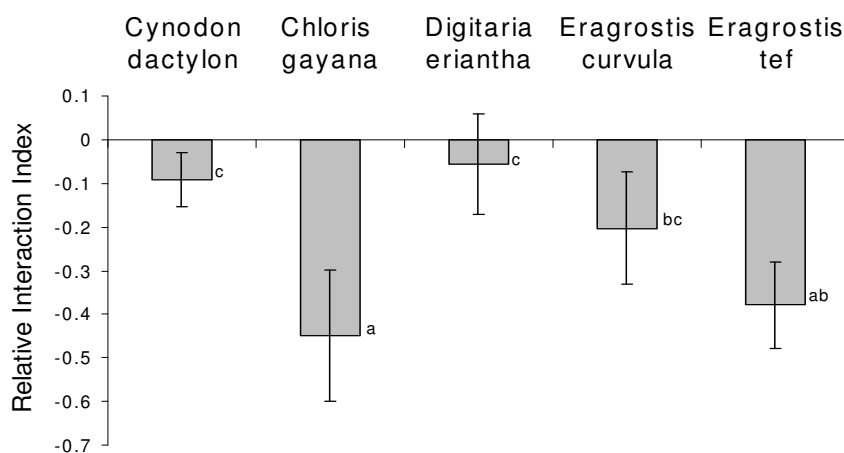


**Figure 4.6:** Competitive effect (based on dry matter yield) of neighbour species on target species under high nutrient conditions. Bars with letters common are not significantly different (p>0.05) using the least significant difference value of 0.149. Vertical lines represent the 95% confidence intervals.

The neighbour affects the growth and competitive ability of the target species in the high nitrogen treatments (p<0.001, Table 4.4) as shown in Figure 4.7. *Chloris gayana* and *E. tef* had the largest competitive effect on all species (length of species bars). *Cynodon dactylon* and *D. eriantha* had limited affect on the growth on the target. *Eragrostis tef* was relatively uniformly affected by the neighbour species but *C. dactylon* shows the least competitive effect. *Cynodon dactylon* affected the growth of all the target species the least but its growth was only severely affected by *C. gayana* and *E. tef*, showing that even though it is a poor competitor its growth is not always adversely affected.
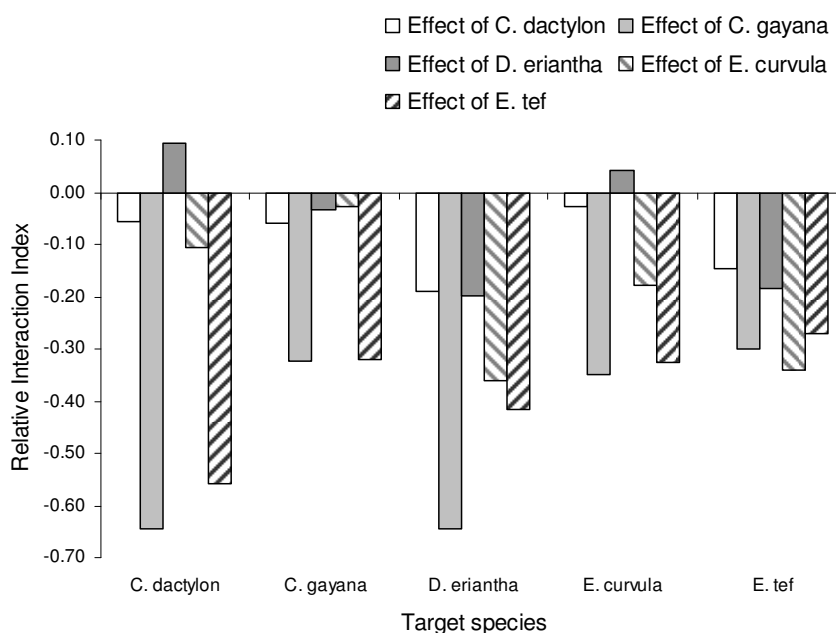


**Figure 4.7:** Comparison of the competitive effect that each species had on the growth of each target species

## 4.4 Discussion

The seedlings can be split into two classes in accordance to their competitive ability. Under low nutrient conditions seedlings of *E. curvula* and *E. tef* are the better competitors as they were affected the least by competition by the neighbour. Under high nutrient conditions *C. gayana* and the two *Eragrostis* species were the best competitors as they were least affected by competition from other species and reduced the growth of the neighbours the most.

*Chloris gayana* was the only species which showed a significant change in its competitive ability with a change in nitrogen availability. The decreased nitrogen

levels inhibited its competitive ability to such a degree that the competitive pressure imposed by the species that are poor competitors (*C. dactylon* and *D. eriantha*) was sufficient to significantly reduce its growth. Ultimately, this species is capable of utilizing resources better than *C. dactylon* and *D. eriantha* when they are available.

Rice *et al.* (1960), Parrish and Bazzaz (1982) and Tilman (1986) showed that there is a positive correlation between the abundance of pioneer species and nitrogen availability. Pioneers use the resources which are available with greater efficiency than climax species, probably because the pioneer species need to absorb an adequate amount of resources for growth and reproduction from a resource limiting environment. Climax species, however, may use a similar amount of resources as pioneers in a given year but the proportion that they require, relative to their availability, is lower. Referring back to Figure 4.1, species A would be a later successional species whilst species B a pioneer if the "environmental resource 1" was referring to nitrogen. Monaco *et al.* (2003) and Paschke *et al.* (2000) showed that low levels of nutrients are less detrimental to perennials than annuals as annuals are usually some of the first species to invade an area.

The only annual in this experiment, *E. tef*, was one of the most competitive species under low nutrient conditions as it was affected least by competition in the low nutrient treatments. As an annual, and pioneer, it has rapid preliminary growth rate to ensure its survival in the highly competitive environment in terms of nutrients available. The RII values for the response of *E. tef* to competition in the low nutrient environments (-0.334) (Figure 4.3) and the high nutrient environments (-0.248) (Figure 4.4) shows that the competitive pressure imposed onto seedlings by *E. tef* is higher in the low nutrient conditions (i.e. the RII is more negative) as there is greater competition for nitrogen. *Eragrostis tef* is not a "clear winner" in terms of being the best competitor in the high nutrient treatments (Figure 4.4) and an increase in pressure from the other species as the nutrient availability increases retards its competitive effect. Therefore, in low nutrient environments *E. tef* is able to assimilate sufficient nutrients for survival better than the other species tested making it a superior above-ground competitor and retards the growth of other species. In the high nutrient environment, however, other species

are able to compete better against *E. tef* as they are able to take advantage of the additional resources better.

It would be interesting to see what would have occurred in the low nutrient treatments had the seedlings been left to grow longer. There may have been a higher long term survival of the slower growing species as they would require less resources, at any point in time, than the faster growing species because of their decreased growth rates. Campbell *et al.* (1992) demonstrated that species with strong root thrust and slow growth are able to conserve resources and tolerate a nutrient shortage leading to that species dominating in low nutrient environments. In the case of *C. gayana* there is a fast growth so it does well in the high nutrient environments but is not conservative enough to compete against the slow growth of the species that are adapted to the low nutrient environments.

Through experimentation it is evident that not all seedlings have the same competitive ability, even when high levels of nutrients are available for increased growth rates. *Eragrostis curvula* and *E. tef* are highly competitive as seedlings, and depending on the nutrient availability, so is *C. gayana*. *Cynodon dactylon* and *D. eriantha* on the other hand did not compete well even when grown against each other (low competitive pressure). In restoration, or revegetation practices, these two groups of species should not be grown with each other as the highly competitive seedlings will out compete, or decrease the probability of survivorship, of the poorer species. The planting of a high number of species to increase biodiversity could, potentially, lead to a decreased biodiversity if the wrong species are planted with each other.

A method, or model, for arranging species in the landscape that will decrease the competition imposed on the seedlings but increasing the potential biodiversity is needed. There are, however, more environmental factors acting on seedling survival than just nutrient availability and competition, as suggested by Tilman (1985) when determining growth isoclines (Figure 4.1). To increase the robustness of such a model further aspects into the ecology of grass seedlings needs evaluation.

# Chapter 5: The affect of shade and nutrients on the competitive interactions of *Digitaria eriantha* (Steud.) seedlings

Authors on this paper are:
Miss M. J. Ellis, Professor K. P. Kirkman and Mr. C. D. Morris

This chapter is in the format of a paper intended for submission to the African Journal of Range and Forage Science. There will be some information overlap within the dissertation.

# Chapter 5: The affect of shade and nutrients on the competitive interactions of *Digitaria eriantha* (Steud.) seedlings

## 5.1 Introduction

Shading by grass canopies can influence the germination of seeds and can affect the establishment success of seedlings (Morinaga 1926, Tiedemann *et al.* 1971, Patterson 1980, Silvertown 1980, Williams 1983, van der Toorn and Pons 1988, Van Auken and Bush 1990, Walters *et al.* 1993, Belsky 1994, Jutila and Grace 2002, and Lenssen *et al.* 2003). Even though the effects of shading on germination and seedling growth is well documented it is still relatively unclear what influence shading, and the corresponding increase in nutrients (Tiedemann 1970), has on the competitive interactions of grass seedlings.

The growth of a plant is regulated by the amount of energy gained during photosynthesis and lost through respiration (i.e. metabolic activities) (Amthor 1984). If there is a decrease in the photosynthetic rate due to shading then there is a decrease in the amount of energy that is available for growth once the energy requirements for metabolic activities have been met. The grassland canopy can reduce soil surface light by more than 50% (Van Auken and Bush 1990) hampering the photosynthetic ability of the plants in the under-story. Tiedemann *et al.* (1971) showed that when there is more than 20% shading seedling vigour is decreased. Additionally, under high shading levels the grass stems become thinner and longer (etiolation) in order for the individual to grow above the object shading out the light, culm production is reduced, flowering lags and the width of the leaf blade increases with an increase in the level of shading to maximise light interception. In effect, there is a morphological plasticity in grasses which occurs when individuals of a particular species are exposed to varying environmental conditions (Robinson and Rorison 1988).

What is apparent from the literature is that the response of grasses to shading is species specific (Morinaga 1926, Tiedemann *et al.* 1971, Patterson 1980, Silvertown 1980, Williams 1983, van der Toorn and Pons 1988, Van Auken and Bush 1990, Walters *et al.* 1993, Belsky 1994, Jutila and Grace 2002, and Lenssen *et al.* 2003). If a species is not tolerant of shade then its initial growth rate will be temporarily increased to grow above the light impeder as quickly as possible (Walters *et al.* 1993). This will have a negative effect on the plant, such as a

decreased root growth rate (Tiedemann *et al.* 1971) and delayed reproduction as it will take longer to build up the required energy reserves. Patterson (1980) noted similar trends, namely, a significant reduction in total plant dry weight, specific leaf area and the dry weight of the roots, leaves and rhizomes when the grass was shaded. Belsky (1994) found that the stomatal apertures of shade dominant grasses were reduced to decrease water loss when shaded.

Higher nutrient levels are frequently found under tree canopies as there is an increased litter deposition by falling leaves and increased nitrogen rich urea deposition through droppings of roosting birds and resting animals (Belsky 1994). Nutrients facilitate an increase in the seedlings growth rate (biomass and height) (Tilman 1986) and can alter competitive relationships (Chapter 4). It would be expected, therefore, that a trade off between limiting resources is evident in species that are dominant in open grasslands or under canopies. Referring to Figure 5.1, if "environmental resource 1" were nitrogen availability and "environmental resource 2" were light availability, species A would be found under vegetation canopies as there is high nitrogen but low sunlight. Species A's trade-off is adapting to low light in exchange for high levels of nutrients to facilitate its growth.
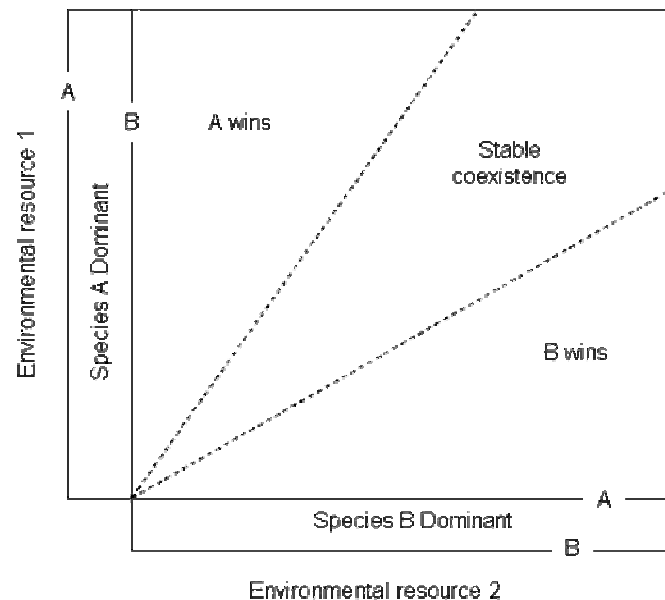


**Figure 5.1:** Growth isoclines as presented in Tilman (1985) represent the competitive outcome between two species competing for two environmental resources.

The aim of this study was to determine what affects shading, nutrient level and seedling density had on the growth of *D. eriantha* seedlings. *Digitaria eriantha* was used as it is sensitive to competition (Chapter 4) and is commonly found under vegetative canopies (Veenendaal *et al.* 1993). The specific objectives were to evaluate:

1. the affect of shading on the growth of the *D. eriantha* seedlings,
2. the interaction of shading and nitrogen availability on the growth of *D. eriantha* seedlings,
3. how competition among *D. eriantha* seedlings is affected by different environmental conditions (light and nutrients).

Physiological information about *D. eriantha* can be found in Chippendall (1959), Clayton *et al.* (2006 onwards), Gibbs Russell *et al.*(1991), Tainton *et al.* (1990) and Zacharias (1990).


**5.2 Material and Methods**

Permanent, 1.5m by 2.5m shade enclosures have been erected at the Neil Tainton Arboretum, on University of KwaZulu-Natal's Life Science Campus. They are constructed from varying densities of black shade cloth creating plots with 0%, 55%, 70%, 85% and 93% shading. Each plot is fitted with drip irrigation hoses which are placed into individual pots.

The design of this experiment was a split-plot design where the whole plot was shade, with five levels of shading. Sub-plots comprised of three levels of nitrogen where 50ml of 20% (2.14mg nitrogen), 60% (6.43mg nitrogen) and 100% (10.71mg nitrogen) Hoagland's solution was applied to the respective treatments on a weekly basis. Hoagland's solution is a nitrogen rich liquid fertilizer consisting of major elements of calcium nitrate, potassium nitrate, monopotassium phosphate and magnesium sulphate. The minor elements in the solution are boric acid, manganese chloride, zinc sulphate, copper sulphate, molybdic acid and FeEDTA (Hoagland and Arnon 1950).

There were three levels of competition: A seedling was grown without competition (Figure 5.2a), was exposed to the competitive pressure from four seedlings (five seedlings per pot) (1250 seedling/m$^2$) (Figure 5.2b) and eight seedlings (nine seedlings per pot) (2500 seedling/m$^2$) (Figure 5.2c). The seedlings were placed 2cm apart. Each treatment combination was placed in separate 20cm

diameter pots and set in a completely randomised fashion within each of the corresponding shade enclosures. There were 6 shading replicates.

The density of seedlings was selected according to a study carried out by Cottam and Curtis (1956) which showed that even at a density of 2084 seedlings per square meter there was still very limited competition between seedling. They also commented that most seedling experiments have artificially inflated densities but, because the overall aim of this study is to better understand seedling competitive interactions, the seedling density needs to be higher than those occurring naturally for sufficient competitive interactions to take place.
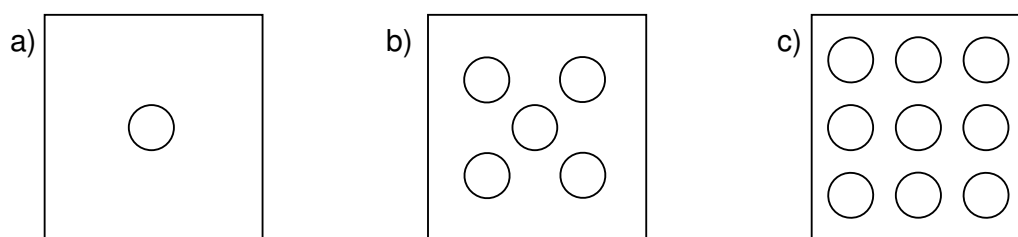


**Figure 5.2:** Diagrammatic representation of the seedling density designs. Seedlings were planted at densities of 1, 5, and 9 seedlings per pot, represented as Figures a, b, and c respectively.

Within each of the competition combinations (as seen in Figure 5.2) three seeds were placed in each seedling growth position in order to ensure that at least one seedling would establish in each position. If more than one seed germinated per position then the youngest seedling was removed from the pot.

The spatial position of the pot in the enclosure was noted as the pots that were placed near the enclosure access point may have received more sunlight and the seedling growth may have been affected. The pot position was added to the model as a covariate.

The intention was to have 3 levels of seedling density (competition) but rain drop action, caused by the shade cloth structures funnelling water onto the soil, resulted in uneven displacement of seedlings in the pots. The seedlings were no longer uniformly distributed and the closer the seedlings were to each other the higher the competitive effect of the seedlings on each other. The position of the seedlings in the pot and the degree of clumping of the seedlings (spatial distribution) was recorded as a covariate. The "degree of seedling clumping" was

recorded as 1) the seedlings were dispersed evenly from each other (similar to original position), 2) they were generally separate from each other with some clumping, 3) they were generally clumped with some separated and 4) they were all clumped tightly together (less than 2cm from each other).

There was also high seedling mortality through raindrop action, particularly in the high shading treatments where there was a higher water funnelling effect. The number of seedlings in each pot were counted at the end of the experiment and separated into 5 competition classes. These classes were (1) 1 - 2 seedlings, (2) 3 - 4 seedlings, (3) 5 - 6 seedlings, (4) 7 - 8 seedlings and (5) 9 - 10 seedlings. These competition classes were used in all analyses instead of the three densities originally applied (i.e. 1, 5 and 9 seedlings per pot). In some instances, when there was severe seedling displacement and clumping, it was not possible to identify how many seedlings were growing in the pot. Seedling density was only determinable at harvesting. As a result four of the pots had 10 seedlings established; this was incorporated into competition class 5.

The experiment ran for 17 weeks from the 17 April to the 18 August 2008. On the 18 August 2008 the seedlings were harvested and the number of seedlings that were present in the pot was noted as well as the degree of seedling clumping and the position of the pot in the shade enclosure. The harvested seedlings were dried at $60^{o}$C for 3 days. In each pot the dry weight of the seedlings were divided by the number of seedlings in the pot to give the average weight of the seedling per treatment per pot and was expressed as the mean dry weight per seedling per treatment.

An unbalanced ANOVA was used to assess the affect of shading, nutrients and competition on the mean seedling weight. To adjust for several missing treatment combinations resulting from seedling mortality a series of analyses were conducted as follows: (1) shade*competition class, (2) shade*nitrogen, (3) nitrogen*competition class and (4) shade*nitrogen*competition class. Some interactions could not be measured due to 100% seedling mortality. Due to the variability of missing treatments between replicates the unbalanced ANOVA could only calculate predicted log transformed average seedling weights (Payne *et al.* 2006). Since the statistical significance of the model is based on these predicted weights any additional analyses on the data were carried out using the predicted, rather than actual average weights.

All statistics were calculated using Genstat (v 9.1). The average seedling weights were log transformed in order to meet the assumptions of the ANCOVAs.

## 5.3 Results

The covariate of pot position in the enclosure had no significant influence on the model (p>0.05) and was excluded from further analyses. Shade had a significant effect on the growth of the seedlings but competition and nitrogen did not with the respective p-values of <0.001 (Table 5.1 and Table 5.2), 0.266 (Table 5.2) and 0.069 (Table 5.3). The shade by nitrogen, nitrogen by competition and an interaction of all three factors had no significant effect on the growth of the seedlings with p-values of 0.876 (Table 5.1), 0.742 (Table 5.3) and 0.964 (Table 5.4) respectively. Shade by competition was the only interaction that had significant effects on the growth of the seedlings with a p-value of 0.032 (Table 5.2). Although the level of nitrogen did affect the seedling growth in the shade by nitrogen analysis (p = 0.002) (Table 5.1) it did not have any significant effects when analysed in the competition by nitrogen experiment (p = 0.069) (Table 5.3). Nitrogen did not interact with other environmental factors.

**Table 5.1:** Results of Unbalanced Analysis of variance for the dry weight of five grass species under a range of light, nutrient and competition intensities. Analysis for shade x nitrogen interaction.

| Change | d.f.[1] | s.s.[2] | m.s.[3] | v.r.[4] | F pr.[5] |
|---|---|---|---|---|---|
| Shade | 4 | 32.0158 | 8.0040 | 59.34 | <.001 |
| Nitrogen | 2 | 1.6608 | 0.8304 | 6.16 | 0.002 |
| Shade x Nitrogen | 8 | 0.5079 | 0.0635 | 0.47 | 0.876 |
| Residual | 223 | 30.0764 | 0.1349 | | |
| Total | 242 | 66.4303 | 0.2745 | | |

[1] degrees of freedom  [2] sum of squares  [3] mean squares
[4] variance ratio  [5] F probability

**Table 5.2:** Results of Unbalanced Analysis of variance for the dry weight of five grass species under a range of light, nutrient and competition intensities. Analysis for shade x competition interaction.

| Change | d.f.[1] | s.s.[2] | m.s.[3] | v.r.[4] | F pr.[5] |
|---|---|---|---|---|---|
| Shade | 4 | 32.0158 | 8.0040 | 61.36 | <.001 |
| Competition | 4 | 0.6858 | 0.1714 | 1.31 | 0.266 |
| Shade x Competition | 16 | 3.7749 | 0.2359 | 1.81 | 0.032 |
| Residual | 213 | 27.7844 | 0.1304 | | |
| Total | 242 | 66.4303 | 0.2745 | | |

[1] degrees of freedom  [2] sum of squares  [3] mean squares
[4] variance ratio  [5] F probability

**Table 5.3:** Results of Unbalanced Analysis of variance for the dry weight of five grass species under a range of light, nutrient and competition intensities. Analysis for nitrogen x competition interaction.

| Change | d.f.[1] | s.s.[2] | m.s.[3] | v.r.[4] | F pr.[5] |
|---|---|---|---|---|---|
| Nitrogen | 2 | 1.4674 | 0.7337 | 2.70 | 0.069 |
| Competition | 4 | 0.8779 | 0.2195 | 0.81 | 0.521 |
| Nitrogen x Competition | 8 | 1.3944 | 0.1743 | 0.64 | 0.742 |
| Residual | 223 | 60.5213 | 0.2714 | | |
| Total | 242 | 66.4303 | 0.2745 | | |

[1] degrees of freedom  [2] sum of squares  [3] mean squares
[4] variance ratio  [5] F probability

**Table 5.4:** Results of Unbalanced Analysis of variance for the dry weight of five grass species under a range of light, nutrient and competition intensities. Analysis for Shade/(nitrogen*competition) interactions.

| Change | d.f.[1] | s.s.[2] | m.s.[3] | v.r.[4] | F pr.[5] |
|---|---|---|---|---|---|
| Shade | 4 | 32.0158 | 8.0040 | 59.59 | <.001 |
| Shade x Nitrogen | 10 | 2.1687 | 0.2169 | 1.61 | 0.106 |
| Shade x Competition | 20 | 4.5659 | 0.2283 | 1.70 | 0.037 |
| Shade x Nitrogen x Competition | 31 | 2.4060 | 0.0776 | 0.58 | 0.964 |
| Residual | 172 | 23.1045 | 0.1343 | | |
| Total | 242 | 66.4303 | 0.2745 | | |

[1] degrees of freedom  [2] sum of squares  [3] mean squares
[4] variance ratio  [5] F probability

The shade and competition class interaction had a significant effect on the growth of the seedlings (measured as mean seedling dry weight). Shade was the main driving factor influencing the seedling growth of *D. eriantha* and the competitive effect was only evident at the low shading intensities (Figure 5.3). As

the level of shading decreased the affect of competition increased. This was particularly evident in the 0% and 55% shading as the seedlings in the lowest competition classes showed a dramatic increase in growth. The average weight of the seedlings in 0% shade ranged from 0.09g ±0.02g per seedling in the high competition class to 0.26g ± 0.05g per seedling with low competition. In the 55% shading the average seedlings weight was as 0.05g ±0.03g per seedling in the high competition class and 0.14g ±0.07g per seedling with low competition.

In the 70%, 85% and 93% shading there was no significant change in the seedling weight with a change in competition level ($p>0.05$). In the 70% shade the seedling weight varied from 0.06g ±0.01g per seedling to 0.06g ±0.03g per seedling in the high and low competition treatments respectively. In the 85% shade enclosures the weight varied from 0.02g ±0.024g per seedling to 0.03g ±0.014g per seedling in the high and low competition treatments respectively. In the 93% shading the weight varied from 0.02g ± 0.002g per seedling 0.01g ±0.003g per seedling in the high and low competition treatments respectively.

As the level of shading decreased there was an increase in the average seedling weight regardless of the competition intensity ($p < 0.001$) (Table 5.2).
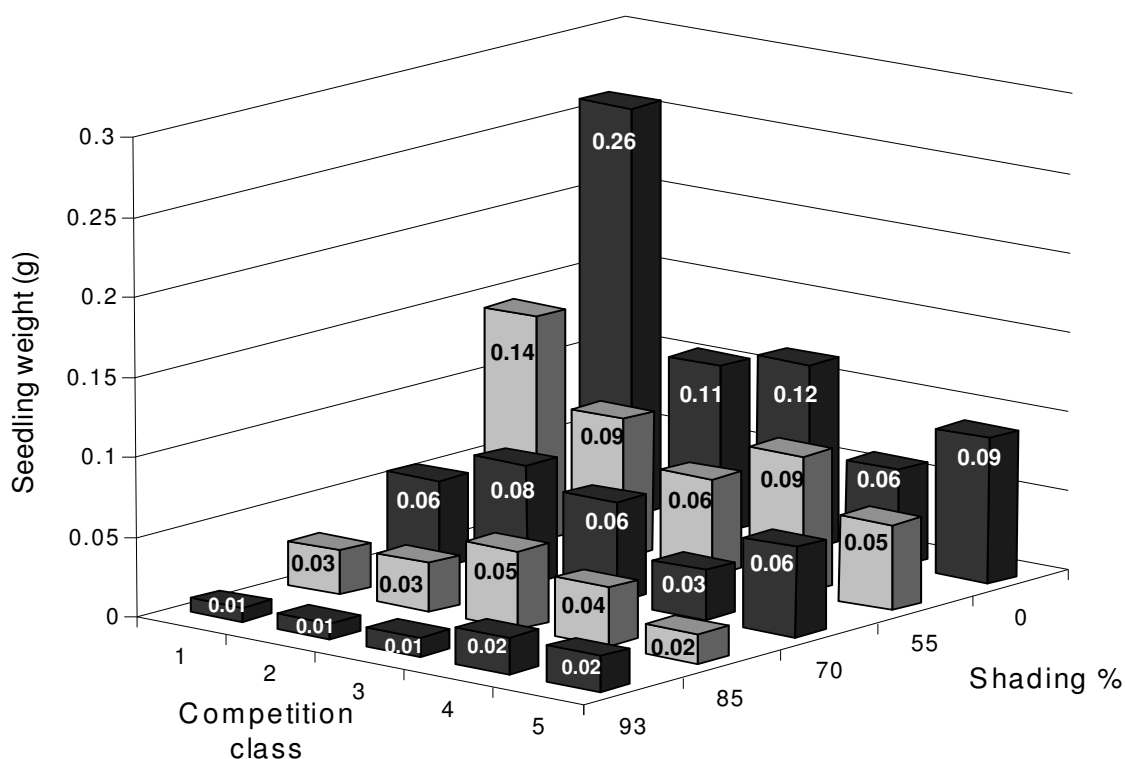
**Figure 5.3:** Relationship between the average seedling weight, percentage shade, and competition level (p = 0.032). Competition class "1" has the least number of seedlings (1-2 per pot) and class "5" has the most number of seedlings (9-10 per pot). The values on the bars represent the predicted average seedling weight (g) as calculated by the ANCOVA.

## 5.4 Discussion

*Digitaria eriantha* demonstrated a low competitive ability against four South African grass seedlings, namely *Chloris gayana*, *Cynodon dactylon*, *Eragrostis curvula* and *E. tef* (Chapter 4). It was expected, therefore, that competition intensity would have a significant effect on the growth of the seedlings, especially when there was an added stress of shading. What was found, however, was that shading had such a large influence on the growth of *D. eriantha* that the seedling weight was reduced from 0.26g ± 0.05g grams per seedling in full light to 0.01g ±0.003g grams per seedling in 93% shade at competition class 1 (pots containing 1-2 seedlings). The level of competition only had a significant effect when there was high light availability (between 0% and 55% shading). If competition was the main factor influencing *D. eriantha*'s growth then the same trends evident in the

0% and 55% shade classes would be evident in all the shade enclosures i.e. an increase in the seedlings mass with a decrease in competition, especially in the lowest level of competition.

Environmental factors may affect the growth of a plant independently, or they may interact with each other so that one factor reduces the impact of another because it has a stronger relationship with the species (Lenssen *et al.* 2003). In other words, species that are tolerant of environmental influences could be independently affected by any two environmental factors. But, for species that are intolerant of certain environmental factors the effects of a second factor would be reduced because of the dominance of the first factor as the stress factor. In this experiment the dominating stress factor is reduced light availability and even though competition significantly decreases the growth of *D. eriantha* seedlings (Chapter 4) it is not evident in this experiment until there was sufficient light for the seedling growth. Subsequently, there is a hierarchy of limitations acting on the growth of *D. eriantha* seedlings and the primary limitation is related to shading. A subsequent limitation of the seedling growth was caused by competition and the seedling growth was not influenced by nitrogen availability. It is not clear why nutrient availability did not affect seedling growth in the low shading, low competition and high nutrient treatments. A positive seedling growth was expected with low levels of shading and/or competition and high levels of nutrients.

Several studies (e.g. Veenendaal *et al.* 1993) have noted that there was an abundance of *D. eriantha* and *Panicum maximum* plants under tree canopies and not in open grasslands. Tiedemann (1970) concluded that these species grew under canopies because of the increased nutrient levels (nitrogen, calcium, potassium and phosphorus (Belsky 1994)) which convey a growth advantage that over-rides the need for high levels of light. It was surprising to find that nutrients had no effect on *D. eriantha* seedling growth when it was expected that the level of nutrients would have a higher significant effect than the levels of competition and shading.

At a seedling level, the theory put forward by Tiedemann (1970) does not hold true for *D. eriantha*. Increased nutrient levels did not reverse the decreased growth rate found with high shading intensities. A possible explanation for the findings of Veenendaal *et al.* (1993) is with an increase in sunlight in the open grassland there is an increase in the competition from surrounding species (Olff *et al.* 1994)

and *D. eriantha* seedlings may not establish as they are not good competitors (Chapter 4). Under the canopy, however, all established species are not achieving their optimal growth rates (Tiedemann *et al.* 1971) decreasing the overall competition intensity and allowing poor competitors, such as *D. eriantha*, to survive, even thought it has a high requirement for light.

A similar response to shading was seen in black grama (*Bouteloua eriopoda*) (Tiedemann *et al.* 1971). It was concluded that black grama failed to adapt to canopy shade, and the intolerance of high shade levels was more important to growth and competitive ability than the additional nutrients available under a canopy. This reasoning could be transferred to *D. eriantha*'s response as the stress caused by the low levels of light was enough to over-ride the advantage that is usually gained by an increase in nutrients availability. It would appear that the argument put forward by Lenssen *et al.* (2003), that there are interactions of growth limiting factors, holds true as there was a decreased response of *D. eriantha* seedlings to nitrogen availability when the seedlings are shaded.

As mentioned previously, there are morphological changes which can occur within a species that allow it to compete, or grow better, in low light environments. Some observations made during the experiment were that the seedlings grown in the highest shade enclosures (70%, 85% and 93%) were tall and spindly (etiolated), they did not produce tillers and the leaves were placed up along the culm. Presumably, this was to gain height and grow above the source of shading. In the low shade enclosures (0% and 55%) the seedlings were short and flat with broad leaves (high specific leaf area) and tillering spreading along the soil surface. Similar trends were identified by Wiseman *et al.* (2002) when studying the affects of pre-planting treatment on the growth of *Themeda triandra* and *Tristachya leucothrix*. In treatments which were poisoned (using Roundup[©]), or cleared using a tractor drawn scraper, there was a significant increase in the number of tillers of the transplanted seedlings compared to the burning treatments and the control. Additionally, they found that the mean height of the highest leaf was significantly less in the treatments with the most tillering. By growing against the ground, rather than vertically when light was not limiting, the seedlings were taking up more space in the pot. If another individual happened to grow near the seedlings the level of shading would be reduced because the "intruder" would not shade out the entire area. Additionally, having large, flat leaves increases the amount of light

interception increasing the potential photosynthetic rate and, therefore, growth rate (Wright and Westoby 2001). The change in growth form indicates that *D. eriantha* is shade intolerant. The growth structures are preventing or decreasing the shading experienced by the individual.

Monk and Gabrielson (1985) demonstrated that shade is the most important factor limiting plant biomass, number of species and individual species performance in a grassland. Additionally, they demonstrated that species respond differently to shading and that shade-intolerant species are almost completely out-competed by shade-tolerant species within two years of establishment when grown in 55% shade. *Cynodon dactylon* was the only species which demonstrated an increased biomass in shade conditions when a significant reduction in biomass was measured in the other species. Tiedemann *et al.* (1971) noted that all species grow best in full sunlight but also identified a varied response of species to shading. Two of the species studied showed a non-significant reduction in growth, one species *Trichachne californica* showed a five fold reduction in growth and *Bouteloua eriopoda* an eight times reduction in growth. Additionally, Tiedemann *et al.* (1971) measured the physiological adaptations of these grasses to shading and noted that three of the grasses, *T. californica*, *Setaria macrostachya* and *Muhlenbergia porteri* are capable of adapting to shade. For example, they had significantly increased leaf lengths. The leaf length of *B. eriopoda*, however, did not change when grown in the shade suggesting that it did not adapt to conditions of reduced light. Patterson (1980) found similar physiological adaptations to shade when studying the response of *Imperata cylindrica* to shading. Unfortunately, Monk and Gabrielson (1985), Tiedemann *et al.* (1971) and Patterson (1980) did not assess the effect of nutrient on the growth of these species when shaded.

There is scarce information regarding the role that shading has on the growth of grass seedlings, this is especially evident when evaluating the effect of shade in conjunction with other growth limiting factors, such as nitrogen and competition. There is some evidence, however, that the effect of shade on grass growth is dependent on the level of water and nutrients available to the plant (Ludwig *et al.* 2001). When there is sufficient water and nutrients for optimal growth then shading will have a negative affect on the grasses' growth compared to grass growing in full sunlight. The decrease in dry mass yield under shade will be proportional to the level of shading (Robinson 1991, Wilson and Wild 1991, Cruz 1997). These

effects are most evident when growth is limited by nutrient availability under full light (Wilson and Wild 1991). In the dry season, however, shading from the canopy vegetation decreases the rate of soil water evaporation and the growth of the herbaceous layer increases (Ludwig *et al.* 2001). Unfortunately Ludwig *et al.* (2001) did not assess the species specific responses to varying ratios of light and nutrients. These trends were not evident when examining *D. eriantha* seedling growth as the growth was always negatively effected by shading regardless of the availability of nutrients in full sun and under shade. The alternative explanation is that the seedlings were assimilating enough nitrogen for optimal growth at the lowest nitrogen concentration treatment (20% Hoagland's solution) and an increase in available nitrogen was not beneficial for increasing the seedlings growth rates and competitive ability.

In conclusion, shading is a primary stress factor for *D. eriantha* seedlings and significantly hampers the seedlings growth. Exposing the seedlings to additional stress factors (such as low nutrient availability and high competition levels) whilst the seedlings are shaded will have no additional affect on the seedlings growth. Evidence suggests that *D. eriantha* is an obligate heliophyte, which means that it is not capable of physiologically adapting to growing under heavy shading (Tiedemann *et al.* 1971).

It is clear that the relationship between light and nutrient availability and competition is not straight forward. It is, therefore, imperative that the species specific response of seedlings to shading is known prior to selecting a species for rehabilitation. For example, *D. eriantha* should not be used for over sowing established vegetation. Getting seedlings of *D. eriantha* to establish with pre-established vegetation is not likely because of its low competitive ability (Chapter 4) and intolerance of shading. On the other hand, *C. dactylon* adapts well to shading (Monk and Gabrielson 1985) and is a relatively good competitor and can, therefore, be used to over sow vegetation.

# Chapter 6: The response of seeds and seedlings of five South African grasses to different concentrations of smoke-infused water

Authors on this paper are:
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This chapter is in the format of a paper intended for submission to the African Journal of Range and Forage Science. There will be some information overlap within the dissertation.

# Chapter 6: The response of seeds and seedlings of five South African grasses to different concentrations of smoke-infused water

## 6.1 Introduction

Through experimentation it was discovered that plant-derived smoke produces a chemical, or a combination of chemical compounds, that increases the rate and success of seed germination  (Harker 1959, cited by Kulkarni *et al.* 2007) and seedling vigour (root and shoot extension rate) (Blank and Young 1998, Light and Van Staden 2003, Sparg *et al.* 2005, Van Staden *et al.* 2006, Kulkarni *et al.* 2007). The compound, 3-methyl-2H-furo[2,3-*c*]pyran-2-one, is a  butenolide compound that has been isolated from plant smoke (Van Staden *et al.* 2004) and is believed to be one of the compounds which acts on the seed, in a similar manner to the plant hormone auxin (Jain *et al.* 2008).

The smoke from a variety of carbon based compounds (including tissue paper) promotes germination showing that the active butenolide compound can be found in the smoke of any carbon based substance (Jager *et al.* 1996). Different plants species, however, produce smoke with different germination inducing potentials. Jager *et al.* (1996) demonstrated that the smoke produced from four plant species effected the germination of Grand rapids lettuce seeds differently. Likewise, Baxter *et al.* (1995) showed that *Themeda triandra* did not show the same germination response to smoke derived from 27 different species.

Butenolide is highly active and works at very small concentrations (Van Staden *et al.* 2004). Dilutions between 1:100 and 1:1000 of a particular batch of smoke-infused water had significant effects on seedling growth and germination percentage (Drewes *et al.* 1995, Light *et al.* 2002, Light and Van Staden 2003, Van Staden *et al.* 2004, and Kulkarni *et al.* 2007). Baldwin *et al.* (1994) showed that less than $1^{-12}$g of the compound is required to stimulate the germination response of *Nicotiana attenuata*.

Artificially increasing the germination rate of seeds and increasing the seedling vigour may be beneficial for the rehabilitation. Increasing germination success means that less seeds will be required to reseed an area. Likewise, increasing the seedling vigour, and therefore time to seedling establishment and seedling survival

will also decrease the number of seeds required. Conversely, removing the need for germination cues by treating seeds with smoke-infused water may be detrimental to seedling survival. The seedling may be forced to establish in sub-optimal growth conditions and, if this occurs, the seedling is more dependent on its cotyledonous reserves. When treated with smoke-infused water, which increases seedling vigour, there is a decrease in the efficiency of nutrient and resource use. In effect, the cotyledonous reserves may be exhausted before the environmental conditions around the seedling improve.

The purpose of the study was to investigate if the "smoke affect" is evident in South African grasses, especially since some grasses have a naturally high germination rate and fast growth. Experiments were conducted to determine the affect of varying concentrations of smoke-infused water on 1) the germination success and 2) seedling vigour measured as shoot and root growth.

## 6.2 Materials and Methods

The smoke-infused water was made using grass collected at the University of KwaZulu-Natal's Ukulinga Research Farm (Murray road, Pietermaritzburg). The grass was burnt in an iron furnace and the smoke was pulled through a double layered pipe into approximately 75 liters of water using a reversed compressor system. The smoke was contained in the inner pipe and the outer pipe acted as a cooling system allowing water to flow around the inner pipe. The process of burning grass and sucking the smoke through water continued for three days until the water was a dark golden-yellow colour. The species used in the following experiments were *Cynodon dactylon*, *Chloris gayana*, *Digitaria eriantha*, *Eragrostis curvula* and *Eragrostis tef*. Physiological information about these species can be found in Chippendall (1959), Clayton *et al.* (2006 onwards), Gibbs Russell *et al.*(1991), Tainton *et al.* (1990), Tainton (1999) and van Oudtshoorn (2002)

*6.2.1. The effect of smoke-infused water concentration on grass seed germination*

The purpose of this experiment was to determine if smoke-infused water affects the germination rate and percentage germination of the five grass species. There were six smoke-infused water concentrations applied to each species, namely 1:2, 1:10, 1:100, 1:500, 1:1000, 1:5000 and a control where seeds were only exposed to distilled water. A secondary objective of the experiment was to evaluate the

affect of rinsing the seeds after exposure to smoke-infused water. Seeds which were treated with smoke-infused water were either rinsed with distilled water after being soaked in the smoke-infused water for 1.5 hours (rinsed treatment) or they were immersed in smoke-infused water for the duration of the experiment ("not rinsed" treatment). Rinsing was included as a treatment to simulate a rainfall event after the seeds were exposed to the smoke-infused water. Additionally, there is evidence that suggests that rinsing a seed after exposure to a high concentration of smoke-infused water may remove the germination inhibitory affect (Light *et al.* 2002).

Experimental evidence suggests that grass seeds (perennial and annual ryegrass) imbibe most of the water required for germination within the first four hours of imbibition but a high percentage of this water was absorbed within the first two hours (McWilliam *et al.* 1970). Likewise, corn kernels showed the highest water absorption rate was in the first five hours of imbibition with approximately half the required water for germination absorbed within the first one and a half hours (Laria *et al.* 2006). Additionally, the time that seeds should be soaked in the smoke-infused water is dependent on the smoke-infused water concentration and the species being tested. Light *et. al.* (2002) demonstrated that, for Grand Rapids lettuce seeds, the time of imbibition could be 1 hour at high concentrations (1:100) or up to six hours at low smoke-infused water concentrations (1:1000). The time of 1.5 hours was chosen for this experiment as a wide range of smoke-infused water concentrations were being tested and any effect of the smoke-infused water on the grass seeds germination success should be evident after this imbibition time.

In total there were 13 treatments that were applied to each species (6 smoke-infused water concentration treatments which were either rinsed or not rinsed, and the control). Twenty five seeds of each species, for each treatment, were placed in a petri dish that was lined with Whatman's #2 filter paper. There were six replications per treatment combination per species resulting in a total of 390 dishes. The position of the petri dishes in the germination chamber was randomised. The germination chamber temperature fluctuated between $25^{o}C$ and $30^{o}C$ (with no light) from 25 February 2008 to 21 April 2008 (8 weeks). Germination was assessed weekly and seeds which had germinated (the hypocotyl was visible) were counted and removed.

Because the data were expressed as percentage germination per petri dish the data were arcsine square-root transformed to normalize and restrict the confidence limits to between 0 and 100% (McDonald 2008). An ANOVA was performed on the transformed data to determine the effect of smoke-infused water concentration and rinsing on germination success. The calculated means were then back transformed into percentages in order to present the data in a more meaningful, practical format. Five percent least significant difference values calculated in the ANOVA were used to determine the significant interactions within the analysis. All analyses were done using GenStat v9.1.

The seeds from the control were neither rinsed nor not rinsed and a comparison of germination percentage of both rinsing treatments was required against the controls germination percentage. The control was removed as a "concentration treatment" and integrated into the ANOVA syntax formula as (control/(treatment*concentration))*species).

### 6.2.2 The effect of smoke-infused water concentration on grass seedling vigour

The seedling vigour experiment was carried out in a greenhouse at the University of KwaZulu-Natal's Neil Tainton Arboretum (Life Science Campus). The trial took place for 24 days from the 29 September 2008 to the 22 October 2008. Seeds of the five species used in the previous experiment were treated with three different concentrations of smoke-infused water, namely 1:1000, 1:500 and 1:2 as well as a control (soaked in water). The seeds were soaked in their respective concentrations for two hours then two seeds of the same treatment and species were placed in a petri dish. The number of seedlings per petri dish was kept to a minimum so that each seedling could be identified and its growth accurately tracked. It would have been difficult to accurately identify the individual seedlings if there were more than two seedlings per dish since the seedlings moved around the petri dish as they grew (the root thrust caused the seedling's position to shift) and they were also displaced by water drop action.

The petri dishes had holes burned through the bottom to allow for water drainage and were then lined with Whatmans #2 filter paper. There were eight replications per treatment combination (species x concentration), and 16 seedlings per species per treatment. The petri dishes were placed on a mist-bed (in a

randomised order) and sprayed with a fine mist for 30 seconds every 20 minutes to keep them moist.

The length of the root (sum of the rootlet lengths) and shoot (hypocotyl length and the sum of the leaf lengths) were recorded daily for the duration of the trial. Repeated measures ANOVAs were run on the full data set to examine the effects of treatment on the pattern of growth over time. Because of the variability in time to germination a subset of the data, the growth of a seedling after 10 days was also examined. This was done to standardise the time of growth so that a direct comparison can be made within species.

General ANOVAs were carried out on the 10 measurement data set followed by a Tukey's test for unequal number of samples (not all the seeds for each treatment germinated). The aim of this experiment was to determine if there was a difference in seedling growth between treatments, within a species. The growth of each species were analysed independently.

## 6.3 Results

*6.3.1 The effect of smoke-infused water concentration on grass seed germination*

Statistical analysis on the seed germination percentage after treatment with smoke-infused water revealed that two treatment combinations had significantly different germination percentages compared to the control. The two treatment combinations were the interaction of treatment (rinsing) and smoke-infused water concentration (p=0.003) and the interaction of treatment (rinsing) and species (p=0.006). These interactions are depicted as control x treatment x concentration and control x treatment x species in Table 6.1. In the control x treatment x concentration interaction the only significant difference in the germination percentage from the control was in the 1:2 non rinsed treatments where there was a decrease in the average germination from 45.9% ±1.614 (control) to 36% ±1.614 (Figure 6.1). The main reason for the decrease in germination at 1:2 non rinsed treatment was because of *E. tef*, as indicated in Figure 6.2 (control x treatment x species interaction). Further analysis indicated that there was a significant decrease in the germination of *E. tef* when treated with 1:2 concentration and not-rinsed (p < 0.001) (Figure 6.3). This was the only control x treatment x concentration interaction which was significant in species independent analyses (Table 6.1).

In general, germination of *E. curvula* and *E. tef* was high (>60 %) even without treatment from smoke-infused water. There was a significant positive response of *E. curvula* to rinsing after exposure to smoke-infused water with average germination increasing from 64.9% ±3.609 to 78.8% ±1.473 (Figure 6.2) even though there was no significant response to any particular concentration (p = 0.323) (Table 6.1). There was a significant negative overall response of *E. tef* to not being rinsed after exposure to smoke-infused water, decreasing the average germination percentage from 97.8% ±3.609 to 91.1% ±1.473 (Figure 6.2). This overall decrease in germination when the seeds were not rinsed was due to the significant decrease in germination percentage when the seeds of *E. tef* were treated with the 1:2 concentration and not rinsed (62.6% ±3.68) (Figure 6.3). For *C. dactylon*, *C. gayana* and *D. eriantha* there was no difference in the germination percentage when the seeds were treated with smoke-infused water or whether they were rinsed or not (Figure 6.2).

**Table 6.1:** Results of Analysis of variance for the Arcsine transformed germination percentage of 5 grass species treated with six concentrations of smoke-infused water. The treatments were either rinsed or not rinsed the seeds after smoke-infused water imbibition.

| Source of variation | d.f.[1] | s.s.[2] | m.s.[3] | v.r.[4] | F pr.[5] |
|---|---|---|---|---|---|
| Control | 1 | 0.06 | 0.06 | 0.00 | 0.978 |
| Species | 4 | 162627.1 | 40656.79 | 520.28 | <.001 |
| Control x Treatment | 1 | 46.36 | 46.36 | 0.59 | 0.442 |
| Control x Concentration | 5 | 596.82 | 286.47 | 1.53 | 0.181 |
| Control x Species | 4 | 670.92 | 284.23 | 2.15 | 0.075 |
| Control x Treatment x Concentration | 5 | 1432.36 | 119.36 | 3.67 | 0.003 |
| Control x Treatment x Species | 4 | 1136.94 | 167.73 | 3.64 | 0.006 |
| Control x Concentration x Species | 20 | 2039.63 | 101.98 | 1.31 | 0.173 |
| Control x Treatment x Concentration x Species | 20 | 1812.54 | 90.63 | 1.16 | 0.288 |
| *C. dactylon*: Control x Treatment x Concentration | 5 | 219.21 | 43.84 | 1.30 | 0.276 |
| *C. gayana*: Control x Treatment x Concentration | 5 | 341.50 | 68.30 | 0.94 | 0.461 |
| *D. eriantha*: Control x Treatment x Concentration | 5 | 269.7 | 53.9 | 0.40 | 0.849 |
| *E. curvula*: Control x Treatment x Concentration | 5 | 451.60 | 90.32 | 1.19 | 0.323 |
| *E. tef*: Control x Treatment x Concentration | 5 | 1962.90 | 392.58 | 5.43 | <.001 |

[1] degrees of freedom [2] sum of squares [3] mean squares
[4] variance ratio [5] F probability
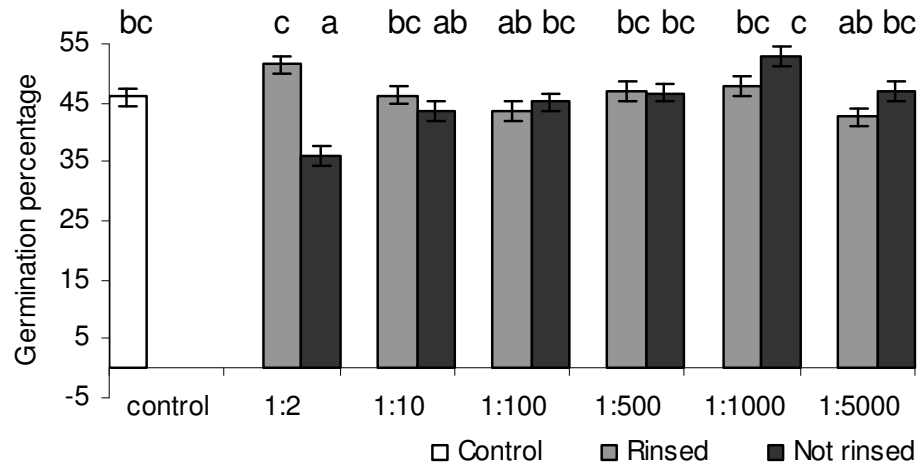
**Figure 6.1:** The effect of smoke-infused water concentrations and rinsing on the mean germination percentage (back arcsine square-root transformed) of five grass species (p = 0.003). Bars with letters in common are not significantly different using calculated least significant value of 4.491. Vertical lines represent the standard errors.
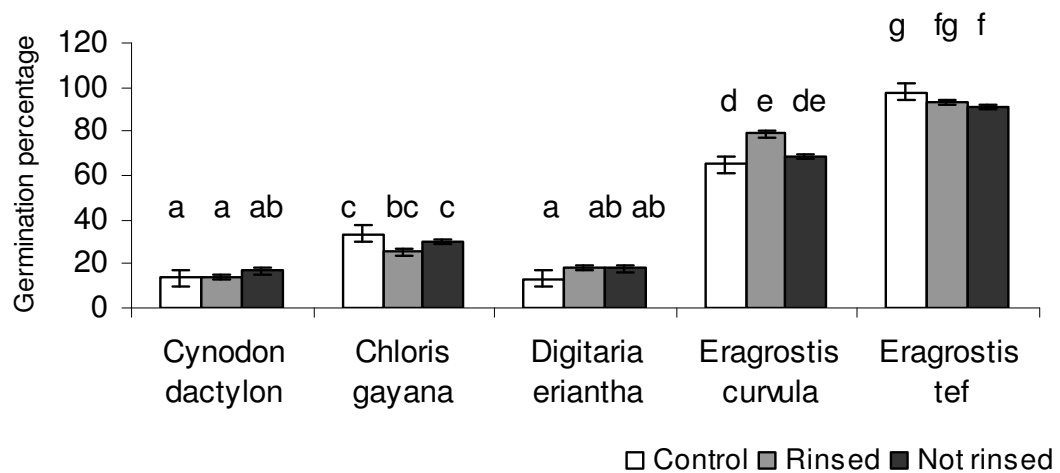


**Figure 6.2:** Smoke-infused water and rinsing on the mean germination percentage of 5 species (back arcsine square-root transformed) (p = 0.006). Bars with letters in common are not significantly different as calculated by the least significant difference value of 7.669. Vertical lines represent the standard error.
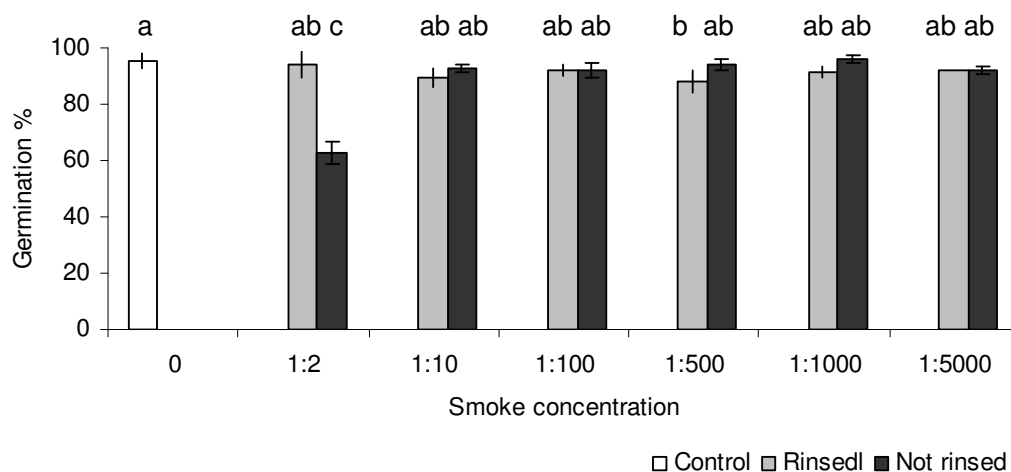
**Figure 6.3:** Germination percentage of *Eragrostis tef* when treated with varying concentrations of smoke-infused water, with and without rinsing treatments (p < 0.001). The grey bars represent the rinsed treatments and the black bars are for the non-rinsed treatments. The vertical lines represent the standard errors. Bars with letters in common are not significantly different using calculated least significant value of 9.812.

*6.3.2. The effect of smoke-infused water concentration on grass seedling vigour*

The analysis of variance to determine differences of within-species growth when treated with four concentrations of smoke-infused water for the full growing period of up to 22 days could not be carried out due to different growth periods of each seedling measured. When the number of days of growth was limited to 10 days the ANOVA showed that the growth of *C. dactylon*'s roots and *E. curvula*'s shoots and roots had significant differences in its length between concentration treatments (p = 0.024, p < 0.001 and p = 0.037 respectively) (Table 6.2). The shoot of *C. dactylon* and the shoots and roots of the other three species (*C. gayana*, *D. eriantha* and *E. tef*) did not show a significant change in growth between smoke-infused water concentration treatments (p > 0.05).

Repeated measures ANOVAs were performed on *C. dactylon*, *C. gayana*, *D. eriantha*, *E. curvula* and *E. tef* to determine if smoke-infused water affected the growth pattern of the seedlings' roots and shoots for the full growth period of 22 days and the 10 day data set. This analysis is additional to the previous ANOVAs as they only showed differences in the final length. The pattern of the root and shoot growth will indicate at what time the compounds began to affect the seedling's development and for how long a growth advantage is experienced.

There were no significant changes in the growth rates and growth patterns of *C. gayana*, *D. eriantha* and *E. tef* seedlings when treated with smoke-infused water (p>0.05). The data analyses for these three species did not continue further.

*Cynodon dactylon* (root and shoot) showed a significantly different growth pattern with a change in concentration over time for the full data set and when measuring the first 10 days growth (p < 0.001) (Table 6.3a and Table 6.4a respectively). After 7 days of growth the control's shoot growth pattern was significantly different from the 1:1000 concentration and ten days after germination the growth pattern of the control was significantly different from treated seedlings of all concentration (Figure 6.4a and Figure 6.5a), even though the actual average shoot lengths were not significantly different (p = 0.145) (Table 6.2 and Table 6.5a). At 12 days after germination the growth pattern of the control was significantly different from the seedlings treated with the 1:2 concentration (Figure 6.4a) but the seedlings' shoots were approximately the same length of the treated seedlings. The growth rate of the seedlings shoots, when treated with the 1:2 concentration of smoke-infused water, showed an increased growth rate after 8 days growth which was still increasing after 12 days (Figure 6.4a).

When the seeds of *C. dactylon* were treated with smoke-infused water diluted at 1:1000 there was a significant increase in the growth of the root, after 10 days growth, when compared to those treated with 1:2 and 1:500 concentrations (Table 6.5a, Figure 6.4b). The root length of seeds treated with the 1:1000 concentration increased faster than the other treated seedlings six days after germination (Figure 6.4b and 6.5b) and continued to have a significantly different growth pattern till 11 days after germination. At this time the root growth of the seedlings treated with the 1:1000 concentration and the 1:2 concentration were not significantly different (Figure 6.4b) as there was a rapid increase in the roots of the 1:2 concentration treatment after 9 days growth (Figure 6.4b). Twelve days after germination the roots treated with the 1:2 concentration were significantly longer than the seedlings pre-treated with the 1:1000 concentration and the control (Table 6.5a).

The repeated measures analysis for *E. curvula* shows that the growth pattern for the shoot and root after 10 days growth (p > 0.001 and p = 0.006 respectively) and full the full data set (p > 0.001) are significantly different between treatment concentrations (Table 6.3b and Table 6.4b respectively). The final length of the shoot in the 1:1000 treatment was significantly lower than the other treatments (p

< 0.001) (Table 6.2b and Table 6.5b) due to a growth rate that was noticeably slower than the other treatments after 10 days growth (Figure 6.5c). Prior to this the growth pattern of the 1:1000 concentration was significantly different to the 1:2 concentration treated seedlings after seven days growth (Figure 6.5c). Unlike the growth of the roots and shoots of *C. dactylon*, there was no delayed increase in the growth of *E. curvula* in the 1:2 treatments.

The roots of *E. curvula*, when pre-treated with 1:500 concentration of smoke-infused water, showed increased growth compared to the other treatments (Figure 6.5d). Six days after germination the difference in root length, compared to the other treatments, was significantly higher (Figure 6.5d). After 10 days, however, the only significant difference in growth was a lower root length of the 1:1000 treatment (Figure 6.4d) which was significantly different to the control (Table 6.5b).

**Table 6.2:** Results for the analysis of variance for growth (cm) of the roots and shoots of two grass species after 10 days growth.

a) *Cynodon dactylon*

|  | Shoot growth after 10 days | | | | | Root growth after 10 days | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Source of variation | d.f.[1] | s.s.[2] | m.s[3] | v.r.[4] | F pr.[5] | d.f. | s.s. | m.s. | v.r. | F pr. |
| Concentration | 3 | 1.88 | 0.63 | 2.05 | 0.145 | 3 | 18.4 | 6.12 | 4.06 | 0.024 |
| Residual | 17 | 5.18 | 0.3 | | | 17 | 25.6 | 1.51 | | |
| Total | 20 | 5.96 | | | | 20 | 28.7 | | | |

b) *Eragrostis curvula*

|  | Shoot growth after 10 days | | | | | Root growth after 10 days | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. | d.f. | s.s. | m.s. | v.r. | F pr. |
| Concentration | 3 | 4.71 | 1.57 | 8.48 | <.001 | 3 | 9.65 | 3.22 | 3.12 | 0.037 |
| Residual | 38 | 7.04 | 0.19 | | | 38 | 39.18 | 1.03 | | |
| Total | 41 | 9.97 | | | | 41 | 45.55 | | | |

[1] degrees of freedom  [2] sum of squares  [3] mean squares
[4] variance ratio  [5] F probability

**Table 6.3:** Results for the repeated measures ANOVA for the growth (cm) of the shoots and roots of two grass species over a period of 22 days.

a) *Cynodon dactylon*

| Source of variation | Shoot growth over time | | | | | Root growth over time | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d.f.[1] | s.s.[2] | m.s.[3] | v.r.[4] | F pr.[5] | d.f. | s.s. | m.s. | v.r. | F pr. |
| Subject stratum | | | | | | | | | | |
| Conc | 3 | 2.82 | 0.94 | 1.26 | 0.3 | 3 | 34 | 11.3 | 1.94 | 0.14 |
| Residual | 50 | 37.2 | 0.74 | 15.3 | | 51 | 299 | 5.86 | 15 | |
| Subject x Time stratum | | | | | | | | | | |
| Time | 11 | 151 | 13.7 | 281 | <.001 | 13 | 1088 | 83.7 | 215 | <.001 |
| Time x Conc | 31 | 5.29 | 0.17 | 3.51 | 0.03 | 31 | 57.7 | 1.86 | 4.78 | <.001 |
| Residual | 370 | 18 | 0.05 | | | 354 | 138 | 0.39 | | |
| Total | 465 | 94.9 | | | | 452 | 592 | | | |

b) *Eragrostis capensis*

| Source of variation | Shoot growth over time | | | | | Root growth over time | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d.f. | s.s. | m.s. | v.r. | F pr. | d.f. | s.s. | m.s. | v.r. | F pr. |
| Subject stratum | | | | | | | | | | |
| Conc | 3 | 24.7 | 8.24 | 3.03 | 0.04 | 3 | 126 | 42.1 | 2.75 | 0.05 |
| Residual | 50 | 136 | 2.72 | 20.7 | | 52 | 796 | 15.3 | 13.1 | |
| Subject x Time stratum | | | | | | | | | | |
| Time | 13 | 279 | 21.4 | 163 | <.001 | 16 | 4109 | 257 | 219 | <.001 |
| Time x Conc | 39 | 15.3 | 0.39 | 2.98 | 0.001 | 45 | 198 | 4.41 | 3.77 | <.001 |
| Residual | 492 | 64.8 | 0.13 | | | 510 | 597 | 1.17 | | |
| Total | 597 | 301 | | | | 626 | 2527 | | | |

[1] degrees of freedom  [2] sum of squares  [3] mean squares
[4] variance ratio  [5] F probability

**Table 6.4:** Results for the repeated measures ANOVA for the growth (cm) of the shoots and roots of two grass species over a period of 10 days.

*a) Cynodon dactylon*

| Source of variation | Shoot growth over time | | | | | Root growth over time | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d.f.[1] | s.s.[2] | m.s[3] | v.r.[4] | F pr.[5] | d.f. | s.s. | m.s. | v.r. | F pr. |
| Subject stratum | | | | | | | | | | |
| Concentration | 3 | 2.82 | 0.941 | 1.6 | 0.2 | 3 | 34.3 | 11.43 | 3 | 0.039 |
| Residual | 50 | 29.3 | 0.587 | 14.6 | | 51 | 194 | 3.807 | 12 | |
| Subject x Time stratum | | | | | | | | | | |
| Time | 9 | 74.7 | 8.301 | 207 | <.001 | 9 | 420 | 46.71 | 147 | <.001 |
| Time x Concentration | 27 | 3.61 | 0.134 | 3.33 | 0.002 | 27 | 37.7 | 1.395 | 4.39 | 0.001 |
| Residual | 358 | 14.4 | 0.04 | | | 339 | 108 | 0.318 | | |
| Total | 447 | 74.4 | | | | 429 | 440 | | | |

*b) Eragrostis curvula*

| Source of variation | Shoot growth over time | | | | | Root growth over time | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d.f. | s.s. | m.s. | v.r. | F pr. | d.f. | s.s. | m.s. | v.r. | F pr. |
| Subject stratum | | | | | | | | | | |
| Concentration | 3 | 11.7 | 3.9 | 2.08 | 0.12 | 3 | 36.4 | 12.1 | 2.04 | 0.12 |
| Residual | 50 | 93.7 | 1.87 | 37.2 | | 52 | 310 | 5.96 | 16.1 | |
| Subject x Time stratum | | | | | | | | | | |
| Time | 8 | 94 | 10.4 | 207 | <.001 | 9 | 333 | 37 | 100 | <.001 |
| Time x Concentration | 2 | 4.85 | 0.18 | 3.57 | <.001 | 27 | 30.1 | 1.12 | 3.02 | 0.01 |
| Residual | 367 | 18.5 | 0.05 | | | 354 | 131 | 0.37 | | |
| Total | 456 | 119 | | | | 445 | 600 | | | |

[1] degrees of freedom  [2] sum of squares  [3] mean squares
[4] variance ratio  [5] F probability

**Figure 6.4:** The effect of smoke-infused water concentration (1:1000, 1:2, and 1:500) on the growth patterns of *Cynodon dactylon*'s (CDA) shoot (a) and root (b) growth and *Eragrostis curvula*'s (ECU) shoot (c) and root (d) growth over a period of 22 days.
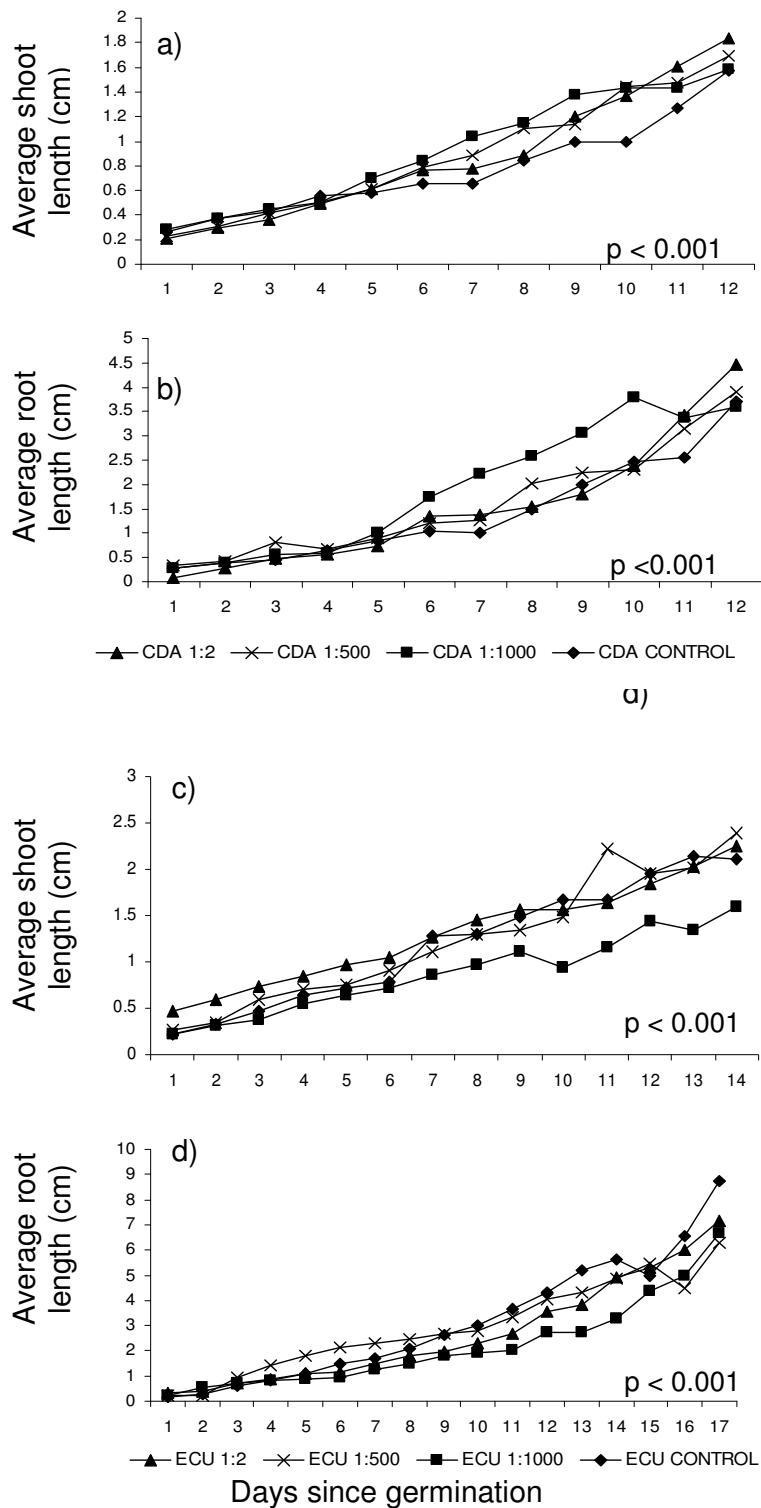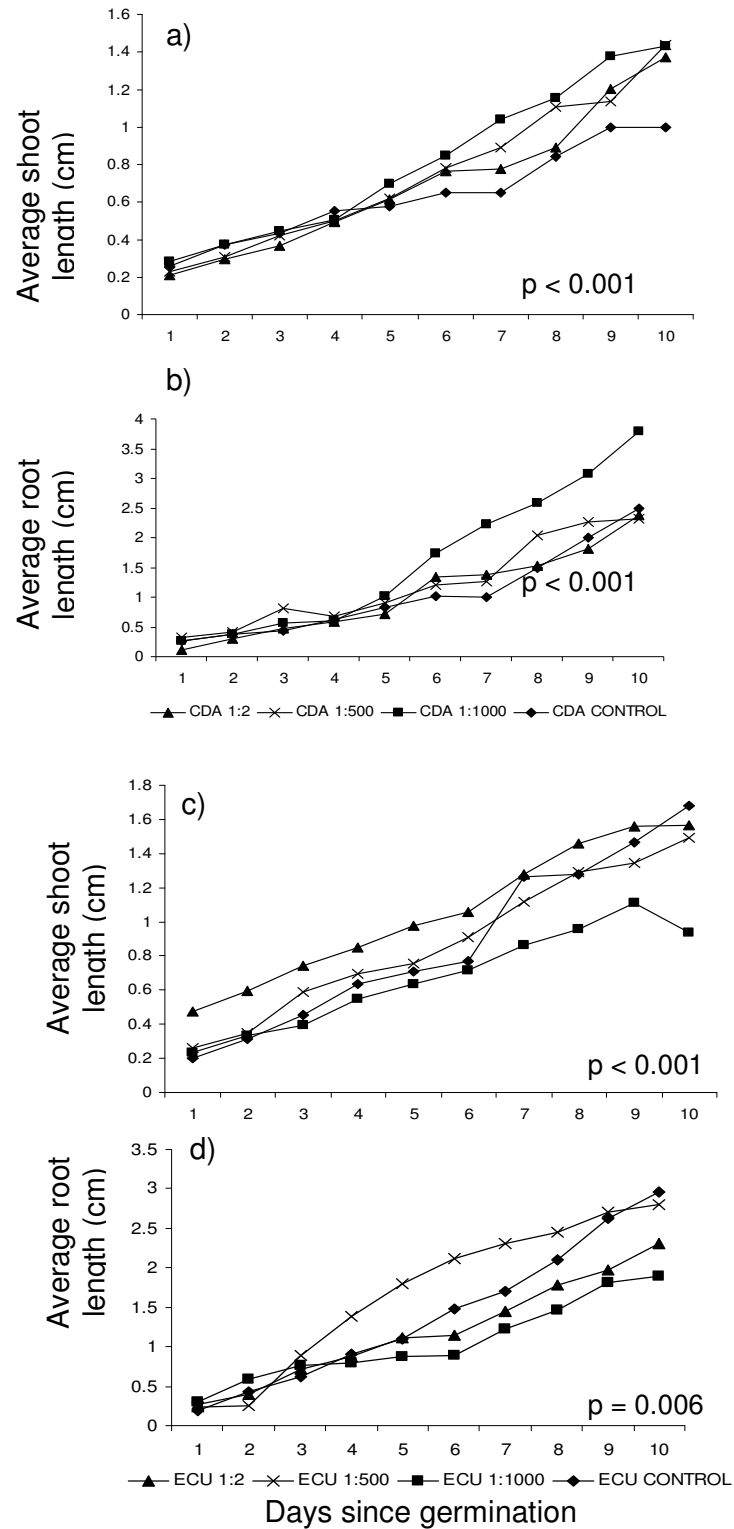
**Figure 6.5:** The effect of smoke-infused water concentration (1:1000, 1:2, and 1:500) on the growth patterns of *Cynodon dactylon*'s (CDA) shoot (a) and root (b) growth and *Eragrostis curvula*'s (ECU) shoot (c) and root (d) growth over a period of 10 days.

**Table 6.5:** Results for the post-hoc comparisons (Tukey's test for unequal sample size) for the growth (cm) of the roots and shoots of two grass species over a period of 10 days. "Yes" indicates there is a significant difference in root or shoot length between the respective concentrations ($p < 0.05$), "No" indicates that there is not ($p > 0.05$).

a) *Cynodon dactylon*

| | Shoot Growth | | | | Root Growth | | | |
|---|---|---|---|---|---|---|---|---|
| Concentration | 0 | 1:2 | 1:500 | 1:1000 | Concentration | 0 | 1:2 | 1:500 | 1:1000 |
| 0 | | No | No | No | 0 | | No | No | No |
| 1:2 | No | | No | No | 1:2 | No | | No | Yes |
| 1:500 | No | No | | No | 1:500 | No | No | | Yes |
| 1:1000 | No | No | No | | 1:1000 | No | Yes | Yes | |

b) *Eragrostis curvula*

| | Shoot Growth | | | | Root Growth | | | |
|---|---|---|---|---|---|---|---|---|
| Concentration | 0 | 1:2 | 1:500 | 1:1000 | Concentration | 0 | 1:2 | 1:500 | 1:1000 |
| 0 | | No | No | Yes | 0 | | No | No | Yes |
| 1:2 | No | | No | Yes | 1:2 | No | | No | No |
| 1:500 | No | No | | Yes | 1:500 | No | No | | No |
| 1:1000 | Yes | Yes | Yes | | 1:1000 | Yes | No | No | |

## 6.4 Discussion

### 6.4.1 The effect of smoke-infused water concentration on grass seed germination

Smoke-infused water had no consistent effect on the germination of the seeds of *C. dactylon*, *C. gayana*, *D. eriantha*, *E. curvula* or *E. tef*. There are three possible reasons for low germination response of seeds to the different concentrations of smoke-infused water.

Firstly, the time that the seeds were exposed to the smoke-infused water may not have been long enough for sufficient butenolide compound to be absorbed by the seed. Light *et al.* (2002) showed that Grand rapids lettuce seeds require an imbibition time of up to 6 hours with a 1:1000 concentration of smoke extract to promote an increased germination. Alternatively, an increased concentration of smoke-infused water would decrease the soaking time i.e. imbibition time was decreased to one hour for a 1:100 concentration. In this experiment the seeds were soaked for 1.5 hours; this may have been too short for sufficient absorption of the smoke compound in the low concentration treatments. There should,

however, have been a change in the germination percentage at the concentrations higher than 1:1000 (ie: 1:2, 1:10 and the 1:100) if smoke-infused water does affect germination of the grass seeds. Some of the seeds were constantly exposed to the smoke-infused water (not rinsed treatments) so insufficient imbibition time does not explain why there was limited response in the non rinsed treatments.

Secondly, the undiluted smoke water could have been too weak or too strong for there to be any effects. Drewes *et al.* (1995), Light *et al.* (2002),  Light and Van Staden (2003), Van Staden *et al.* (2004)  and Kulkarni *et al.* (2007) showed that the butenolide compound is highly effective at extremely low concentrations (<1:1000) whilst Jain *et al.* (2008) demonstrated that the concentration of the butenolide compound for callus production (in soybean) was $10^{-18}$ to $10^{-10}$M and $10^{-16}$M for root production. Baldwin *et al.* (1994) experimentally concluded that less than $1^{-12}$g of this compound is required per seed to stimulate the germination response of *Nicotiana attenuata*. So, in all probability, with such a large range of concentrations used in this experiment, a germination response is expected. A decreased germination percentage of *E. tef* seeds between the control (95.33% ±8.86) and the 1:2 (not rinsed) treatments (62.66% ±3.68) (Figure 6.3) shows that at this concentration the smoke-infused water is a germination inhibiter. There was, however, no evidence of an increased germination response at the lower concentrations. If the smoke water concentration was higher than the germination inducer levels then rinsing the seeds after imbibition should remove the germination inhibiting stimulus. This was not evident in this experiment (Figure 6.2). Additionally, if the smoke water concentrations were too high there would have been a general decrease in germination success when compared to the control. This too was not evident in this experiment (Figure 6.1 and 6.2). The absence of increased germination in *E. tef* with treatment of the smoke-infused water could be because the germination rate of *E. tef* is high without the influence of the butenolide compound.

Finally, it is possible that the butenolide compound found in smoke does not have a stimulatory effect on the germination of grasses, particularly the grass species examined. The indifferent response of seeds to treatment of smoke-infused water is not uncommon. Figueroa *et al.* (2009) demonstrated that 4 of 23 species tested (both exotic and indigenous to Chile) showed a response to smoke, 3 of which only showed a response when treated with a combination of smoke-

infused water and heat shock. Similarly, Thomas *et al.* (2007) demonstrated that 6 of the 18 species tested did not respond to treatment by smoke-infused water unless exposed to heat shock. An additional two species did not respond to heat, smoke-infused water or a combination of the two. It is possible that, because grasses have adapted to fire in the landscape, the species that were studied may need a combination of "heat shock" and the smoke butenolide compound for any growth advantage to be evident.

Ghebrehiwot *et al.* (2008) demonstrated that *E. tef* increased germination percentage by 35% when treated with the butenolide compound ($10^8$M) in conditions with decreased soil water potential (-0.49MPa). Therefore, the germination percentage of *E. tef* may not be affected by smoke-infused water when germination conditions are optimal, but under stressful conditions germination can be forced with treatment. Light *et al.* (2009) argue that this is beneficial to agriculture as there is potential for crop establishment to be increased in drought years. This, however, may not be the case. Ecologically enforced dormancy in seeds is important for insuring successful establishment of seedlings. Forcing seeds to germinate in adverse conditions could lead to decreased seedling establishment and survival (Olff *et al.* 1994).

*6.4.2 The effect of smoke-infused water concentration on grass seedling vigour*

The pre-treatment of seeds of *C. dactylon*, *C. gayana*, *D. eriantha*, *E. curvula* and *E. tef* with varying concentrations of smoke-infused water gave varying responses in terms of the seedling's shoot and root growths. There was no significant effect of the smoke-infused water on the growth of *C. gayana*, *D. eriantha* and *E. tef*, but there was some variable response in the growth of *C. dactylon* and *E. curvula.*

In general, the effect of the smoke-water concentration of 1:1000 on the growth of the roots and shoots of *C. dactylon* and *E. curvula* was evident within the first six days of growth. The difference between the two species was that in *C. dactylon* the affect was positive but in *E. curvula* it was negative. Additionally, the 1:2 concentration only appeared to affect the growth of *C. dactylon*'s shoots and roots (Figure 6.4a and Figure 6.4b) and not *E. curvula*'s. These results clearly indicate that the effect of the smoke-infused water on the growth of the seedling's roots and shoots is species specific. The response is not limited to the concentration

which affects the root and shoot's growth but there also seems to be a growth stimulatory and inhibiting effect.

Several studies have indicated that there may be a negative interaction between butenolide and light; similar to the affect that light has on the root stimulatory properties of auxin. Kulkarni *et al.* (2007) showed that when three *Acacia* species seedlings were treated with smoke-infused water, in dark conditions, there was a significant increase in the shoot and root length, seedling vigour and seedling mass but there was limited success when the seeds were exposed to permanent light or alternating light and dark periods. Likewise, Van Staden *et al.* (2006) found that there was a significant increase in the growth of tomato, okra, and bean seedlings when grown in complete darkness, but there was no change in the growth of the seeds that were exposed to a 16:8h light/dark cycle from the control. The non-significant response of *C. gayana*, *D. eriantha* and *E. tef* seedlings to pre-treatment with smoke-infused water may be because they were grown under natural light/dark regimes. Likewise, the non-significant response of *E. curvula*'s root and shoot to the smoke-water concentrations of 1:2 and 1:500 may also be due to the light/dark cycles present. There is no explanation in current literature for a decreased root and shoot growth of seedlings which were pre- treated with smoke-infused water as the seed.

Treating seeds with a concentration of smoke-infused water that will promote a decreased seedling growth rate may be beneficial for rehabilitation practices. Treating fast growing, highly competitive species with such a concentration may decrease the growth rate enough to allow a co-existence with the weaker competitive seedling long enough for them to establish, thereby promoting higher species diversity in the landscape. Additional advantages can be inferred onto the weaker species if treated with a concentration of smoke-infused water that promotes rapid seedling growth. For example: Chapter 4 showed that *C. dactylon* is a weak competitor at a seedling level whilst *E. curvula* is a relatively strong competitor. Pre-treating *C. dactylon* seeds with the 1:2 concentration of smoke-infused water will promote increased root growth and possibly shoot growth. Planting these seeds with *E. curvula* seeds which have been pre-treated with the 1:1000 concentration (which decreases root and shoot growth) may allow for co-establishment of these two species in the landscape.

What is evident from these experiments is the variable response of these grass species to pre-treatment with smoke-infused water. Additional investigation into the long term affects of the growth trends evident in the first two weeks of growth on the seedling's establishment success and survival probability is necessary. Once the growth advantage imposed onto the established seedling is identified it can be determined if the treatment of seeds prior to sowing is advantageous for rehabilitation practices.

# Chapter 7: Implications of experimental findings for rehabilitation

The primary goal for restoration is to increase the ecosystem functionality in the degraded area by means of decreasing soil loss, increasing water holding properties, and increasing the vegetative biodiversity. By determining which species are ecologically compatible at the seedling stage a mix of species can be selected to increase the biodiversity and rate of system recovery of a restoration area. The aim of this dissertation was to evaluate the response of grass seedlings to three growth stimuli namely, nutrients, light and smoke-infused water, and their response to competition. Consequent experimentation identified those species which are capable of forming a stable, biodiverse grassland community in order for the goals of restoration to be achieved.

## 7.1 Implications of seedling competition experiments for rehabilitation

There are distinct differences in the competitive ability of the five South African grass seedlings. There are good competitors such as *E. curvula, E. tef* and *C. gayana* and those that are not, *C. dactylon* and *D. eriantha*. There is also some evidence that it is the species that the seedling is competing against that influences the outcome of the competitive interaction (Figure 4.6). It may, therefore, be beneficial to consider a "two-phase" or "multi-phase" restoration plan which could be initiated with the focus on developing a restored area which is species-diverse. Parmenter and MacMahon (1983) showed that, at a mine reclamation site in Wyoming (USA), the time of planting and spatial pattern of the seedlings were important in the development of community structure, vegetation age structure and soil dynamics. In other words, by adding seeds at different times to a revegetation area the direction that the community interactions will drive the final community composition can be manipulated.

There are several ways that this two- or multi-phase restoration can be achieved. Manipulating the time and/or space that certain species are planted (in accordance with the community assembly theory (Section 2.2.1.3), or manipulating the nutrient levels in a patchwork manner, may allow for increased patchiness of the species distribution in the area and, therefore, decrease competition between species and increase the species diversity. There are several factors which determine the communities progression to a stable state, the primary one being

climate, which can not be controlled for. Subsequent determinants: nutrient availability, management and utilization of the area (Westoby *et al.* 1989) can all be incorporated into a two- or multi-phased restoration plan.

### 7.2.1. Manipulate the time at which species are planted

1. Large stands of the competitive species could be planted and allowed to grow till they dominate the rehabilitation area and there is increased functionality (nutrient cycling, water and soil retention). Using species from this experiment *E. curvula, E. tef* and possibly *C. gayana* could be used.

2. Once these species have established, patches of vegetation within the rehabilitation area are removed. Several methods could be employed depending on the environmental conditions. For example, burning small patches, herbicide treatments or manual removal of vegetative growth (Jutila and Grace 2002).

3. These patches are re-seeded with the weaker competitor species e.g. *C. dactylon*, *D. eriantha* and possibly *C. gayana*. These species are sown at low densities to decrease competition and avoid density dependant mortality (Zeide 1987).

4. Depending on the nutrient availability in the rehabilitation areas *C. gayana* may be planted as part of the initial mix of species or as part of the secondary species mix. In high nutrient environments it will be planted with the primary species mix, in low nutrient environments the secondary.

5. In order to encourage the species from the patches to disperse into the inter-patches of the "strong competitors" management intervention may be required. The aim would be to decrease canopy cover of the surrounding vegetation for decreased light competition (especially with species that are negatively affected by light such as *D. eriantha*). By doing so the "low competitor" sward may establish more rapidly and species not used in the rehabilitation project may have a better chance of invading the area when competition from the surrounding vegetation is reduced.

### 7.2.2. Manipulate spatial separation of the seedlings

1. Planting patches or strips of strong competitor's (*E. curvula, E. tef* and possibly *C. gayana*) will improve the landscape functionality by promoting

    soil stability, water infiltration and nutrient cycling relatively quickly. The strips act as a sink for any resources that may have been lost to the area prior to planting the vegetation (Rottle and Thruong 2005).

2. Between these areas the less competitive species (e.g. *C. dactylon*, *D. eriantha* and possibly *C. gayana*) are sown at a lower density promoting their establishment and allowing space for other naturally occurring species to invade.

3. Depending on the nutrient availability in the rehabilitation areas *C. gayana* may be planted as part of the vegetation strips or as part of the species mix between the strips. In high nutrient environments it will be planted with the vegetative strips, in low nutrient environments the inter-strip species mix.

### 7.2.3. Manipulating the fertility gradient

1. The method for manipulating the fertility gradient is similar to that described above with respect to manipulating the time at which seeds are planted, except high levels of nutrients are added to the area. The nutrients can be any in any form (organic or inorganic) as long as they are high in essential minerals for plant development such as nitrogen, phosphorus and potassium.

2. Once ecosystem functioning has increased the addition of nutrient is stopped. The vegetation patches for the "second phase" are cleared and replanted with the less competitive species.

Another option would be to sow all the species at the same time and apply fertilizer conservatively to patches in the landscape. Additionally, the erection of small shading enclosures will promote a patchwork of dominating species. The species which dominate these areas will be fast growing and do not conservatively use nutrients. They will dominate initially but as the slow growing, nutrient conservative species develop there could be a shift in species dominance. In order to manipulate the rate at which the dominance of species changes within the area the time between nutrient addition, concentration of the fertilizer and shading intensities can be adjusted.

Hardy (2008, pers. comm.) mentioned that *D. eriantha* pastures are often invaded by fast growing species such as *E. curvula* and *E. tef*. A revegetation technique subsequently suggested was planting large stands of the least

competitive species, such as *C. dactylon*, *D. eriantha* and potentially *C. gayana* and allowing natural succession to take place. The more competitive and opportunistic species, such as *E. curvula* and *E. tef*, will naturally move into and occupy the area.

Janssens *et al.* (1998) showed that low levels of nitrogen are associated with higher levels of species diversity. Weiher *et al.* (1998) explains this using the converse relationship: with an increase in fertility there is a greater above ground competition which leads to a greater mean nearest neighbour distance. They showed that there is a negative relationship between mean nearest neighbour and species diversity. Alternatively, Davidson (1964) showed that adding fertilizers to disturbed areas decreases the probability of old field succession. Old field succession is unique to previously mismanaged or abandoned farm lands where vegetation cover is dominated by a small selection of species for several decades (Cramer *et al.* 2008).

Unfortunately in most rehabilitation areas, as in mining and severely eroded areas, the nutrient levels are very low and there are only limited species that can establish there. Soils of the humid grasslands of South Africa have approximately 2% organic carbon but some soils, for example soils with a high clay content, can have up to 5% organic carbon. After processes such as open cast mining the carbon levels drop to less than 0.5% leading to a depletion of organic nitrogen (Mentis 2006). In order to promote initial establishment in these conditions essential minerals need to be added to the soils.

Jutlia and Grace (2002) showed that, in coastal prairie grasslands, areas that have had plants removed with the least disturbance to the soil (e.g. using a herbicide) resulted in seedlings with the greatest biomass. There was also a significantly higher seedling biomass when the grass was hayed (cut and removed) compared to other treatments. Either of these two methods (herbicide treatment or haying) would be appropriate for removing patches of vegetation when preparing the land for the "second phase" of the restoration process.

When using multi-phase restoration it may be desirable to allow for one or two flowering events as this would improve the seed bank of the area. Conversely, allowing a seed fall will form a seed bank of the strong competitors and any disturbance (such as clearing patches for the "second phase") may induce a flush of these species which may not be desirable. It may be beneficial to employ both

of these techniques of delayed or enhanced flowering at different stages of the revegetation process. Bearing in mind the generalizations which have emerged during the development of the community assembly theory (Section 2.2.1.3) (Case 1991), keeping the number of highly competitive species low will decrease the invasibility of the species into the patches of the poor competitors. Similarly, a high number of poor competitors may be beneficial to the dispersal of these species into the areas where the strong competitors have established.

Allowing grazing early or taking cattle off the land late may delay flowering in some species. For example, *Themeda triandra* has an elevated apical bud from which the culm extends and is susceptible to damage through grazing (Cheplick 1998). Allowing cattle on the area whilst the apical bud is elevated will decrease the probability of flowering (Cheplick 1998). Additionally, frequent defoliation of the established grasses decreases the development of large tufted grasses (O'Connor 1994, Mentis 2006) which promotes bare patches around the tuft through the increased use of the localised resources and shading out of smaller vegetation. The gaps that develop through hoof action could be advantageous as they provide a niche for seeds to germinate, especially if the veld has been over-sown to promote diversity (Walker *et al.* 2004). Another advantage to having cattle grazing the area is that there is an increase in nitrogen turnover through defecation and urination (Belsky 1994, Mentis 2006). Westoby et al. (1989) indicated that grazing pressure will decrease the rate at which natural succession takes place but, in this scenario, grazing is also altering the successional path and the final community structure.

In addition to monitoring the rehabilitation project's success in relation to the vegetative structure of the reference site (Section 2.2.3.1) it is important to monitor the vegetative progression within, and between, the patches and inter-patches of the rehabilitated landscape. Monitoring sites which overlap the patches and inter-patches of the various revegetation phases will aid in determining if there are transitional zones between the two. For example, is there evidence that the "weaker competitors" have dispersed out of the patch into which they were planted and are they growing with the stronger competitors? If not, what management plans need to change to allow this dispersal to occur?

Treatment of grass seedlings, particularly the ones studied in these experiments, with smoke-infused water will not be advantageous for rehabilitation

practices in terms of increasing germination success. Additionally, there is no evidence that high concentrations of the smoke-infused water can be used to delay germination of vigorous species to allow for initial germination and establishment of the weaker competitors. There is, however, a possibility that seeds pre-treated with the 1:1000 or 1:2 concentration (of this particular batch of smoke) will produce seedlings of increase or decreased vigour for some grass species. This effect of the smoke-infused water on seedling growth can be manipulated to increase the landscape species diversity.

## 7.2 Further research

The most important research to follow these experiments is field trials which test the suggestion of a "two-phase" or "multi-phase" restoration practice. There are often several factors that are not adjusted for when working with controlled experiments that will have a significant impact on the outcome of the actual rehabilitation process. These factors need to be identified and corrected for before implementation of these management suggestions. Other aspects in seedling growth and development, such as the affect of litter cover on increasing moisture and decrease light, grazing (and at what age is a sward can be grazed without debilitating effects), precipitation level, daily temperature fluctuations and soil toxicity (especially in mine rehabilitation), should be considered when determining the growth, development and competitive ability of the species desirable for rehabilitation of degraded areas. Additionally, environmental stimuli not tested in these experiments, such as rainfall and soil structure, may override the influence of the factors tested.

Each rehabilitation situation is unique in terms of the environmental conditions present and local species that are desired for the project. These factors need to be considered when looking at species mixes. There should, however, be general trends within plant communities on which this model of a multi-phased restoration plan can be superimposed.

It is important to increase the knowledge of aspects of seedling ecology in other species, especially those that are desirable for restoration projects. Other species which have been used successfully for rehabilitation of mine sites in South Africa are *Panicum repens, Cenchrus ciliaris* and *Urochloa* species (Mentis 2006).

Ultimately, it depends on the species that are found in the local habitats that need to be examined for competitive ability and rehabilitation suitability.

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## Appendix 1: Average seedling weight of five South African grasses under varying competition combinations and nitrogen availability levels.

**Table A1.1:** Average seedling weight (grams) and proportionate change of seedling weight compared to the control treatment's growth for five South African grasses. The seedlings were grown under varying competition combinations and low nitrogen availability conditions.

| Target | Neighbour | Average seedling weight (g) | Proportion change |
|--------|-----------|----------------------------|-------------------|
| C. dactylon |  | 0.027 | 1.000 |
| C. dactylon | C. dactylon | 0.008 | 0.301 |
| C. dactylon | C. gayana | 0.008 | 0.277 |
| C. dactylon | D. eriantha | 0.020 | 0.754 |
| C. dactylon | E. curvula | 0.011 | 0.415 |
| C. dactylon | E. tef | 0.009 | 0.328 |
| C. gayana |  | 0.088 | 1.000 |
| C. gayana | C. dactylon | 0.025 | 0.282 |
| C. gayana | C. gayana | 0.024 | 0.276 |
| C. gayana | D. eriantha | 0.036 | 0.403 |
| C. gayana | E. curvula | 0.017 | 0.190 |
| C. gayana | E. tef | 0.037 | 0.421 |
| D. eriantha |  | 0.100 | 1.000 |
| D. eriantha | C. dactylon | 0.030 | 0.304 |
| D. eriantha | C. gayana | 0.024 | 0.240 |
| D. eriantha | D. eriantha | 0.030 | 0.298 |
| D. eriantha | E. curvula | 0.079 | 0.789 |
| D. eriantha | E. tef | 0.019 | 0.195 |
| E. curvula |  | 0.111 | 1.000 |
| E. curvula | C. dactylon | 0.120 | 1.083 |
| E. curvula | C. gayana | 0.033 | 0.295 |
| E. curvula | D. eriantha | 0.047 | 0.425 |
| E. curvula | E. curvula | 0.053 | 0.478 |
| E. curvula | E. tef | 0.046 | 0.412 |
| E. tef |  | 0.036 | 1.000 |
| E. tef | C. dactylon | 0.033 | 0.900 |
| E. tef | C. gayana | 0.019 | 0.513 |
| E. tef | D. eriantha | 0.018 | 0.500 |
| E. tef | E. curvula | 0.020 | 0.558 |
| E. tef | E. tef | 0.023 | 0.618 |

**Table A1.2:** Average seedling weight and proportionate change of seedling weight compared to the control treatment's growth for five South African grasses. The seedlings were grown under varying competition combinations and high nitrogen availability conditions.

| Target | Neighbour | Average seedling weight | Proportion change |
|---|---|---|---|
| *C. dactylon* | | 15.280 | 1.000 |
| *C. dactylon* | *C. dactylon* | 12.410 | 0.812 |
| *C. dactylon* | *C. gayana* | 1.660 | 0.109 |
| *C. dactylon* | *D. eriantha* | 15.470 | 1.012 |
| *C. dactylon* | *E. curvula* | 7.650 | 0.501 |
| *C. dactylon* | *E. tef* | 3.040 | 0.199 |
| *C. gayana* | | 36.090 | 1.000 |
| *C. gayana* | *C. dactylon* | 21.940 | 0.608 |
| *C. gayana* | *C. gayana* | 13.490 | 0.374 |
| *C. gayana* | *D. eriantha* | 31.860 | 0.883 |
| *C. gayana* | *E. curvula* | 27.100 | 0.751 |
| *C. gayana* | *E. tef* | 17.900 | 0.496 |
| *D. eriantha* | | 15.060 | 1.000 |
| *D. eriantha* | *C. dactylon* | 7.330 | 0.487 |
| *D. eriantha* | *C. gayana* | 2.150 | 0.143 |
| *D. eriantha* | *D. eriantha* | 5.680 | 0.377 |
| *D. eriantha* | *E. curvula* | 4.150 | 0.276 |
| *D. eriantha* | *E. tef* | 3.900 | 0.259 |
| *E. curvula* | | 17.270 | 1.000 |
| *E. curvula* | *C. dactylon* | 10.110 | 0.585 |
| *E. curvula* | *C. gayana* | 5.220 | 0.302 |
| *E. curvula* | *D. eriantha* | 12.660 | 0.733 |
| *E. curvula* | *E. curvula* | 8.090 | 0.468 |
| *E. curvula* | *E. tef* | 5.050 | 0.292 |
| *E. tef* | | 4.880 | 1.000 |
| *E. tef* | *C. dactylon* | 4.700 | 0.963 |
| *E. tef* | *C. gayana* | 2.030 | 0.416 |
| *E. tef* | *D. eriantha* | 3.650 | 0.748 |
| *E. tef* | *E. curvula* | 3.320 | 0.680 |
| *E. tef* | *E. tef* | 3.020 | 0.619 |

**Appendix 2: Mean germination percentages of five South African grasses when treated with varying concentrations of smoke-infused water, with and without rinsing.**
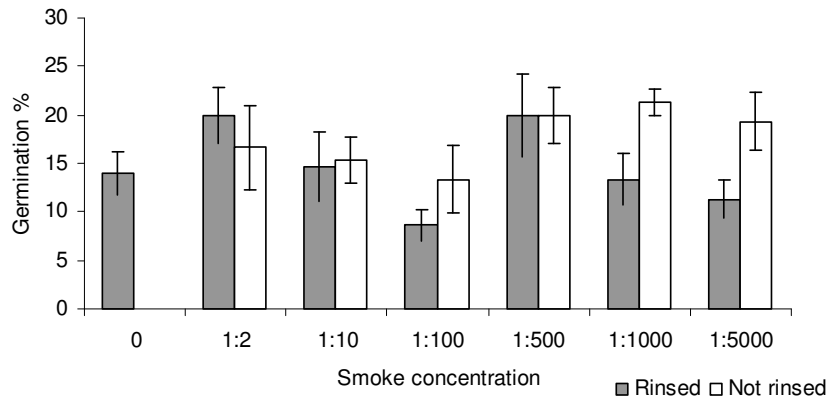


**Figure A2.1:** Germination percentage of *Cynodon dactylon* when treated with varying concentrations of smoke-infused water, with and without rinsing treatments (p = 0.276). The grey bars represent the rinsed treatments and the white bars are for the non-rinsed treatments. The vertical lines represent the standard errors.
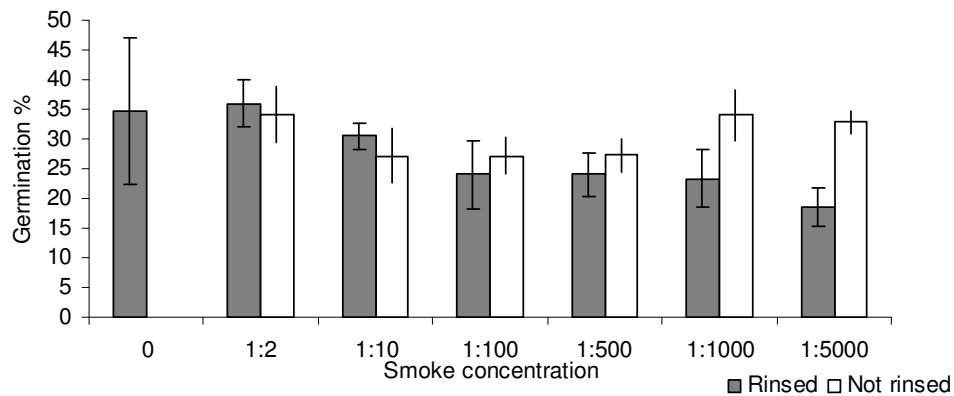


**Figure A1.2:** Germination percentage of *Chloris gayana* when treated with varying concentrations of smoke-infused water, with and without rinsing treatments (p = 0.461). The grey bars represent the rinsed treatments and the white bars are for the non-rinsed treatments. The vertical lines represent the standard errors.

**Figure A2.3:** Germination percentage of *Digitaria eriantha* when treated with varying concentrations of smoke-infused water, with and without rinsing treatments (p = 0.849). The grey bars represent the rinsed treatments and the white bars are for the non-rinsed treatments. The vertical lines represent the standard errors.
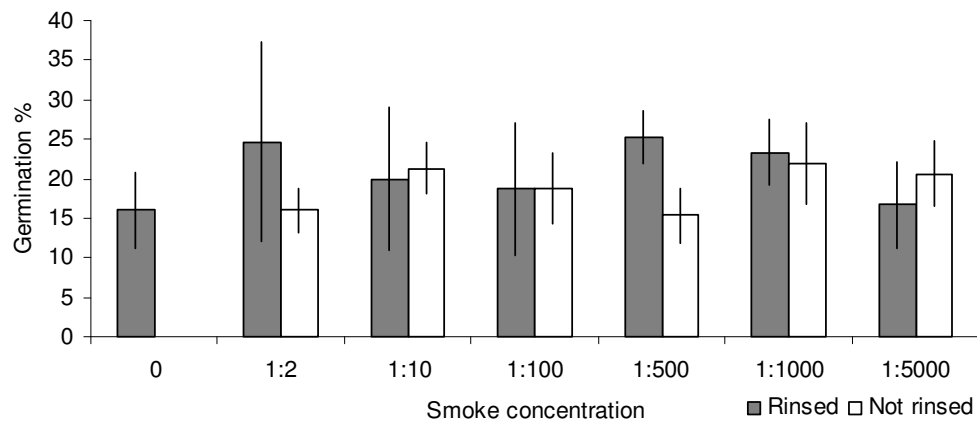


**Figure A2.4:** Germination percentage of *Eragrostis curvula* when treated with varying concentrations of smoke-infused water, with and without rinsing treatments (p = 0.323). The grey bars represent the rinsed treatments and the white bars are for the non-rinsed treatments. The vertical lines represent the standard errors.
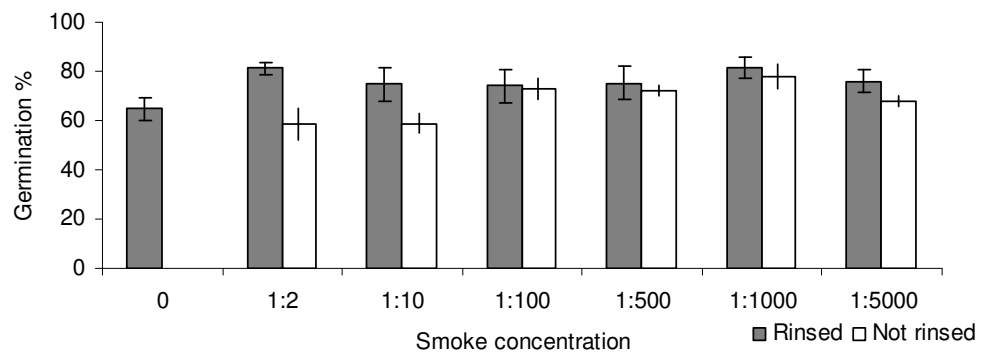
## Appendix 3: Mean shoot and root lengths of grass seedlings of three species pre-treated with three concentrations of smoke-infused water.

**Table A3.1:** Mean shoot and root length (cm) for the seedling of three grass species after treatment with four concentrations of smoke-infused water and a maximum of 22 days growth. Letters adjacent to lengths show significant difference in length within species (along rows).

| Species | Factor | s.e.[1] | 0 | | 1:1000 | | 1:500 | | 1:2 | |
|---|---|---|---|---|---|---|---|---|---|---|
| C. dactylon | Shoot | 0.135 | 1.003 | a | 1.235 | b | 1.313 | b | 1.168 | b |
| C. dactylon | Root | 0.368 | 2.18 | c | 2.8 | d | 2.71 | de | 2.41 | ce |
| E. curvula | Shoot | 0.162 | 2.154 | f | 1.445 | g | 2.15 | fh | 1.886 | h |
| E. curvula | Root | 0.74 | 5.53 | i | 3.07 | j | 6.51 | k | 4.98 | i |
| E. tef | Shoot | 0.178 | 1.927 | l | 1.927 | l | 2.021 | l | 1.823 | l |
| E. tef | Root | 1.245 | 7.92 | m | 5.43 | n | 8.02 | m | 7.65 | m |

[1] standard error

**Table A3.2:** Mean shoot and root length (cm) for the seedling of three grass species after treatment with four concentrations of smoke-infused water and 10 days growth. Letters adjacent to lengths show significant difference in length within species (along rows).

| Species | Factor | s.e.[1] | 0 | | 1:1000 | | 1:500 | | 1:2 | |
|---|---|---|---|---|---|---|---|---|---|---|
| C. dactylon | Shoot | 0.135 | 1.003 | a | 1.235 | b | 1.313 | b | 1.168 | b |
| C. dactylon | Root | 0.368 | 2.18 | c | 2.8 | d | 2.71 | de | 2.41 | ce |
| E. curvula | Shoot | 0.149 | 1.265 | f | 0.896 | g | 1.112 | h | 1.571 | i |
| E. curvula | Root | 0.264 | 2.37 | j | 1.65 | k | 2.12 | jl | 2.09 | l |
| E. tef | Shoot | 0.34 | 2.33 | m | 1.27 | n | 1.99 | mo | 1.8 | o |
| E. tef | Root | 0.086 | 1.002 | p | 0.992 | pq | 1.036 | pq | 0.907 | q |

[1] standard error