THE ROLE OF FIRE AND FIRE-RELATED FACTORS ON GERMINATION AND GROWTH OF GRASSLAND SPECIES

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Submitted in fulfillment of the requirements for the degree of

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Research Centre for Plant Growth and Development School of Biological and Conservation Sciences University of KwaZulu-Natal, Pietermaritzburg Campus

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THE ROLE OF FIRE AND FIRE-RELATED FACTORS ON GERMINATION AND GRASSLAND SPECIES GROWTH

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Regular consultation took place between the student and ourselves throughout the investigation. We advised the student to the best of our ability and approved the final document for submission to the Faculty of Science and Agriculture Higher Degrees Office for examination by the University appointed Examiners.

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ABSTRACT

Fire, natural or of anthropologic origin is a recurrent phenomenon in South African mesic grasslands. The species composition of these grasslands is sensitive to fire frequency and there exists a permanent fire-species relationship syndrome. The shift follows a general trend where, in the absence of fire for longer periods, the native grass species, possibly the most economically important grass species, give space to relatively less desirable and invasive species accompanied by significant decline in basal cover. Though much is known as to how the floristic composition of the grassland changes in response to fire, the underlying mechanisms responsible for changes in plant vigour and species composition are not fully understood. There exists limited information on germination requirements of the local flora and how recruitment of the species is influenced by fire, fire-related factors and the interaction of these factors among themselves is virtually unknown.

Since the discovery by **DE LANGE and BOUCHER (1990)**, plant-derived smoke is being widely studied as an important germination cue and the theme has been the subject of intense experimental research and theoretical work. Germination studies conducted on South African Fynbos, Californian Chaparral, and Australian species have illustrated the widespread ability of plant-derived smoke in promoting germination of species from both fire and non-fire prone habitats. Studies have also showed that plant-derived smoke plays a significant role in vegetative growth of many plants including horticultural and agricultural crops, though mechanisms of smoke action in enhancing germination and promoting plant growth are still under active research. This study sought to gain insight into the role of fire and fire-related cues and other related factors on germination and seedling growth of key grass species from fire-prone grassland in South Africa. In this study, various investigations were conducted on different aspects related to smoke-induced seed germination. Furthermore, in depth examinations were

conducted on the effects of fire-related factors such as smoke, heat, soil and nutrients on seed germination and seedling growth of representative key mesic grassland species from South Africa.

Laboratory-based germination and vigour experiments were conducted using smoke solutions. The aim was to examine if the strong fire-species relationship syndrome we encounter in post-fire mesic grassland in South Africa is related to the effect of plant-derived smoke on germination and seedling growth. In addition, the interaction of plant-derived smoke solutions with temperature was examined by incubating seeds at a range of temperatures. Treating seeds with smoke-water and butenolide, the germination rate (GR) and final germination percentage (FGP) were greater in 3 of the 6 species. *Themeda triandra* Forssk. and *Tristachya leucothrix* Trin. ex Nees showed the greatest response, with final germination increased from 43% to 67% and 35% to 63% respectively. These smoke solutions have also significantly enhanced germination in *Eragrostis tef* (Zucc.) Trotter from 62% to 80%. The degree of responsiveness varied from species to species and across different temperatures. Findings from this study suggested that plant-derived smoke and its interaction with temperature significantly influence the germination and seedling growth of the South African mesic grassland species, which can further impact on the grassland composition.

To characterise the role of plant-derived smoke on certain economically important seed traits, the effect of smoke-water and a smoke-isolated butenolide on seed germination and seedling growth of *Eragrostis tef* (grass species which has moderately good tolerance to east African drought) was investigated at a range of temperatures, light conditions and osmotic potentials. Smoke-water (1:500 v/v) and butenolide (10^{-8} M) treatment of the seeds increased percentage germination, seedling vigour and imbibition from high to low osmotic potential. These results suggested that smoke solutions have characteristics with overriding effects toppling stresses exerted from high temperature and low osmotic potential, thereby widening the temperature and moisture zone over which germination can occur. Furthermore, these findings

suggested that the mode of smoke action in promoting seed germination is likely to be linked to the role of smoke in facilitating water uptake by seeds (imbibition).

A simple and rapid bioassay was implemented to detect the germination activity of extracts from soils in pre/post-burn status. Soil samples taken from burnt, unburnt and adjacent plots at depths of 0-2, 2-4, 4-6 and 6-8 cm before and after burning mesic grassland in South Africa were analysed for germination activity over an eight-week period. Soil samples were extracted using dichloromethane and bioassayed using Grand Rapids lettuce (*Lactuca sativa* L.) achenes. The Grand Rapids lettuce seeds exhibited several-fold greater germination percentages when treated with extracts from burnt soil compared to the other plots. The magnitude of such an activity declined through time since burn. The Grand Rapids lettuce seeds also showed significantly greater germination percentage when treated with unburnt soil extracts compared to the control (distilled water) which indicates the existence of other factors controlling germination in unburnt soil. Germination percentage was negatively influenced in the adjacent plots which reflected the inhibitory role of smoke on adjacent plots neighbouring the burn. Findings from this study suggested that the germination activity of a burning plant-material (smoke) indeed diffuses into the soil and its persistence declines with time. Furthermore, the findings implied that fire and smoke may significantly influence the germination of the soil seed bank of mesic grassland in South Africa.

The effect of smoke solutions of various concentrations and their interaction with soil macronutrients NPK in influencing seedling growth of three selected grass species namely, *Eragrostis curvula, Panicum maximum* and *Themeda triandra* was investigated. These grass species were selected on the basis of the contrasting response they previously showed to smoke treatments. Results showed that the interaction between species, treatment and nutrients for seedling vigour index was highly significant ($P < 0.001$). In the absence of NPK elements, the smoke solutions generally enhanced seedling growth in *Themeda triandra*, while suppressing seedling vigour indices of *Eragrostis curvula* and *Panicum maximum*. It is ecologically significant that not only

smoke but also its interaction with soil macronutrients appears to be important in structuring the post-fire regeneration and colonisation processes.

To examine the effect of fire-associated cues of smoke and high temperature on germination and seedling emergence from the mesic grassland soil seed bank in South Africa, and disentangle responsible factors, the effect of heat and smoke-water on seedling emergence and the resultant effect on biomass production was examined in a greenhouse tray experiment over 90 days. Soil seed bank samples obtained from 0-5 cm top soil were treated with smoke-water solution (SW 1:500 v/v), heat (H = 100 °C for 15 min) and heat + smoke-water (H = 100 °C for 15 min + SW = 1:500 v/v) combined. Significantly, the highest number of seedlings emerged from soil seed bank samples treated with heat + smoke-water combined followed by smoke-water alone. Heat + smoke-water treatment of soil seed bank samples also significantly enhanced the rate of seedling emergence and total dry biomass production. Heat treatment by itself was not statistically different from the control in many respects. Generally, these results suggest that both heat and smoke are important germination cues and play a curial role on germination and seedling emergence from the mesic grassland soil seed bank. A greater number of fire-loving *Themeda triandra* seedlings emerged from soils treated with smoke, though this was not statistically significant. Seedling emergence indicators showed that seedling emergence was favoured more by smoke and smoke + heat treatments compared to the control. Seedling growth indicators also showed that soil samples treated with smoke or smoke + heat combined produced significantly higher biomass than untreated samples.

In general, findings from this comprehensive study suggest that plant-derived smoke and its interaction with temperature (heat) and soil nutrients significantly influences seed germination and seedling growth of South African mesic grassland species differently, this can further influence the grassland composition. The association of certain fire-climax species with the re-occurrence of fire can partly be explained due to the association of such species with fire-induced changes in the habitat. Post-fire conditions of low nutrient (NPK) availability, presence of high heat and smoke cues in the habitat may favour the competitive ability of native short and smoke-responsive

species such as *Themeda triandra* and *Tristachya leucothrix* over those characteristic species of fertile habitats such as *Eragrostis curvula* and other non-smoke-responsive species.

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CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AN…………… Ammonium Nitrate

ANOVA……….Analysis of Variance

B………………Butenolide

FGP…………...Final Germination Percentage

GR……………Germination Rate

LAN…………..Limestone Ammonium Nitrate

NPK………….Nitrogen, Phosphorus and Potassium

PEG………….Polyethylene Glycol

SA……………Smoke Aerosol

SW …………...Smoke-Water

UKZN..............University of KwaZulu-Natal

CHAPTER ONE

Introduction

"Great is the art of beginning, but greater is the art of ending"

Henry Wadsworth Longfellow (1807 – 1882)

1.1 SMOKE AS A GERMINATION CUE

The history of fire and smoke on earth is as old as the earth itself. Hence, fire has been part of the ecosystem for millions of years, and its role as a major evolutionary force on shaping global vegetation pattern is one of the oldest observations of the natural world. The role of fire in the origin, maintenance and distribution of terrestrial plant communities has also been well recognised since long **(CLEMENTS, 1916; PHILLIPS, 1931; SOUSA, 1984; GLENN-LEWIN and VAN DER MAAREL, 1992)**. It has been commonly assumed that certain plant species are frequently eliminated by the physical heat of burning which changes the relative frequency of species occurrence, and new plant species adapted to fire are introduced into burned ecosystems **(TAYLOR, 1973; KREFTING and AHLGREN, 1974)**. Fire also creates opportunities for exotic plant species to establish successfully in a foreign habitat, and to outperform dominating native species. Since the early 1990,s however, fire has been identified as hosting an important chemical germination cue (i.e. **smoke**) **(DE LANGE and BOUCHER, 1990)**, and hence smoke-stimulated germination as a new factor influencing seed germination, seedling establishment and colonisation processes has received

1

considerable global attention **(KEELEY et al., 1985; BOND and VAN WILGEN, 1996**; **KEELEY and KEELEY, 1987; KEELEY and FOTHERINGHAM, 2000; CLARKE and FRENCH, 2005)**. Benchmark studies conducted on South African Fynbos species **(BROWN, 1993a; BROWN, 1993b)** reported that plant-derived smoke triggers seed germination of certain species. It is fascinating topic which holds great potential for applications in various fields of plant science **(LIGHT and VAN STADEN, 2004)**. Certainly, the discovery has provided new opportunities for promoting germination and seedling growth in agriculture, horticulture, habitat restoration and weed control programmes. It is ecologically significant that smoke-stimulated germination is also serving as a remarkable tool for scientific investigation into the action of fire in ecosystems. Presently, smoke has been shown to induce germination in a comprehensive and indicative selection of species known to be **smoke-responsive** species **(DIXON et al., 2009)**. This indicates that not all species are responsive to smoke or the degree of the responsiveness may differ from species to species and across a wide-range of environmental settings. Therefore, such a notion may further influence concepts of fire-induced ecological succession, competitive interaction and hence plant community organization **(BROWN and VAN STADEN, 1997; LIGHT and VAN STADEN, 2004)**.

1.2 FIRE IN THE SOUTH AFRICAN MESIC GRASSLANDS

South Africa is a land of fire and smoke of natural or anthropologic origin **(SCOTT, 1971)**. The eastern part of the country is particularly prone to natural fires (lightening) with one of the highest lightening incidences in the world **(MARNY and KNIGHT, 1986)**. Frequent and/or predictable fires occur in South African grassland and savannas; consequently, fire is an integral part of these ecosystems **(SCOTT, 1971; TAINTON, 1978)**. The present study focused on one of the most fire-dependant/fire influenced biomes in South Africa, known as southern Tall Grassland **(ACOCKS, 1988)**. South African mesic (fire-climax) grasslands owe their very origin to fire which occur in a large part of the higher-lying eastern region

of the country, supporting a rich flora of high conservation interests (**Figure 1.1**). These grasslands support substantial quantities of livestock and wildlife, which are the primary subsistence of a large portion of the indigenous population and for commercial purposes **(FOTHERINGHAM, 1981)**.
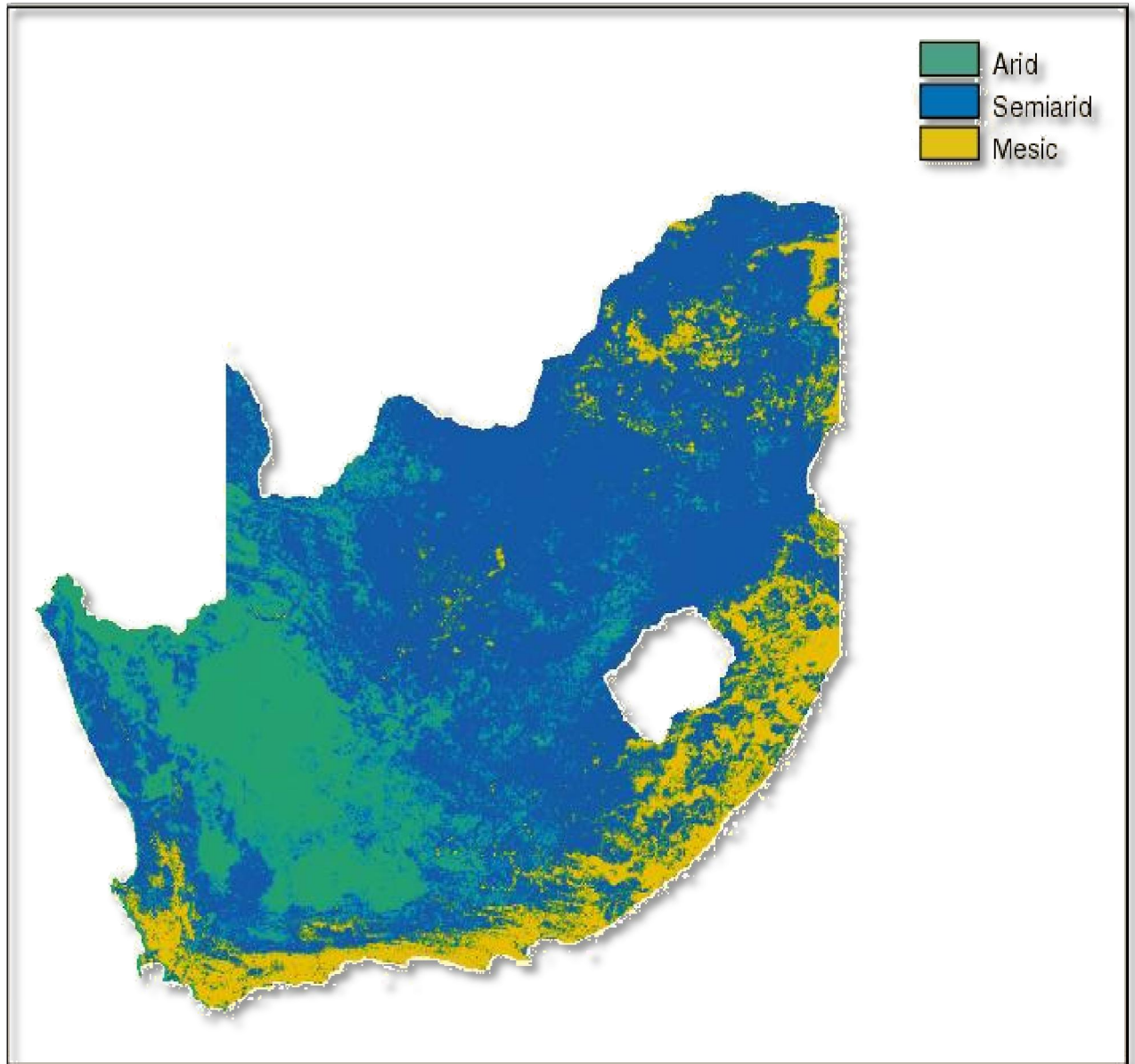
Figure 1.1: **Map of South Africa showing the distribution of the arid, semi-arid and mesic areas (ACOCKS, 1988).**

The grassland community under discussion is mainly composed of perennial herbaceous species with sparsely situated *Acacia* trees. The original dominant grass species are inherently short species largely composed of *Themeda triandra*, *Heteropogon contortus* and *Tristachya leucothrix* **(ACOCKS, 1988)**. The species composition of the grassland is however, sensitive to fire frequency and intensity that fire tolerant/favoured species are often encountered (to be discussed further in **Chapter 3**). Therefore, an understanding of the mechanisms responsible for such fire-induced changes in species composition is essential for the ecology and successful management of the grasslands.

The principal objective of this study has been promotion of predictive understanding of the role of fire and fire-related factors mainly light, heat, nutrients and smoke on grassland species germination and growth. While the role of fire on vegetation dynamics is one of the best studied subjects, previous studies have resulted in contradictory understanding, with some reporting that fire has negative impacts on germination **(ZACHARIAS et al., 1988; DREWES et al., 1995; MUCUNGUZI and ORYEM-ORIGA, 1996)**, whilst others reported stimulatory effects **(BOND and VAN WILGEN, 1996; KEELEY and FOTHERINGHAM, 2000; VAN STADEN et al., 2006)** and still others reporting neutral or no effects **(ADKINS and PETERS, 2001; BUHK and HENSEN, 2006)**. Therefore, if fire and smoke are to be useful as management tools, promotion of predictive understanding of the effects across a wide range of species and environmental setting is needed. In addition to this, promoting the current knowledge on smoke as a germination cue and as a new factor in plant growth was one of the major goals in this study.

1.3 AIMS AND OBJECTIVES

The overall aim of this study was to provide some answers to questions related to the role of fire and fire-related factors mainly smoke on grassland species germination and growth. This was achieved through different investigations on aspects related to fire, smoke, soils and grass species germination. The specific objectives of this study were the following:

1. Improve our understanding of the eco-physiological effect of fire and smoke on grassland species germination and growth, and its implications for mechanisms regulating the dynamics of species and vigour change in response to fire (burning);

2. Provide a means of developing models for predicting the dynamics of key grass species and grassland structure in relation to the eco-physiological effects of fire and smoke on plant growth and development and;

3. Promotion of the existing knowledge on smoke as a germination cue and as a factor in plant growth;

1.4 GENERAL OVERVIEW

Germination requirements and germination rate are amongst the most important seed traits associated with plant fitness and are key components in the ecology and evolution of plant life histories **(HARPER, 1977)**. The importance of seed germination as a plant trait leading to successful establishment and persistence of individual plants and populations is well discussed by **VAN DER VALK (1992)**. Germination is a complex process which is governed and influenced by many factors and slight changes in the physical and chemical environment can lead to different outcomes. Hence, it is often argued that germination ecology of species cannot be fully understood by laboratory-based studies alone **(BASKIN and BASKIN, 1998)**. This notion partially holds true, as there are many more unforeseen factors which influence germination *in situ*. The reverse notion is also available in that in plant communities, often slight differences in the physiological characteristics and life history features of the species involved and the diverse nature of successional environments can influence trends in succession replacements **(GRIME, 1979; TILMAN, 1988)**. Due to this fact, designing and analysing ecological experiments in the field as tests of ecological theory are often

viewed as insurmountable and challenging **(VAN DER VALK, 1992)**. Hence, experimental laboratories are often used as instruments for investigating ecological questions not easily answered through the analysis of field data **(HARPER, 1977; GRIME, 1979; VAN DER VALK, 1992; BAZZAZ, 1996)**.

The research presented in this thesis brings together a variety of different laboratory, greenhouse and field-based studies related to the subject of "the role of fire and fire-related factors on germination and growth of grassland species". Although in some respects the experiments may seem unrelated, together they begin to provide some insight towards a greater understanding of the role of fire and smoke on germination and seedling growth of some representative grassland species which are currently used as livestock feed and/or directly or indirectly for human consumption.

Chapter 2 provides a comprehensive literature review on the topic of fire mediated vegetation changes and the role of smoke as a germination cue. This Chapter also attempts to cover the most recent advances in the role of smoke in different fields of science i.e. agriculture, horticulture and ecological studies. This Chapter concludes with a short discussion on the ecological significance of smoke-stimulated germination and some important considerations for vegetation ecology.

Chapter 3 presents results from the experiments conducted on the effects of plant-derived smoke solutions and the interaction with temperature on germination and seedling growth of six common grassland species from mesic grassland in South Africa. The results from this experiment showed that smoke solutions and its interaction with temperature significantly influence the germination and seedling growth of South African mesic grassland species which in the long-run can impact on grassland composition **(GHEBREHIWOT, KULKARNI, KIRKMAN and VAN STADEN, 2009)**.

Chapter 4 provides results from an experiment which was conducted to test the role of smoke solutions on germination and seedling vigour of *Eragrostis tef* (Zucc.) Trotter under high temperature and low osmotic potential. The purpose of this

study was to understand the mode of smoke action in facilitating germination. The results from this experiment demonstrate that smoke-water and butenolide treatment of *Eragrostis tef* seeds significantly improved germination and seedling growth. This study also revealed that smoke solutions play a significant role in facilitating water uptake by seeds (imbibition) and override stresses exerted from sub-optimal temperature and low osmotic potential. Importantly, this Chapter gives evidence that smoke-water and butenolide are able to widen the environmental range over which germination can occur **(GHEBREHIWOT, KULKARNI, KIRKMAN and VAN STADEN, 2008)**.

Chapter 5 presents the results of the "lettuce seed bioassay" conducted to assess the effect of fire (burning) in altering the soil-smoke chemistry and the biological activity of burnt soil extracts on the germination of Grand Rapids Lettuce seeds. The aim of this study was to examine the germination activity of smoke residue in soils following a fire event. Results from this experiment showed that fire (burning) affects the soil smoke chemistry with regards to the germination activity of smoke which can further influence the germination of seeds contained at a moderate depth in the soil seed bank. **(GHEBREHIWOT, KULKARNI, LIGHT, KIRKMAN and VAN STADEN, 2010)**.

Chapter 6 presents the results of an experiment conducted to assess the role and interactive effect of smoke solutions of various dilution levels with soil macronutrients i.e. nitrogen (N), phosphorus (P) and potassium (K) on seedling growth of three grass species of contrasting response to smoke treatments. Results showed that irrespective of nutrient (NPK) presence, the smoke solutions of various dilution levels enhanced seedling growth of *Themeda triandra*. Seedling growth in *Eragrostis curvula* was more responsive to the presence or absence of soil nutrients (mainly N). Seedling growth of *Panicum maximum* was negatively influenced by the interaction of the smoke solutions with soil macronutrients. Findings from this study demonstrate that the interaction of plant-derived smoke with soil nutrients is an important factor in species growth **(KULKARNI, GHEBREHIWOT, KIRKMAN and VAN STADEN, 2010)**.

Chapter 7 provides the results of greenhouse experiment conducted to assess the effects of plant-derived smoke and heat on soil seed bank stimulation of mesic grassland in South Africa. Findings from this study highlight the potential role of plant-derived smoke in stimulating the soil seed bank of the mesic grassland and the potential use of plant-derived smoke in speedy restoration of degraded grasslands **(GHEBREHIWOT, KULKARNI, KIRKMAN and VAN STADEN 2010)**.

Chapter 8 (General discussion and conclusion) and **Chapter 9** (References) are followed by three appendices compiled from the surveyed literature. **Appendix 1** provides a list of *Poaceae* species which showed a positive germination response to smoke treatments. In contrast, **Appendix 2** provides a list of *Poaceae* species which did not show a positive germination response to any type of smoke treatment. **Appendix 3** shows electro-microscopic photos of the grass seeds used in this study. These electro-microscopic photos demonstrate the difference in size, shape and the nature of the seed coat of the seeds used in this study.

CHAPTER TWO

Literature review

"Vegetation is the basic instrument the creator uses to set all of nature in motion "

Antoine-Laurent de Lavoisier (1743–1794)

2.1 INTRODUCTION

Fire is a common disturbance regime world-wide, which derives processes that induce vegetation change through direct effects on biota and/or indirect effects on the environment **(SOUSA, 1984; WESTOBY et al., 1989; BOND and VAN WILGEN, 1996; BUHK and HENSEN, 2006)**. In fire-prone ecosystems and through the evolutionary time scale, many plants have evolved adaptations that protect them from being extirpated by fire or allow them to survive, thrive, and even require fire for survival **(EVERSON and TAINTON, 1984; SOUSA, 1984; EVERSON et al., 1988; BOND and VAN WILGEN, 1996)**. Species adaptations to fire can be thought of as the evolution of morphological and behavioural traits which allow for **reproduction** and the **continuance** of species. Regeneration strategies related to fire include morphological and physiological traits such as the production of propagules (**seeds, tillers and rhizomes)** capable of **surviving**, **germinating**, **sprouting** and **flowering** following after a fire event **(DANCKWERTS, 1984; ZACHARIAS et al., 1988; EVERSON, 1994)**. Hence, promotion of the understanding we have on how **seed germination** and **seedling establishment** of the local flora (as important plant traits in community structure) are influenced by fire and fire-related factors is of paramount significance. Indeed,

knowledge of the regeneration mechanisms and their interrelation with ecological factors such as fire, smoke and nutrients is essential for the interpretation of the high spatio-temporal variability found in post-fire species performance (**BOND and VAN WILGEN, 1996; BUHK and HENSEN, 2006; CHAKRABORTY and LI, 2009)**.

The aim of the review is firstly, to give a concise overview on subjects related to the role of fire and fire-related factors that influence seed germination, and consequently play a significant role in shaping plant communities. It is generally true that such a topic may inevitably host the entire question of fire ecology and fire-induced ecological succession. In plant ecology, succession is a broad topic and hence discussions around the subject may radiate out into endless directions. On account of the broadness of the subject and in the interest of the study under discussion therefore, this review discusses narrowly on the most relevant issues related to the ecological role of fire and fire-related factors which cause vegetation patterns and the underlying mechanisms governing plant succession. Secondly, it was desirable that smoke as a germination cue and a new factor in plant growth the current applications of smoke-biotechnology in various fields of agriculture, horticulture and ecological studies, and the suggested possible modes of smoke action in facilitating germination and promoting growth are sufficiently discussed. This review concludes by raising some importance considerations on issues linking the discovery of the plant-derived smoke germination cue and its ecological implications for the development of a theoretical framework of vegetation ecology.

2.2 THE ROLE OF FIRE IN PLANT SUCCESSION

It has been recognised for centuries that apparently repeatable patterns in plant community composition and structure in relation to climate, topography, soils and various forms of ecological disturbances is common in nature (**CLEMENTS, 1916)**. In other words, plant communities are not stable assemblages; they are rather dynamic both in time and space. The mechanisms responsible for such spatial and temporal changes in the community composition are commonly thought to be

related to the physiological tolerance of the plants to various physical and chemical factors (e.g. soil pH, salinity, aridity, fertility, biomass removal, grazing) and competition with other species. On the other hand, ecological disturbances (perturbation) of different forms which impact both the plant and the environment (resource flux) may play a significant role in structuring plant communities **(BAZZAZ, 1983; SOUSA, 1984)**.

Hence, plant communities are established through gradual development and for each community a characteristic pattern in which a definite stage (sere) can be recognised. Such a gradual progressive and at times retrogressive development of vegetation through a series of different plant groupings has been termed succession **(CLEMENTS, 1916)**. In simple terms, succession is a universal phenomenon of changes in the abundance and dominance of different species over time. These changes can involve species replacements, shifts in population structure, and changes in the availability of resources such as light, soil moisture and soil nutrients **(GRIME, 1979; TILMAN, 1988; BAZZAZ, 1996)**. Succession is divided into two distinct forms which are recognised as: primary and secondary succession. Primary succession occurs in a situation when plants gradually emerge in areas not previously vegetated because of lack of soil development, e.g. when lichens colonise a bare parent material such as a rock surface. In contrast, secondary succession occurs in areas previously vegetated but have had the pre-existing vegetation destroyed due to disturbance(s). The major causes of secondary succession are fires, cultivation, landslides, volcanic eruptions, floods and wind storms among others. Consequently, depending on the type, intensity and frequency of the causes, succession takes different forms **(SOUSA, 1984; PICKETT et al., 1987)**. Though the full array of ecological disturbances that lead to a successional change is countless, the most influential and best studied disturbance regimes worldwide are those associated with **wildfires**. Fire is a type of **chemical disturbance** caused by the rapid combustion of biomass of an ecosystem, often causing shifts in species composition of plant communities **(SOUSA, 1984; BAZZAZ, 1996; BUHK and HENSEN, 2006)**. Fires destroy part or all of the plant community and leave a barren landscape open to new growth,

vegetation, and re-colonization of plant species **(TROLLOPE, 1984; BAZZAZ, 1996)**. The germination of seeds on the surface and/or from the soil seed-banks is thought to be enhanced by fire **(BOND and VAN WILGEN, 1996; KEELEY and FOTHERINGHAM, 2000)**.

The ecological role of fire as a disturbance factor and a cause in successional change has been recognised since the turn of the last century. **CLEMENTS (1916)** considered fire as one of the natural sources initiating succession. He viewed fire as a retrogressive process that sets back the sequential development of vegetation to the climax. Hence, **CLEMENTS (1916)** considered the post-fire ecological recovery process which manifests in a regenerating vegetation as a type of secondary succession. Succession is a very broad topic in plant ecology which evolves many processes **(RAYNAL and BAZZAZ, 1973; GRIME, 1979)**. As such fire ecology (fire-induced succession) is an extensive topic which encompasses complex interaction between the major products of fire (heat, light, smoke, nutrients and moisture). The complexities of the processes of succession, such as seed dispersal, germination and survival, leading to the establishment and persistence of individual plants and populations are well discussed in the literature **(e.g. VAN DER VALK, 1992, BAZZAZ, 1996)**.

2.2.1 Mechanisms of plant succession: how important is seed germination?

Mechanisms of vegetation regeneration and dynamics (succession) are thought to be the efficient causes that contribute to vegetation change **(PICKETT et al., 1987)**. For several decades, ecologists have been speculating and hotly debating about the mechanisms of plant succession **(CLEMENTS, 1916; MCINTOSH, 1980; PICKETT et al., 1987; TILMAN, 1988)**. The scientific literature around the subject contains an extensive work done in identifying the general and unifying causes of vegetation change **(MCINTOSH, 1980; MCCOOK, 1994; BAZZAZ, 1996)**. Despite the large volume of work, there is yet no single universal and unifying cause or model of plant succession. This is because each habitat has its own unique aspects of environmental circumstances and each plant species has its own

unique physiological and morphological features which makes it difficult to find one unifying generalisation of the concept **(TILMAN, 1988)**. General tendencies in vegetation change however, involve the dominance of species with greater **maximum size**, **better germination**, **vigorous establishment and growth (GRIME, 1979; MCCOOK, 1994; BAZZAZ, 1996)**. One of the most popular models of succession is that of **PICKETT et al. (1987).** These authors in their attempt to systematically model plant succession, suggested a three-level hierarchy that completely enumerates all causes of vegetation succession. In this three-level causal hierarchy of vegetation succession; the highest level defines the general conditions or causes of succession. The intermediate-level consists of ecological factors or processes describing the causes of the highest level. In the intermediate-level factors such as **germination** and **establishment** together with **seed availability**, **seed dispersal mechanisms**, **life history strategies**, **mode of reproduction**, **germination requirements**, and **response to environmental stress factors** are considered as important in fire-induced vegetation change **(PICKETT et al., 1987)**.

Often, questions arise if seed germination and related traits are responsible for the observed patterns of plant distribution in the post-fire habitat **(MCINTYRE et al., 1999; BUHK and HENSEN, 2006; BUHK et al., 2007; CHAKRABORTY and LI, 2009)**. Studies conducted on monitoring and predicting post-fire successional changes of various vegetation types reveal that in many instances, difference in seed germination in response to fire may cause major differences in colonisation and establishment success of species which leads to the creation of vegetation patterns **(BUHK and HENSEN, 2006; BUHK et al., 2007; CHAKRABORTY and LI, 2009)**. In particular, **fire-stimulated germination and recruitment** as an effectual mechanism in species change has been demonstrated as being important for several vegetation types worldwide **(GO´MEZ-GONZA´LEZ et al., 2008)**.

2.2.2 Why do we need to predict vegetation change in response to disturbances?

Understanding and quantifying revegetation in response to disturbance is one of the major goals for basic research and ecological restoration, conservation and rehabilitation purposes **(GRIME, 1979; SOUSA, 1984; GLENN-LEWIN and VAN DER MAAREL, 1992; BAZZAZ, 1996)**. Disturbances are commonly thought to come through fires, wind storms, landslides, volcanic eruptions, diseases, cultivation, overgrazing, climate changes and many other forms of human actions which interfere with the normal functioning of a given biological system **(VAN ANDEL and VAN DEN BERGH, 1987)**. Such disturbances are believed to modify trends in ecological succession through changes in the environment and within the plants themselves **(GRIME, 1979; VAN ANDEL and VAN DEN BERGH, 1987; GRUBB, 1988)**. Disturbances differ in their type (i.e. physical or chemical), extent, duration, magnitude and space **(SOUSA, 1984; PEET, 1992)**. Hence, depending on the intensity and timing thereof, species diversity and population growth oscillate through factors linked to demographic processes such as seed production, dispersal, perdition, germination characteristics, stand establishment and adult plant longevity **(GRIME, 1979; BAZZAZ, 1996; CZARNECKA, 2004)**.

Predictive understanding of plant community response to fire (burning) is the basis for sound management which in turn greatly relies on our understanding of the patterns and processes of disturbance-mediated vegetation change **(GLENN-LEWIN and VAN DER MAAREL, 1992; BAZZAZ, 1996)**. Predicting community composition from life history patterns such as **seed germination** and **growth response to environmental changes** is a useful tool and common in ecological studies **(GRIME, 1979; BAZZAZ, 1996)**. Identifying traits that may be responsible for species response to various changes in the environment are thought to facilitate the generalization of results from specific studies to a larger range of environments **(MCINTYRE et al., 1999)**.

2.2.3 Seed germination and plant community organization

A seed contains an embryonic plant in an inactive condition and seed germination is a process by which inactive seed begins to sprout and grow into a seedling under the right growing conditions. It is a complex physiological process which is governed by a specific moisture level, light conditions, temperature fluctuations and concentration of certain gasses **(GRIME, 1979; RICE, 1984; VAN DER VALK, 1992)**. More importantly, seed germination can be affected by several fire-related factors such as heating temperature **(ZACHARIAS et al., 1988)**, ash **(HENIG-SEVER et al., 1996; REYES and CASAL, 1998)**, nitrogen availability **(BRONCANO et al., 1998; PÉREZ-FERNÁNDEZ and RODRÍGUEZ-ECHEVERRÍA, 2003)**, plant-derived smoke **(KEELEY and FOTHERINGHAM, 1998; PÉREZ-FERNÁNDEZ and RODRÍGUEZ-ECHEVERRÍA, 2003)**, and ethylene **(NE'EMAN et al., 1999)**.

Different species of plants have different requirements and strategies for germination and successful establishment of seedlings. The significance of germination strategies in community composition has been discussed by **GRUBB (1977)**; **PICKETT and WHITE (1985)**; **PICKETT and BAZZAZ (1978)**, and **GRIME (1979)**. Seed germination requirements of species determine the distribution of adult plants in the landscape. Especially, in predominantly grass dominated communities, germination is believed to be a major determinant of species composition and hence community structure **(GULMON, 1992)**. Often, species which germinate early in the growing season are thought to pre-empt resources in a patch and thus establish competitive dominance **(ROSS and HARPER, 1972; RICE, 1984; BAZZAZ, 1996)**. Therefore, germination patterns may influence the subsequent interactions among plants.

The use of germination characteristics in predicting community change is well reviewed by **BAZZAZ (1996)**. According to **BAZZAZ (1996)**, predicting community change from a single germination or growth response to environmental changes such as moisture and temperature fluctuation would not be a useful

parameter. However, physiological response of species to the combined effect of temperature, soil disturbance and fire (smoke) would predictably determine the composition of a given community.

2.3 THE ROLE OF FIRE IN SEED GERMINATION

Fire is a major environmental selective force that controls many structural and functional properties of plant communities, and is a key factor initiating and terminating plant succession **(SOUSA, 1984)**. Fire as disturbance factor directly or indirectly affects plant growth, survival and reproduction and also influences seed germination and seedling dynamics **(BOND and VAN WILGEN, 1996; KEELEY and FOTHERINGHAM, 2000)**. A phenomenon common to nearly all grassland communities worldwide is the flush of new growth that follows a burn **(WHELAN, 1995; SNYMAN, 2005)**. Post-fire increases in germination and seedling emergence may be due to the high resource availability and/or low competition after a fire, but it may also be explained by **fire-stimulated germination** (i.e., dormancy breakage) **(KEELEY et al., 1985)**. **Fire-stimulated germination** is attributable to many fire-related variables. From the literature surveyed, the most commonly cited variables are; a) the **heat** from fire, b) the **charred wood** left on the ground after fire c) the greater **light** intensity that would reach the seeds once the canopy has burned **d)** nitrogenous compounds **(KEELEY et al., 1985; KEELEY, 1987; KEELEY and KEELEY, 1987),** and **smoke (BROWN, 1993a; BROWN, 1993b; VAN STADEN et al., 2000)**. Hence these variables may act, individually or in combination, as a cue (s) for stimulating the post-fire seed germination **(KEELEY et al., 1985)**.

2.3.1 Post-fire regeneration

Post-fire regeneration as an issue related to fire frequency, fire intensity (i.e. heat release), fire type (i.e. crown vs. ground fire), fire season (i.e. timing or season) or fire size (i.e. spatial coverage) in combination with certain climatic factors and other

abiotic conditions on plant composition is of challenging complexity **(VAN STADEN et al., 2000**; **BUHK et al., 2007)**. In its simplest and general form however, post-fire recovery depends on plant characteristics (e.g. resprouters or seeders) and on the meteorological conditions before the onset of fire **(BOND and VAN WILGEN, 1996; BUHK et al., 2007)**. In perennial grasses, post-fire regeneration mechanisms are basically of two types: **vegetative sprouting** and /or soil and/ or **canopy stored seed banks (PIERCE and COWLING, 1991; DALLMAN, 1998; KEELEY and FOTHERINGHAM, 2000)**. In the post-fire season, **sprouting** occurs either from aerial plant parts or undamaged underground nodes. However, non-sprouting and annual species that are killed by fire can only be established from seeds and mainly from the seed bank. Hence, species persistence requires the ability to maintain a high number of viable seeds and high tiller density with live leaves **(DANCKWERTS, 1984; EVERSON et al., 1988)**. Grass species of different genotypes differ in their ability to resprout following fire **(GRIME et al., 1988)**. Resprouting ability depends upon the anatomy and morphology of the plants, pre-disturbance size, intensity, frequency of disturbance and habitat productivity **(GRIME et al., 1988; SOUSA, 1984; KEELEY et al., 1999)**. It is also suggested that, resprouting success following defoliation greatly depends upon the ability of the species to accumulate carbohydrate reserves in below-ground organs **(DANCKWERTS, 1984; WOLFSON, 1999)**. In the mesic grassland of South Africa, some species survive by post-fire resprouting, but have minimal post-fire seedling recruitment **(DANCKWERTS, 1984; EVERSON, 1994)**. Other species have adults that do not survive fires, and post-fire germination and seedling establishment is required for post-fire regeneration **(DANCKWERTS, 1984; WHELAN, 1995; BOND and VAN WILGEN, 1996)**.

2.3.2 Effects of fire on seed germination

Fire-stimulated germination is found in many plant species from both fire-prone and non fire-prone ecosystems worldwide **(KEELEY and FOTHERINGHAM, 2000; VAN STADEN et al., 2000)**. Post-fire germination may be triggered by many

factors directly or indirectly related to fire. Some of the best studied and most frequently cited factors are the direct effects of heat shock and/or the release of chemicals from smoke, charred wood **(BOND and VAN WILGEN, 1996; KEELEY and FOTHERINGHAM, 2000; VAN STADEN et al., 2000)**. The indirect effects include changes in conditions of light availability, soil moisture potential, soil temperature, changes in nutrient status (nitrogen) and post-fire changes in soil microorganisms. In addition to these, the combined role of post-fire increases in physical space availability, increased resource availability, reduction in seed predators creating favourable conditions for enhancing germination and seedling establishment are well recognised **(BOND and VAN WILGEN, 1996; KEELEY and FOTHERINGHAM, 2000)**. Many researchers have carried out experiments that monitor germination after seed exposure to at least one of the above fire-related factors. Often, different intensities or doses of these treatments are applied and hence results may differ depending on the intensities, doses and concentrations used. In general however, the effects of fire on seed germination can be divided into two broad categories which are diagrammatically represented in **Figure 2.1** and will be discussed further. These two categories are the physical and chemical effects of fire.
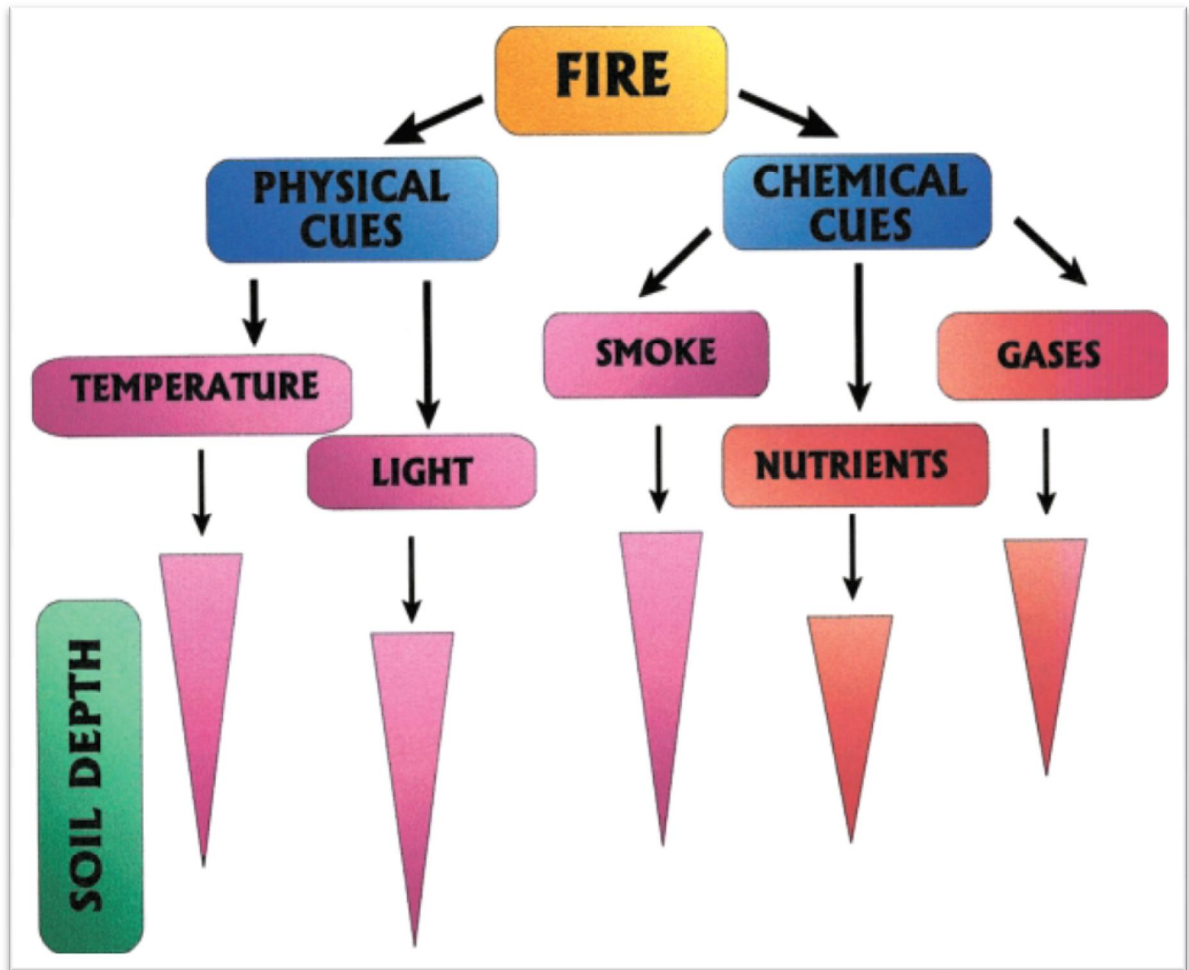
Figure 2.1: Diagram showing the relationship between the physical and chemical cues from fires within the seed environment (Diagram taken from VAN STADEN et al., 2000).

The scientific literature contains a great volume of literature identifying the net effect of each of the factors outlined in **Figure 2.1** on germination. However, at present there is no clear indication to what extent these factors may be linked or interactive in influencing seed germination **(VAN STADEN et al., 2000)**. Furthermore, there is limited experimental evidence of fire-cued germination and disentangling the role and importance of heat and/or smoke as germination cues for various plant communities worldwide **(FIGUEROA et al., 2009; MOREIRA et al., 2010)**.

2.3.2.1 Physical effects of fire on germination

The physical factors associated with fire are most commonly referred to the **heat shock** and post-fire increases on **light** quantity and quality **(VAN STADEN et al., 2000)**. An increase in soil temperature is often experienced due to the direct heat following a fire event which exposes the surface to better illumination **(SAVAGE and VERMEULEN, 1983; ANDERSSON et al., 2004)**. These increases in soil temperature and net radiation are most apparent on the top soil (0-5 cm), where seeds of most grass species occur in seed banks **(EVERSON, 1994)**. The magnitude of such increases in soil temperature depends on soil depth **(BRADSTOCK et al., 1992; ANDERSSON et al., 2004)** and fire behaviour as head or back fire **(TROLLOPE, 1984; SNYMAN, 2005)**, which is also related to fuel type, fuel load, and fuel moisture content **(TROLLOPE, 1984; CHAFFEY and GRANT, 2000)**. Hence the effect of fire on seeds can be lethal or stimulatory depending on the location of the seeds as either on the surface or buried **(ZACHARIAS et al., 1988; SNYMAN, 2004; SNYMAN, 2005; SHACKLETON, 2007)**.

The role of heat in breaking dormancy, related to properties of the outer covering structures of seeds, is often reported **(BEWLEY and BLACK, 1982)**. The effect of heat shock on germination of seeds, particularly hard-seeded species such as leguminous, woody and herbaceous species is well studied **(JEFFERY et al., 1988; DIXON and BARRETT, 2003; REYES and TRABAUD, 2009)**. These studies investigated the effect of heat shocks on germination and, in many of the species studied, heat enhanced germination **(GONZALEZ-RABANAL and CASAL, 1995)**. In heat-stimulated germination, *Cistaceae, Ericaceae, Fabaceae, Malvaceae Pinaceae, Proteaceae* and *Rhamnaceae,* **(CHRISTENSON and MULLER, 1975; VAN DE VENTER and ESTERHUIZEN, 1988; KEELEY and FOTHERINGHAM, 2000; MOREIRA et al., 2010)** are the most represented families. These studies differed with respect to the degrees of the temperature treatments applied, often ranging between 70-120 °C, and the duration of heat exposition ranging between 3-10 min. Such heat-shock stimulated germination is

widespread in species from the Mediterranean Basin **(THANOS et al., 1992; GONZALEZ-RABANAL and CASAL, 1995; REYES and TRABAUD, 2009; MOREIRA et al., 2010)**, Californian Chaparral **(KEELEY et al., 1985; KEELEY, 1987; KEELEY, 1991)**, Australia Kwongan/Mallee **(BELL et al., 1993)**, South African Fynbos **(JEFFERY et al., 1988; PIERCE and COWLING, 1991; BROWN, 1993a, BROWN and BOTHA, 2004)** and other fire-prone ecosystems. These studies collectively suggest that the dry heat generated from fire may break dormancy of hard-seeded species by providing a heat-pulse that stimulates the embryo of seeds directly **(MARTIN et al., 1975; JEFFERY et al., 1988; BASKIN and BASKIN, 1998)**, subsequently facilitating embryo imbibitions by rupturing the seed coat layer and leading to radicle expansion. The quest for dry or moist heat has also been a subject of great debate in the literature. **MARTIN et al. (1975)** compared whether the germination of 18 hard-seeded *Leguminosae* species is influenced by moist or dry heat treatment. These authors have shown that the presence of moisture combined with heat was optimum for germination rather than dry heat alone. Similarly, **HERRANZ et al. (1998)** tested the effect of dry heat on seed germination of seven Mediterranean *Leguminosae* species. These authors found that treating seeds with dry heat (90-120 °C) significantly increased germination in many of the species tested. It is generally suggested that there should be an important linkage between heat and moisture availability in stimulating germination **(MARTIN et al., 1975)**.

Post-fire environments are typified by the removal of litter, shrubs and canopies which subsequently increases the amount of light (and heat) reaching the soil surface. The amount of light which is received on the ground surface basically depends on the vegetation type, and the nature of the fire itself **(TROLLOPE, 1984; SNYMAN, 2005)**. The significance of light as a factor in seed germination is well recognised **(GRIME, 1979; GRIME et al., 1981; BAZZAZ, 1996)**. Germination of many herbaceous perennial seeds is cued to light and such a quality determines the subsequent fate of seeds to germinate under different levels of light or shading **(OJIMA et al., 1994; BAZZAZ, 1996).** Studies by **FLINT and MCALISTER (1937)** and **BORTHWICK et al. (1952)** showed that the responsiveness of seeds to

changes in illumination is mediated by the presence phytochrome (a compound which absorbs light) in seeds. Hence germination depends upon the ratio of red: far-red in the light impinging upon the seed **(GRIME et al., 1981)**. Sunlight filtered through a leaf canopy may be depleted in red light which causes germination inhibition **(GRIME, 1979; GRIME et al., 1981; BAZZAZ, 1996)**. Vegetation removal (mowing) increases the red:far-red light ratio at the soil surface, which subsequently causes increases in germination **(SILVERTWON, 1980)** and changes in growth patterns **(THOMPSON and HARPER, 1988)**.

2.3.2.2 Chemical effects of fire on germination

Post-fire environments are also typified by changes in the chemical properties of the soil **(RAISON, 1979)**. Phytomass combustion produces many volatile and non-volatile chemical cues that potentially influence canopy and soil-stored seeds. From the literature surveyed, some of the most researched chemical cues associated with fire are **ethylene, nitrogenous compounds** (**nitrites, nitrates** and **ammonia)**, **charred wood** and **ash**. The role of **ethylene** as a possible cue in regulating dormancy release and germination is well researched **(e.g. KĘPCZYŃSKI, 1985; SUTCLIFFE and WHITEHEAD, 1995; VAN DE VENTER and ESTERHUIZEN, 1988; BASKIN et al., 2003)**. This compound has been shown to promote seed germination in some species **(KĘPCZYŃSKI and KEPCZYŃSKA, 1997; BASKIN and BASKIN, 1998)**. Hence, it was largely believed that ethylene is the active compound in smoke that stimulates seed germination **(SUTCLIFFE and WHITEHEAD, 1995)**. In an attempt to identify the active germination compound in smoke, **JÄGER et al. (1996)** investigated the effect of smoke, octanoic acid and ethylene on stimulating germination of Grand Rapids lettuce seeds. They found that the germination compound in smoke was neither octanoic acid nor ethylene. According to **BASKIN et al. (2003)**, ethylene only serves as an indirect signal that indicates water availability. **Charred wood** also appears to be an important factor in promoting germination following a fire **(WICKLOW, 1977)**. The germination of the Californian Chaparral annual

Emmenanthe penduliflora was stimulated by placing seeds near to charred stems of a shrub *Adenostoma fasciculatum* **(WICKLOW, 1977)**. Subsequently, charred wood stimulated germination has been demonstrated in a large number of Matorral species. However, recent report by **BUHK and HENSEN (2006)** shows that ash and charred wood treatment of 21 Mediterranean species did not show any significant effect on seed germination.

Post-fire habitats are also characterised by changes in availability of nitrogenous compounds in the soil **(OJIMA et al., 1994; FYNN et al., 2003)**. The literature shows that the effect of fire on nutrient availability, especially N is less predictable. In the long-term, fire may cause severe N losses due to volatisation and soil leaching **(DEBANO and CONARD, 1978; GROGAN et al., 2000; FYNN et al., 2003)**. In the short-term however, slight increases in available N following a fire incidence are common in nature **(DEBANO and CONRAD, 1978; RAISON, 1979; KNAPP and SEASTEDT, 1986; OJIMA et al., 1994; FYNN et al., 2003)**. Nitrogen is added in the form of ammonium or organic nitrogen which is subsequently converted to nitrate by nitrifying bacteria. Several studies show that such nitrogenous compounds (mainly nitrite and nitrates) are effective in breaking dormancy and/or promoting germination of many species **(BASKIN and BASKIN, 1998; KEELEY and FOTHERINGHAM, 1997)**. Thus, in fire-areas, nitrogen-induced germination has often been interpreted in terms of its relationship to fire **(THANOS and RUNDEL, 1995)**. Consequently, nitrates and ammonium ions in ash have been proposed as germination stimulatory factors **(HENDRICKS and TAYLORSON, 1974; HENIG-SEVER et al., 2000)**. **KEELEY and FOTHERINGHAM (1997; 1998)** reported that nitrogen oxides, and nitric oxide (NO) in smoke induced 100% germination of a California Chaparral annual *Emmenanthe penduliflora* in a manner similar to that of smoke. Though not as effective as smoke, the germination of several Chaparral **(KEELEY and FOTHRINGHAM, 1998)** and Western Australian **(BELL et al., 1999)** species has also responded positive to KNO_3 treatment. However, **LIGHT and VAN STADEN (2003)** examined the effects of two nitrogenous oxides on germination of Grand Rapids lettuce seeds, and neither of these compounds promoted germination,

suggesting that NO was unlikely to be responsible for the smoke-enhanced germination **(LIGHT and VAN STADEN, 2003)**. By the same token, **LUNA and MORENO (2009)** have found no association between nitrates and mean germination enhancement on 53 representative Mediterranean species in Central-Eastern Spain.

The effect of **ammonium ions** in breaking dormancy, promoting germination and seedling emergence is also well recognised **(HENIG-SEVER et al., 2000)**. Often nitrates stimulate germination of many dormant seeds, while ammonium salts are usually ineffective. Ammonium-containing fertilizers such as limestone ammonium nitrate (LAN) and liquid ammonium nitrate (AN) were reported to be effective in stimulating germination and emergence of certain species **(AGENBAG and DE VILLIERS, 1989)**. For instance, ammonium treatment of seeds of *Phacelia grandiflora* and *Salvia mellifera* effectively promoted germination **(THANOS and RUNDEL, 1995)**. Hence, it has been suggested that the germination activity of smoke is due to ammonia or ethylene **(VAN DE VENTER and ESTERHUIZEN, 1988).** However, studies conducted by **VAN STADEN et al. (1995)** and **JÄGER et al. (1996)** have confirmed that neither of these compounds are responsible for the promotive effect of smoke solutions on the germination of the Grand Rapids lettuce seeds.

2.4 GERMINATION RESPONSE TO SMOKE

The use of fire and smoke for various purposes in agriculture is one of the oldest practices in Africa. African farmers have traditionally used fire and smoke in grain drying practices **(MODI, 2002)**. They kept their kernels near a fireplace and believed these methods would improve seed germination and produced healthier seedlings **(PAASONEN et al., 2003; MODI, 2002; 2004).** Besides burning farm lands were commonly thought to produce healthier plants and that fire would kill crop pests and rodents in the soil. In its scientific way however, the role of plant-derived smoke in stimulating seed germination appeared to be important after the benchmark studies by **DE LANGE and BOUCHER (1990)**.

Seed germination in response to fire can be affected by many factors associated with fire **(GRIME, 1979; GOUBITZ et al., 2003)**. In fire ecology, plant responses to heat and nutrient release during and after wildfires are the most researched topics **(DEBANO and CONRAD, 1978; KNAPP and SEASTEDT, 1986; OJIMA et al., 1994)**. Although enhanced germination following a fire incidence has been observed for many species, often the effect was, accredited to the heat effect of fire. For instance, **FISHER (1978)**; **SAVAGE and VERMEULEN (1983)** reported that fire benefits plant growth because of changes in the physical environment rather than in the chemical environment. This indicates that the chemical role of fire and fire-related factors as germination cues had been given little attention. With time however, researchers revealed the existence of multiple germination cues associated with fire which significantly play a role in the post-fire environment **(VAN DE VENTER and ESTERHUIZEN, 1988)**. **VAN DE VENTER and ESTERHUIZEN (1988)** were among the first to report that smoke from fires, independently of heat, can stimulate germination. In other words, high temperatures caused by fire were not necessarily the only factors responsible for the elevated germination in a post-fire environment, and there could be multiple germination cues associated with fire.

2.4.1 The discovery of smoke as a germination cue

The historical background of fire-stimulated germination dates back to 1935. Indeed, as early as 1935, **LEVYNS** reported that germination of seeds of a wide variety of highland species was enhanced following a fire. By the same token, **WICKLOW (1977)** reported that chemicals generated by phytomass combustion either from smoke or charred wood cued germination of the post-fire Chaparral annual herb *Emmenanthe penduliflora*. This author suggested that fire was needed to stimulate the germination of Chaparral flora through mechanisms involving seed coat scarification and/or the removal of alellopathic inhibitors to germination. **KEELEY et al. (1985)** have observed similar effects of fire-stimulated germination in the Californian Chaparral, however defining which aspect of the fire was the

most important or even crucial to germination was somewhat problematic or unrevealed.

In the 1990's however, it became evident that one of the most striking inducers of germination in the post-fire environment was that of **smoke** itself **(DE LANGE and BOUCHER, 1990; BROWN, 1993a; BALDWIN and MORSE, 1994)**. **DE LANGE and BOUCHER (1990)** demonstrated that smoke in particular was responsible for the germination stimulation of the South African Fynbos species *Audouinia capitata* (Bruniaceae). This discovery raised a number of questions. For example, what is the factor in smoke that induces germination? Is there one or more than one factor? Do all species respond to the factor in smoke? How does smoke interact with other germination cues such as light and temperature? What is the mode of action of smoke-stimulated germination? Do sources of smoke determine the chemical nature of the smoke generated? The answer to these questions was the most sought-after prize in seed germination biochemistry **(VAN STADEN et al., 1995)**. Aqueous extracts of plant-derived smoke indeed contains a mixture of thousands of compounds **(ADRIANSZ et al., 2000)**. Hence, the search for active germination stimulant (s) from a cocktail of thousands of compounds in plant-derived smoke was a daunting task **(VAN STADEN et al., 1995)**. Clearly, an understanding of the mode of action of the active component(s) in smoke required their isolation and identification. The long way travelled and the race for the search and identification of an active germination triggering compound from crude plant-derived smoke is sufficiently reviewed by **LIGHT et al. (2009)**. **FLEMATTI et al. (2004, 2008)** also discuss the details of the various strategies carried out by an Australian research group leading to the isolation of the highly active germination compound (3-methyl-2H-furo[2,3-c]pyran-2-one) from burnt cellulous **(FLEMATTI et al., 2004)** commonly referred to as "**butenolide**" **(FLEMATTI et al., 2004; VAN STADEN et al., 2004)** or more recently termed as "**karrikinolide**" **(NELSON et al., 2009)**.

Smoke contains thousands of compounds and thus the search for active compounds related to germination is still actively researched. Recently, a related

butenolide, 3,4,5-trimethylfuran-2(5*H*)-one (2), which inhibits germination and significantly reduces the effect of the stimulatory butenolide if applied simultaneously, was also isolated from plant-derived smoke **(LIGHT et al., 2010)**. Hence, it is suggested that the interaction of these compounds with opposing actions on seed germination may have important ecological implications in a post-fire environment. The chemical structure of both the stimulatory (1) and the inhibitory (2) compounds are provided below (**Figure 2.2**).
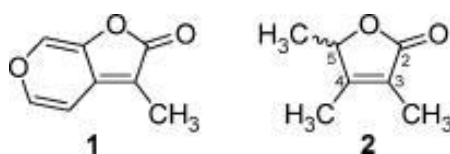
Figure 2.2: Chemical structure of the bioactive compound (3-methyl-2*H*-furo[2,3-c]pyran-2-one) from smoke that promotes seed germination (1) and (3,4,5-trimethylfuran-2(5*H*)-one) that inhibits seed germination (2) (LIGHT et al., 2010).

Since the discovery of smoke as a germination cue, numerous smoke-stimulated germination studies have been conducted on South African, Australian, Californian and Mediterranean basin species using various methods of smoke application **(ROCHE et al., 1997; CLARKE and FRENCH, 2005)**. The commonly used techniques of smoke application involved;

1. The direct exposure of test seeds to different durations of aerosol smoke (SA), which on average, ranged between 3 min to 60 min **(BROWN, 1993b; BAXTER et al., 1994; SPARG et al., 2006)** and using various types of plant material as fuel sources **(BROWN, 1993a; BAXTER et al., 1994; ROCHE et al., 1997)**.

2. The application of plant-derived smoke solutions of various concentration levels ranging from 1:1 to 1:1500 v/v **(VAN STADEN et al., 2000)**.

Germination and seedling vigour responses generally fall into three categories, i.e. positive, negative (inhibited) and neutral **(ADKINS and PETERS, 2001)**. The list of all plant species which responded to smoke treatments is long and hence compiling the whole bunch of numbers was not possible. In this review, the list of the grass species (Poaceae) which have been tested for smoke-stimulated germination are attached as two appendices. **Appendix 1** presents examples of species whose seeds were stimulated by smoke. **Appendix 2** presents examples of grass species which did not show a significant germination response to smoke treatments.

2.4.2 The interaction of smoke and heat in influencing seed germination

Fire-stimulated germination can be the result of one or the interactive effects of smoke with heat and other fire-related factors in the post-fire environment. Despite the popularity of smoke-stimulated germination, few studies suggest that smoke has a limited role in stimulating germination of species from the Mediterranean basin **(BUHK and HENSEN, 2006; REYES and CASAL, 2006; REYES and TRABAUD, 2009)**. Hence, the relevance of heat and/or smoke as a germination cues has been a source of hot debate and is still extensively researched **(FIGUEROA et al., 2009; MOREIRA et al., 2010)**. A few studies have attempted to unscramble the net effect of heat and smoke as independent treatments **(READ et al., 2000; ENRIGHT and KINTRUP, 2001; WILLS and READ, 2002; KEELEY et al., 2005; FIGUEROA et al., 2009; MOREIRA et al., 2010)**. For example, **MOREIRA et al. (2010)** attempted to disentangle the role of heat and smoke as germination cues and have found that heat stimulated the germination of 21 species and smoke only eight species, out of the 30 species studied. In central Chile, **FIGUEROA et al. (2009)** studied whether heat or smoke influenced seedling

emergence of the local Motorral flora. This study provided evidence that heat-shock is responsible for post-fire increases in emergence. **WILLS and READ (2002)** reported that smoke treatment of the soil seed bank resulted in several-fold increases in species richness and seedling density; heat treatment resulted in high mean species richness and seedling density. By the same token, **KEELEY et al. (2005)** obtained three types of responses from seeds treated with smoke and heat, where the germination of certain species was stimulated by smoke, some by heat and some by the combined effect of both smoke and heat. Several other studies have also investigated the combined effect of smoke and heat on germination **(KEITH, 1997; GRANT et al., 1997; MORRIS, 2000; THOMAS et al., 2003; CLARKE and FRENCH, 2005)**. For instance, **KEITH (1997)** showed that germination of *Epacris stuartii Stapf.*, an endangered Australian shrub responded well to the additive effect of heat and smoke rather than only a single treatment alone. Similarly, **MORRIS (2000)** showed that the germination of seven east Australian *Grevillea* species was the highest when exposed to smoke and heat treatments combined compared with either treatment alone. Hence, it can be inferred that for a number of species the effects of smoke and heat on germination can be interactive. A number of studies suggest that there are multiple germination cues and that the stimulatory effects of the cues could be independent and additive or interactive **(DIXON et al., 1995; MORRIS, 2000; DIXON et al., 2009)**.

2.4.3 The interaction between plant-derived smoke and soil nutrients in influencing plant growth

Major soil macronutrients which are essential for plant growth and development include nitrogen (N) potassium (P) and phosphorus (K). These are the nutrients most likely to impact site productivity and vegetation dynamics and therefore are of most interest to ecologists. The effect of fire on major soil macronutrients is well studied. Fire causes slight temporary increases in nitrogen levels in the post-fire habitat **(DEBANO and CONRAD, 1978; RAISON, 1979; KNAPP and SEASTEDT, 1986; OJIMA et al., 1994; FYNN et al., 2003)**. Plant–derived smoke is another

new factor which enhances post-germination vegetative growth of seedlings in a similar way to plant growth-hormones **(SPARG et al., 2005; JAIN and VAN STADEN, 2006; DAWS et al., 2007)**. In other words, smoke affects plant processes through interaction, in some way, with endogenous plant growth regulators **(VAN STADEN et al., 2000)**. However, it remains largely unknown as to how smoke solutions interact with major soil nutrients such as NPK in influencing plant growth. In this study, the growth of three grass species which showed contrasting (i.e. positive, intermediate and negative) response to various smoke treatments were examined in the presence or absence of major soil nutrients (NPK) (see **Chapter 6**).

2.5 POST-GERMINATION EFFECTS OF SMOKE AND BUTENOLIDE

The role of plant-derived smoke and butenolide is not limited within the germination process. Since the discovery of smoke as a germination cue and identification of butenolide as a highly active germination compound in smoke, the effect of various smoke solutions and butenolide on post-germination effects have also been studied **(VAN STADEN et al., 2006; JAIN et al., 2006; JAIN and VAN STADEN, 2006)**. Post-germination processes, often considered as the continued growth and successful establishment of seedlings **(SPARG et al., 2005),** were facilitated due to the effect of smoke and/or butenolide. Several studies show that smoke solutions and butenolide exhibit hormone-like responses in many species and they interact with gibberellins, cytokinins, abscisic acid and ethylene to promote germination and growth **(VAN STADEN et al., 2000)**. Studies conducted on various aspects of growth collectively reveal that many seeds treated with smoke-water or butenolide germinated faster, and of higher percentages with more vigorous seedlings (shoot, root, leaf count) **(SPARG et al., 2005; JAIN and VAN STADEN, 2006; DAWS et al., 2007)**. Examples of the studies which investigated the post-germination effects of smoke and butenolide and the main findings are given in **Table 2.1.**

Post-germination effects of plant-derived smoke may also include its role in overcoming stresses arising from environmental extremes. Crude smoke or butenolide can also widen the environmental range over which germination can occur. Butenolide particularly can fully or partially substitute for the light requirement of seeds during the process of germination **(MERRITT et al., 2006)** and overrides stresses exerted due to sub-optimal temperature **(JAIN et al., 2006)**. These findings have important ecological implication in that, in highly productive late successional environments characterised by high stress from low light and sub-optimal temperatures **(GRIME, 1979; BAZZAZ, 1979)**, crude smoke or butenolide may widen the temperature range over which germination of certain late successional species can occur. Furthermore, this could lead to greater balance among species and avoid situation which could otherwise lead to local extinction of certain species.

Table 2.1: Examples of studies investigating the post-germination effects of plant-derived smoke and butenolide on various aspects of plant growth.

Publication title/ Reference	Author (s)	Main findings on post-germination activities of plant-derived smoke
Smoke stimulated flowering in the fire-lily *Cyrtanthus vertricosus*	**KEELEY, 1993**	Flowering in *Cyrtanthus vertricosus* (Jacq.) Willd. was stimulated by smoke
Root initiation in *Vigna radiata* (L.) Wilczek hypocotyl cutting is stimulated by smoke-derived extracts	**TAYLOR and VAN STADEN, 1996**	Aqueous extracts of smoke, derived from *Themeda triandra,* a fire-climax grass, and *Passerina vulgaris,* a Fynbos plant, stimulated root initiation and development in the hypocotyls of mung beans
Heated substrate and smoke: influence on seed emergence and plant growth	**BLANK and YOUNG, 1998**	Leaf production and leaf elongation of four grass species were significantly greater when treated with plant-derived smoke
Plant-derived smoke solutions stimulate the growth of *Lycopersicon esculentum* roots in vitro	**TAYLOR and VAN STADEN, 1998**	Aqueous extracts of smoke, derived from *Themeda triandra*, a fire-climax grass, and *Passerina vulgaris,* a Fynbos plant, stimulated the growth of primary root sections of tomato roots in suspension culture
Improving seedling vigour of indigenous medicinal plants with smoke	**SPARG et al., 2005**	The vigour index of South African indigenous medicinal plants *Albuca pachychlamys, Merwilla natalensis* and *Tulbaghia violacea* was increased with the application of dry smoke and smoke extract dilutions

Post-germination effects of the smoke-derived compound 3-methyle-2H-furo[2,3-c]pyran-2-one, and its potential as a preconditioning agent	**VAN STADEN et al., 2006**	Smoke-water and butenolide treatment of seeds enhanced shoot length, root length and number of leaves in tomato, okra, maize and bean
A smoke-derived butenolide improves early growth of tomato seedlings	**JAIN and VAN STADEN, 2006**	Post-germinative growth of tomato seedlings (emergence of the radicle and hypocotyls) was significantly improved with butenolide treatment at 10^{-7} M
Stimulation of rice (*Oryza sativa* L.) seedling vigour by smoke-water and butenolide	**KULKARNI et al., 2006**	Smoke-water and butenolide treatments significantly increased shoot length, root length and seedling weight of rice
Aerosol smoke and smoke-water stimulation of seedling vigour of a commercial maize cultivar	**SPARG et al., 2006**	Treating seeds with aerosol smoke showed significant stimulatory effects on seedling vigour of commercially bred maize seeds
Comparative enhancement of germination and vigour in seed and somatic embryos by the smoke chemical 3-methyl-2h-furo[2,3-c]pyran-2-one in *Baloskion tetraphyllum (Restionaceae)*	**MA et al., 2006**	Butenolide promoted development and maturation of somatic embryogenesis providing a potential new class of phyto-active compounds for *in vitro* culture
Butenolide from plant-derived smoke enhances germination and seedling growth of arable weed species	**DAWS et al., 2007**	Butenolide is a highly effective germination and seedling growth stimulant for a range of arable weeds

2.6 POSSIBLE MODE OF ACTION OF SMOKE-STIMULATED GERMINATION

By definition, germination incorporates those events that commence with the uptake of water by the quiescent dry seed and terminate with the elongation of the embryonic axis **(BEWLEY and BLACK, 1994; BEWLEY, 1997)**. The time taken for water uptake (imbibition) depends on many factors related to the nature of the seed coat (testa) and the osmotic potential of the medium used for germination initiation. Under favourable conditions rapid expansion and growth of the embryo culminates in rupture of the covering layers and emergence of the radicle. Radicle emergence is considered as the completion of germination **(GRIME, 1979; BAZZAZ, 1996)**.

Since the discovery of smoke as a germination cue, and the identification of the germination compound in smoke (butenolide), many attempts have been made to understand the mode of action of smoke-aided germination. Though presently, the mode of action of smoke and/or butenolide is not fully known however, there are three postulates proposed as possible mechanisms of smoke action. These are: a) chemical scarification of the seed coat; b) nutrient-aided stimulation of germination; and c) signal-mediated stimulation of germination. Clearly, exposure to smoke treatment has resulted in the collapse of the surface cuticle **(EGERTON-WARBURTON, 1998)**. Though there is no consensus on the site and mode of physiological action of smoke-stimulated germination, it is becoming clearer that smoke may influence seed coat scarification mechanically which facilitates imbibition **(VAN STADEN et al., 2004; LIGHT, 2006)**. Recently, **SOÓS et al. (2009a and 2009b)** investigated the effect of smoke-water on seedling vigour and changes of the global transcriptome in the early post-germination phase in maize. These authors reported that smoke treatment of maize seeds induced stress in the early post-germination phase which leads to better adaptation of the seeds to environmental stress factors occurring during germination and eventually resulting in greater seedling vigour. Some of the possible suggestions of mode of smoke action as a chemical germination cue are sufficiently discussed by **VAN STADEN et al. (2000)** and reviewed by **LIGHT (2006)**.

2.7 CURRENT APPLICATIONS OF PLANT-DERIVED SMOKE IN VARIOUS FIELDS OF PLANT SCIENCES

It has been well recognised that fire and smoke play an important role in traditional African agriculture. By-products of fire such as smoke and ash were commonly used in drying and storing grains **(MODI, 2002; 2004)**. However, the discovery of smoke and identification of butenolide has essentially lead to the application of smoke technology for a variety of agricultural, horticultural, and ecological restoration and conservation programmes **(ROCHE et al., 1997; BOUCHER and MEETS, 2004; LIGHT and VAN STADEN, 2004; VAN STADEN et al., 2006)**. Examples of the current use of plant-derived smoke solutions include agricultural, horticulture, habitat restoration and weed control programmes. Furthermore, smoke is being widely used as a useful tool in various ecological studies in determining the size, extent **(VAN DER VALK and DAVIS, 1978; WARD et al., 1997; FUNES et al., 1999; READ et al., 2000)**, composition **(VAN DER VALK and DAVIS, 1978; ADKINS and PETERS, 2001; COX and ALLEN, 2008; FISHER et al., 2009)**, and seasonal dynamics **(WILLS and READ, 2002; DIXON and BARRETT, 2003; FISHER et al., 2009)** of soil seed banks.

2.7.1 Agriculture

Plant-derived smoke has the potential to be used for a variety of applications related to seed technology in agriculture **(LIGHT and VAN STADEN, 2004)**. Over the last decade, the germination of several agricultural crops **(SPARG et al., 2006; KULKARNI et al. 2006)** has been tested with various smoke solutions using different methods of smoke application. For instance, seeds of rice treated with smoke solutions germinated faster and to higher percentages than controls **(DOHERTY and COHN, 2000; KULKARNI et al. 2006)**. By the same token, **PAASONEN et al. (2003) and MODI (2002, 2004)** investigated the effect of various storage methods on the seed quality of two traditional maize landraces. Storage method which involved smoking improved the germination rate and final germination in comparison to untreated seeds. Furthermore, seeds stored above the fireplace produced significantly more vigorous seedlings, which were heavier and taller, than those from untreated seeds. Thus, smoke treatments could possibly be used to promote

synchronous germination of seeds and to increase the rate of germination of certain agricultural crops. Research efforts are continuously being made on seeds of various agricultural crops on smoke-aided modification of certain economically important traits such as faster emergence, better stand establishment, drought tolerance, earlier flowering and harvesting **(HARRIS et al., 1999)**. In this study, the application of plant-derived smoke solutions in improving seed germination and seedling establishment of a selected cereal crop *Eragrostis tef* has been studied (see **Chapter 4**).

2.7.2 Horticulture

The potential use of plant-derived smoke in improving horticultural crop production has been highlighted by **LIGHT and VAN STADEN (2004)**. One of the preliminarily studies on horticultural crops was by **DREWES et al. (1995)**. These authors reported that light sensitive lettuce seeds (*Lactuca sativa* cv. Grand Rapids) responded positively to treatment with smoke solutions. In a similar study **THOMAS and VAN STADEN (1995)** reported that smoke solutions were effective in breaking dormancy and stimulating germination in a common vegetable crop i.e. celery (*Apium graveolens* L.). In these studies it was revealed that smoke has the ability to substitute for the light requirements of these seeds **(DREWES et al., 1995; THOMAS and VAN STADEN, 1995)**. Due to their rapid germination response (often < than 24 h) and sensitivity to environmental changes, Grand Rapid lettuce is commonly used as a bioassay for the detection of the germination activity of various smoke solutions (see **Chapter 5**).

VAN STADEN et al. (2006) investigated the effects of smoke-water and butenolide on germination and seedling development of three different vegetable crops (tomato, okra and bean) and a commercial maize variety. Seeds from these crops have showed remarkable improvement in shoot length, root length and number of leaves when treated with smoke solutions. Even foliar application of smoke-water and butenolide on germinated seeds of okra and tomato significantly improved seedling growth **(KULKARNI et al., 2007)**. **KULKARNI et al. (2010)** also investigated the effect of smoke-water and butenolide treatment on various growth parameters of onions and tomatoes. Smoke-water treatment resulted in maximum

height, greater number of leaves, and greater stem thickness of tomato plants. Similarly, onion plants treated with smoke–water and butenolide solution had significantly greater above-ground biomass than untreated plants **(KULKARNI et al., 2010)**.

2.7.3 Habitat restoration and conservation

The seed bank is a central topic for plant community restoration and conservation **(VAN DER VALK and DAVIS, 1978; THOMPSON and GRIME, 1979; EVERSON, 1994; BEKKER et al., 1998)**. Seed bank also called diaspore banks **(VON BLANCKENHAGEN and POSCHLOD et al., 2005)** consist of seeds, fruits and vegetative parts which provide an immediate source of propagules for recruitment after disturbance. From a conservation perspective the soil seed bank also preserves seeds of species of a local species pool **(VAN DER VALK and DAVIS, 1978; THOMPSON and GRIME, 1979; KEELEY, 1987)**. Hence, the soil seed bank is thought to provide a memory of the past vegetation and represents the structure of future populations. Though there are different ways by which this is achieved **(e.g. BEKKER et al., 1998; VON BLANCKENHAGEN and POSCHLOD et al., 2005; WILLS and READ, 2007**), the most widely accepted seed bank classification is the one proposed by **THOMPSON and GRIME (1979)**. In this type of classification two types of seed banks are recognised i.e. transient and persistent. The classification takes seed persistence in the soil as major parameter. In the transient viable seeds remain in the soil for less than one year, whereas seeds in the persistent seed bank remain dormant for longer periods.

With the expansion of urban areas, industrialization, mining and construction of new roads, many habitats have become degraded and new revegetation techniques and approaches are required. In such areas, where fires are not desirable, smoke technology can be used to enhance the germination of native species **(VAN STADEN et al., 2000)**. The possible use of smoke technology in habitat restoration has been studied by many **(ROCHE et al., 1997; ROKICH et al., 2002; ROKICH and DIXON, 2007)**. In Australia, pioneering work on smoke as a restoration tool has been done at Kings Park Botanical Gardens **(DIXON et al., 1995; ROCHE et al., 1997)**. This research group has developed a practical

technique using smoke-water for restoration, which is now widely used in broader scales. **ROCHE et al. (1997)** have also used smoke techniques for the rehabilitation of bauxite mine sites in the Jarrah forests in Western Australia. This research group applied aerosol smoke and smoke-water on unmined and mined and broadcasted seeds which resulted in 48-fold increases in germination followed by marked increases in plants species richness in all the areas studied. By the same token, **READ et al. (2000)** assessed the effectiveness of smoke and heat application on soil seed bank germination and re-establishment of native forest in New South Wales, Australia. They found that smoke application increased the density of total emergents, and revegetation depended on the composition of the soil seed bank. A recent study by **ROKICH and DIXON (2007)** suggested that smoke treatments to enhance seed bank germination have marked differences in effectiveness at specific times of the year, as many seeds in the soil seed bank cycle between dormancy states. Another study by **ROKICH et al. (2002)** suggested that smoke-water application on soil surface can be used as an effective strategy for woodland restoration. Smoke solutions can also be used to improve germination of threatened species **(BELLAIRS et al., 2006)**. These studies collectively demonstrate that smoke solutions and aerosol smoke has the potential for the use in sustainable maintenance of pre-existing populations, as well as in the development of effective strategies for the emergence of new populations of species of high conservation interests **(VAN STADEN et al., 2004; BROWN and BOTHA, 2004)**.

2.7.4 Weed control

In arable fields where the majority of weeds are annuals, the seed bank is the prime factor for the renewal of populations and hence the main source of fresh weed infestation **(DESSAINT et al., 1991; ADKINS et al., 2000)**. Therefore, knowledge of the contents of the soil seed bank is of prime importance as it is used to predict the potential weed and alien species infestation **(VAN DER VALK and DAVIS, 1978; DESSAINT et al., 1991; WILLS and READ, 2007)**. One of the main features of weeds is their ability to retain viability and build a persistent seed bank due to dormancy. This situation hampers weed emergence and management and thus promotion of strategies aimed at enhancing maximum germination and emergence

are of great significance **(DESSAINT et al., 1991; ADKINS et al., 2000; STEVENS et al., 2007)**.

Since the discovery of smoke as a germination cue a considerable number of studies have examined the use of smoke as an effective agent in breaking dormancy of weed seeds to deplete arable weed seed banks of troublesome weeds **(ADKINS et al., 2000; READ et al., 2000; KĘPCZYŃSKI et al., 2006; DAWS et al., 2007; STEVENS et al., 2007)**. These studies have shown smoke to be a useful tool for obtaining much higher germination efficiencies of weeds which occupy the surface as well as the soil seed bank. For instance, smoke-water or butenolide treatment of seeds from agricultural weeds such as *Avena fatua* (Wild oats), *Brassica tournefortii* (Wild turnip) and weeds of restoration areas e.g. *Acetosa vesicaria* (ruby dock) has resulted in several-fold greater germination percentages **(ADKINS and PETERS, 2001; KĘPCZYŃSKI et al., 2006; DAWS et al., 2007; DIXON et al., 2009)**. **GRANT et al. (1997)** reported a similar response from the topsoil of 12-year-old rehabilitated bauxite mines, where six of the 13 smoke-stimulated species were weeds. Comparing the effectiveness of crude smoke and butenolide, **DAWS et al. (2007)** reported that butenolide is highly effective germination and seedling growth stimulant for a range of arable weeds rather than the crude smoke. Butenolide may also function as a germination stimulant for seeds of parasitic plants (weeds) such as *Orobanche and Striga* **(DAWS et al., 2008)**. Smoke treatments at lower concentrations were also able to promote the germination of the parasitic weed *Orobanche aegyptiaca* **(BAR NUN and MAYER, 2005)**. These observations collectively suggest that smoke solutions may play an important ecological role in the management and control of introduced weeds in native and arable plant communities. Smoke-aided weed seed bank stimulation is thought to be promising in reducing the cost of herbicides or physical labour needed for weed eradication **(ADKINS and PETERS, 2001)**.

2.8 ECOLOGICAL STUDIES USING SMOKE-STIMULATED GERMINATION

In the last two decades, the use of smoke as an ecological tool for studying the size, composition, distribution and seasonal dynamics of seed banks has been shown for many vegetation types **(BALDWIN et al., 1994; ROCHE et al., 1997; WARD et al.,**

1997; LLOYD et al., 2000; READ et al., 2000; ROKICH et al., 2002; DIXON and BARRETT, 2003). Many of the recent studies involving smoke-aided seed germination demonstrate that various smoke treatments are significant in understanding the size, composition, special and temporal dynamics of the soil seed bank **(ROKICH and DIXON, 2007; DIXON et al., 2009)**. To date, overall, smoke application has resulted in:

- 48-Fold increases in total number of germinants from the soil seed bank;
- 4-Fold increases in species diversity with approximately 1200 species responding under field conditions; and
- Increased germination of more than 80 genera, representing major plant families **(DIXON et al., 2009)**.

STEVENS et al. (2007) were among the first who showed the capability of smoke to stimulate seedling emergence when delivered to the soil seed bank under field conditions. Several other studies have also been conducted using various smoke treatments on soil seed bank samples. These studies were significant in providing essential baseline information for forest restoration, conservation, management and weed invasion control programmes.

2.9 SMOKE-STIMULATED SEED GERMINATION AND SOME IMPORTANT CONSIDERATIONS FOR PLANT ECOLOGY

The greatest potential for the development of plant ecology as a predictive science lies in developing an understanding of the relationship between plant-traits and various forms of disturbances (e.g. fire, grazing, mowing) which alter resources availability (e.g. light, nutrients, pH, moisture, temperature) **(GRIME, 1979; TILMAN, 1988; BAZZAZ, 1996)**. Detailed understanding of how specific plant- traits (e.g. shoot, root, leaf and flower) react to specific environmental influences will enable greater understanding of the species-environment relationship and assist in the development of predictive understanding of how specific environmental changes influence germination, establishment and survival of plant species.

Germination behaviour as an important trait strongly linked to the continued survivorship of plant populations is highly influenced by many forms of disturbances

one of which is fire **(BROWN, 1993a; BROWN, 1993b; KEELEY and FOTHERINGHAM, 1998)**. Disturbance by fire can have a profound effect on the dynamics of plant populations, and influence recruitment, mortality, dominance and distributions **(HARPER, 1977)**. A considerable number of fire-involving germination studies have shown that germination can be effectively stimulated by fire-related cues such as heat **(CLARKE and FRENCH, 2005)**, charate extract **(KEELEY et al., 1985, KEELEY, 1987, KEELEY and KEELEY, 1987)**, aqueous smoke extracts and aerosol smoke derived from burning plant material **(VAN STADEN et al., 2006)**. Smoke solutions of various concentrations have been shown to stimulate seedling emergence **(STEVENS et al., 2007)** and induce vigour (e.g. shoot, root, leaf count) in many species both from fire-prone and non-fire prone habitats, even under stress conditions **(SPARG et al., 2005; VAN STADEN et al., 2006; JAIN et al., 2006)**. Though many species are responsive to smoke (See **Appendix 1**), not all are responsive to smoke (see **Appendix 2)** or at least not to the same degree **(DIXON et al., 1995; READ et al., 2000; DIXON et al., 2009)**. This has lead to the selection of a group of plant species whose germination behaviour can be modified by smoke which are recognised as smoke-responsive species. By contrast, those whose germination behaviour cannot be modified by smoke treatments are designated as non-smoke responsive species **(DIXON et al., 2009)**.

In fire-prone ecosystems it is generally considered that there is "succession after fire'' **(WESTOBY et al., 1989; BOND and VAN WILGEN, 1996; BUHK and HANSEN, 2006; BUHK et al., 2007)**. It is commonly thought that such a succession happens because of the variation in environmental fluxes and extreme events of many kinds caused by disturbance **(BAZZAZ, 1983; SOUSA, 1984)**. On the other hand, some plants may survive the disturbance, and these plants can contribute to the post-fire regeneration. However, it is now widely questioned that beyond the natural variation in seed germination behaviour, resprouting abilities and survivorship, variation in response to smoke may generate a major influence in recruitment dynamics and potential distribution of adult plants **(KEELEY and FOTHERINGHAM, 1998; BOND and VAN WILGEN, 1996; DIXON et al., 2009)**. It is generally true that the trajectory of ecological succession can be modified by the presence or absence of fire and smoke. Therefore, a theoretical ecological framework of fire-induced vegetation dynamics and studies dealing with the

understanding of the post-fire patterns and mechanisms of plant succession essentially need to constitute the role of fire and smoke **(CHAKRABORTY and LI, 2009)**. With regards to this there is not sufficient information documented on how recurrent fires and factors related to fire-regime influenced global vegetation patters during evolution. Hence, future research prospects need to consider the role of smoke and heat-stimulated seed germination as an efficient mechanism in successional change **(PICKETT, et al., 1987; CHAKRABORTY and LI, 2009)**.

CHAPTER THREE

The effect of smoke solutions and temperature on germination and seedling growth of South African mesic grassland species

"Anyone can count the seeds in an apple, but only God can count the number of apples in a seed"

Robert Harold Schuller (1926˜)

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3.1 INTRODUCTION

Fire, natural or of anthropologic origin, is a recurrent phenomenon in the mesic grasslands of South Africa and it has been a major ecological influence since time immemorial **(SCOTT, 1971; TAINTON, 1978)**. Fire consumes nearly all grass cover and post-fire grasslands are marked by a flush of vigorous vegetative regeneration from perennial tufts and new seedling emergence **(EVERSON and TAINTON, 1984; TAINTON, 1999)**. Frequent fires cause a major shift in species composition of the Tall Grassland favouring the survivorship of relatively short to medium height native grasses such as *Themeda triandra* Forssk., *Tristachya leucothrix* Trin. ex Nees and *Heteropogon contortus* (L.) Roem. & Schult. By contrast, infrequent burning or complete absence of fire often results in the replacement of these short species by relatively taller species such as *Aristida junciformis* Trin. & Rupr., *Eragrostis curvula* Nees*, Cymbopogon validus* Stapf ex Burtt Davy and *Alloteropsis semialata* (R.Br.) Hitchc. **(EVERSON and TAINTON, 1984; FYNN et al., 2005)**. Little is known about the germination and regeneration ecology of these species and knowledge of grass

species responses to fire and fire-related factors is generally lacking. Despite the number of studies on grassland fire, South African native grass species are poorly represented in smoke stimulation research and no information exists on the response of the species to plant-derived smoke and their interaction with temperature **(ZACHARIAS et al., 1988; BAXTER et al., 1994)**. This is mainly due to the generally held view that in the South African mesic grasslands most grass reproduction takes place via vegetative reproduction and the contribution from seed germination is minimal **(DANCKWERTS, 1984; EVERSON, 1994)**.

Plant-derived smoke is being widely studied for its role in seed germination and seedling growth of many plant species **(See Chapter 2, Section 2.7; ADKINS and PETERS, 2001; SPARG, et al., 2006; VAN STADEN et al., 2006; KULKARNI et al., 2007)**. In the South African Fynbos **(BROWN, 1993b)**, Californian Chaparral **(KEELEY and FOTHERINGHAM, 1998)** and Australian native grasslands **(DIXON et al., 1995; READ and BELLAIRS, 1999; CLARKE and FRENCH, 2005)**, plant-derived smoke influences the recruitment of grass species differently. Germination enhancement has been shown in several species from different families **(BROWN, 1993a; BROWN, 1993b; ADKINS and PETERS, 2001)** and a biochemically active compound butenolide (3-methyl-*2H*-furo[2,3-*c*]pyran-2-one), isolated from smoke has been shown to enhance seed germination and seedling vigour of diverse plant species **(See Chapter 2, Section 2.5; VAN STADEN et al., 2006; KULKARNI et al., 2007)**. Several studies also reported that the germinative role of smoke solutions is temperature dependant **(KEELEY et al., 1985; GONZALEZ-RABANAL and CASAL, 1995)**.

The main objective of this study was to understand how and which species would positively or negatively respond to smoke and whether these species require a specific temperature to promote germination in the presence of smoke. This study was therefore conducted to determine the effects of plant-derived smoke-water and a smoke-isolated butenolide compound, at different temperatures, on germination and seedling growth of species from South African mesic grasslands.

3.2 MATERIALS AND METHODS

3.2.1 Species, Seed Source and Storage

Six perennial and naturally occurring C_4 grassland species were selected for this study from typical mesic grasslands of South Africa.

Table 3.1: Source and viability of the seeds of six South African mesic grassland species used in this study. (Nomenclature follows ARNOLD and DE WET, 1993).

Species	Common name	Source	Viability (%)
Aristida junciformis Trin. & Rupr.	Wire grass	Top Crop & Wild	90.0
Eragrostis curvula Nees	Love grass	McDonald's	85.5
Hyparrhenia hirta (L.) Stapf	Thatch-grass	Top Crop	30.0
Panicum maximum Jacq.	Guinea grass	Wild	92.0
Themeda triandra Forssk.	Kangaroo grass	Top Crop & Wild	75.0
Tristachya leucothrix Trin. ex Nees	Trident grass	Wild	70.0

The grass species used (**Table 3.1**) are among the most commonly occurring grass species in the mesic grasslands **(ACOCKS, 1988)**. These species were also selected on the basis of their contrasting response to fire frequency **(EVERSON and TAINTON, 1984; FYNN, 2004)**. Mature, naked and husked seeds were collected in 2008 from the wild around Pietermaritzburg (29°40´E and 30°24´S; 755 m a.s.l). The area has a warm sub-tropical climate. Mean maximum summer temperature is around 26.4°C in February, and mean minimum winter temperature is around 8.8°C in July with occasional frost. Mean annual rainfall is 885 mm. All the seeds were from the current year (<1 year old) and were stored at 5°C for 1 month until they were used for germination and seedling growth experiments.

3.2.2 Brief description of the study species

Aristida junciformis Trin. & Rupr. produces a prolific amount of seed allowing it to rapidly colonize overgrazed areas. Its expansion increases as fire frequency decreases and through selective grazing **(VENTER, 1968; VAN ZYL, 1998)**. *Eragrostis curvula* is indigenous to large parts of southern Africa and forms significant proportion of local grassland. Its abundance increases and it replaces *Themeda triandra* in the absence of burning and under conditions of elevated soil nitrogen and phosphorus **(FYNN and NAIKEN, 2009)**. It produces very high numbers of tiny seeds per head (on an average 12000 seeds per plant) and colonizes areas of high fertility **(KIRKMAN and MORRIS, 2003)**. *Hyparrhenia hirta* (L.) has a low seed production and seed viability due to variations in seed setting between plants **(ROBINSON and POTTS, 1950)**. It is a tall robust species commonly found on well-drained soils and often dominates old lands. It is a strong competitor in infertile habitats **(FYNN et al., 2005)**. *Panicum maximum* Jacq. flourishes on fertile soil, is well adapted to a wide variety of environmental conditions and fire does not cause long-term damage **(HARTY et al., 1983)**. *Themeda triandra* Forssk. is a fire-loving species and it is replaced by late successional species in the absence of regular burning **(EVERSON and TAINTON, 1984; O'CONNOR, 1997; FYNN et al., 2005)**. *Tristachya leucothrix* Trin. ex Nees increases in regularly burnt but underutilized grasslands. The seeds used in this study differ in size and shape. For the purpose of showing the difference in size and shape of the seeds, electro-microscopic photos of the six seeds of the grass species used in this study are provided in **Appendix 3**.

3.2.3 Smoke-water and butenolide solutions

A number of studies have described the procedure of preparing smoke-water extract using different plant material **(DE LANGE and BOUCHER, 1990; BROWN, 1993b; JÄGER et al., 1996; BOUCHER and MEETS, 2004; VAN STADEN et al., 2004)**. In this study dry *Themeda triandra* leaf material (5 kg) was burnt in a 20-L metal drum and smoke generated was passed through a glass column containing 500 mL of tap water for 45 min **(BAXTER et al., 1994)**. This smoke-extract was filtered through Whatman No. 1 filter paper and was used as stock solution. The test solution was

prepared by diluting 1 mL of smoke-extract with 500 mL of distilled water (1:500 v/v). A pure butenolide (3-methyl-*2H*-furo[2,3-*c*]pyran-2-one) used in this experiment was isolated from plant-derived smoke-water as described by **VAN STADEN et al. (2004)**. It is important to note that seed germination response to smoke is highly affected by the concentration of the smoke solution used. In this study, smoke-water solution at a dilution level of 1:500 v/v and butenolide compound at a dilution level of 10^{-8} M are tested. This is because, from previous studies **(KEELEY and FOTHERINGHAM, 2000; LIGHT, 2006)**, it was known that smoke-water and butenolide are more effective in stimulating seed germination at the specified concentration levels.

3.2.4 Seed germination and seedling growth experiments

Seeds were placed in 90 mm Petri dishes on two layers of Whatman No. 1 filter paper. There were four replicates of twenty five seeds each. The germination was recorded daily on the emergence of the radicle (0.5 mm) from the seed covering structures or the seed coat. Prior to the germination experiment, viability of the seeds was determined using 1% aqueous solution of tetrazolium salt (2, 3, 5-triphenyl tetrazolium chloride). Three replications of 25 filled seeds were soaked in distilled water for 18 h, subsequently, the lemma and palea were removed and the caryopsis was bisected longitudinally with a razor blade to expose the main structures of the embryo, and half of the caryopsis was immersed in the tetrazolium solution. After 3 h of dark incubation at 25°C, caryopses with completely red stained embryos were scored as viable **(GRABE, 1970)**.

To determine the effect of different constant temperatures, 25 seeds were incubated at 15, 20, 25, 30 and 35°C (16:8 h light:dark) with smoke solutions or distilled water (control). Filter papers were moistened with distilled water or smoke solutions when required. Germinated seeds at different temperatures were retained for 21 days in the same Petri dishes for seedling development. For seedling growth studies, the seeds were also subjected to alternating temperatures (30/15°C) under 16:8 h light:dark. Response variables considered were germination rate (GR), final germination percentage (FGP) and subsequent seedling growth (shoot and root length). Germination rate for each species was calculated by the modified Timson index **(EASTON and KLEINDORFER, 2009)**: Germination rate (% d^{-1}) = $\sum [(G^1/t) +$

$(G^2/t) \cdots + (G^t/t)]$ where G is the percentage of seed germination at 1-day intervals, and t is the total number of days of the germination period. A greater value of G represents a faster germination rate.

3.2.5 Statistical analysis

Percentage germination data were arcsine transformed before analysis. Germination and seedling growth data were subjected to one-way analysis of variance (ANOVA) and Tukey's post-hoc test was used for pair-wise comparison at a 5% level of significance ($P < 0.005$). General analysis of variance (ANOVA) was conducted for main effects and their interactions. Statistical packages used were GENSTAT[®] **(GENSTAT, 2008)** and MINITAB[®] **(MINITAB, 2003).**

3.3 RESULTS

Seed viability, as determined by tetrazolium analysis, varied among the six species of grasses tested, ranging from 30 to 92%. In comparison to the control, smoke-water treatment significantly increased germination rate (GR) and final germination percentage (FGP) of *Aristida junciformis* seeds at 35°C (**Figure 3.1**). At this temperature shoot and root length was also significantly greater than the control (**Figure 3.2**). The seeds of *Eragrostis curvula*, after treating with smoke-water at 30°C, showed a significant increase in FGP compared to the control (**Figure 3.1**). A butenolide treatment significantly increased shoot length at 25, 30 and 30/15°C when compared with the control (**Figure 3.2**). Smoke-water-incubated seeds of *Eragrostis curvula* showed a significant increase in shoot and root length of seedlings only at 30/15°C over the control. Smoke-water-treated seeds of *Hyparrhenia hirta* showed significantly higher GR and FGP at 30 and 35°C and at all other temperatures had a significantly greater shoot and root length than the control with the exception of 15°C (**Figure 3.2**). Although smoke-water and butenolide treatments increased GR and FGP of *Panicum maximum* seeds at all tested temperatures, these results were significantly different to control only at 30 and 35°C (**Figure 3.1**). These treatments exhibited significantly greater shoot and root length at 35°C (**Figure 3.2**). The seeds of *Themeda triandra* when treated with smoke-water at 15, 25, 30 and 35°C showed significantly higher GR and FGP compared to the respective controls (**Figure 3.1**).

Smoke-water also significantly increased shoot and root length of *Themeda triandra* seedlings in comparison to the control at 25, 30 and 35°C (**Figure 3.2**). With the exception of 15 and 20°C, at all other tested temperatures smoke-water-treated seeds of *Tristachya leucothrix* showed significantly higher GR and FGP in comparison to the control (**Figure 3.1**). The shoot and root length were significantly increased by smoke-water treatment when compared with the control at 25, 30 and 35°C (**Figure 3.2**). The overall result of this study shows that there was a significantly positive interaction between species, temperature and treatment for GR and FGP (**Table 3.2**).
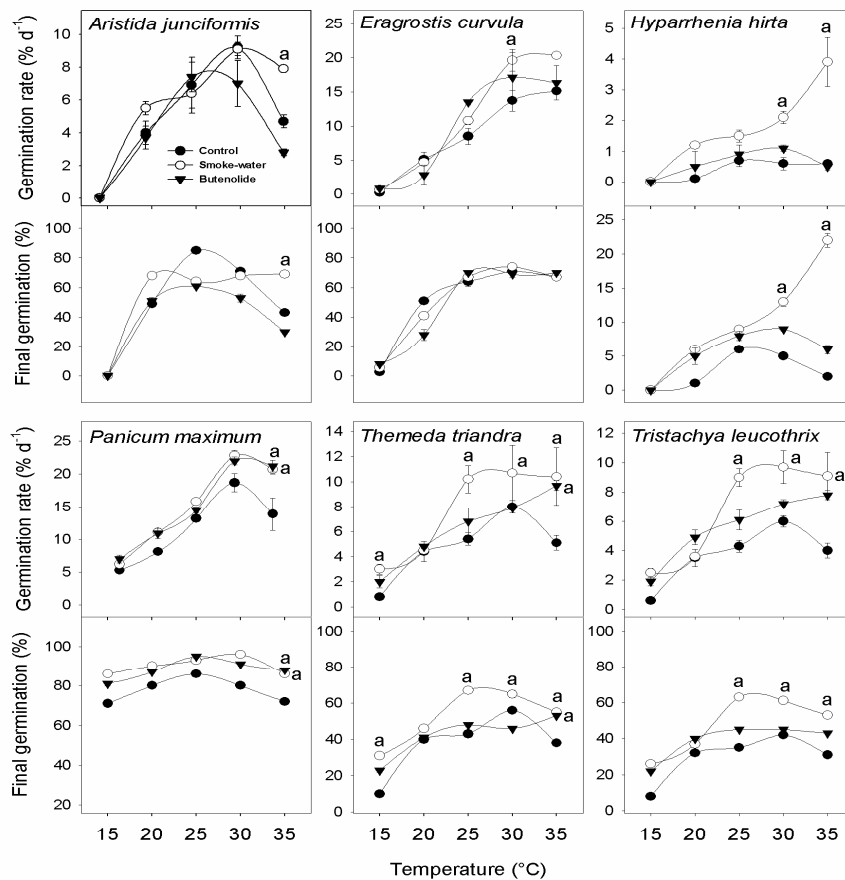
Figure 3.1: Effect of smoke solutions (smoke-water 1:500 v/v and butenolide 10^{-8} M) on seed germination rate and final germination percentage of six mesic grassland species at constant temperatures under 16:8 h light:dark conditions. Symbol (±SE) with letter 'a' is significantly different from control according to Tukey's test (P < 0.001). NS = Non-significant.
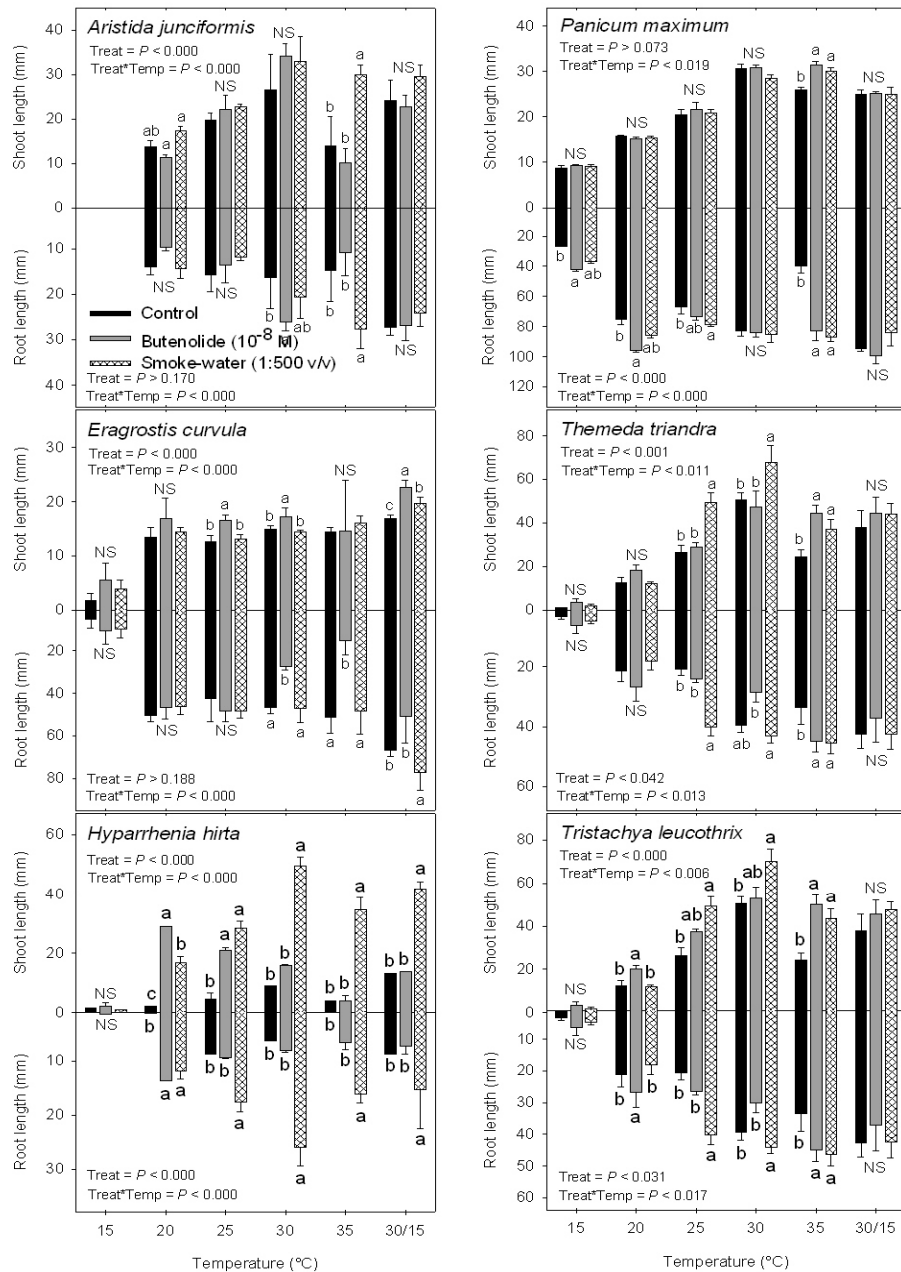
Figure 3.2: Effect of smoke-water (1:500 v/v) and butenolide (10^{-8} M) on shoot and root length (mm) of six mesic grassland species at different temperatures under 16:8 h light:dark conditions. Bar (±SE) with different letter(s) is significantly different according to Tukey's test (P < 0.001). NS = Non-significant; Treat X Temp = Interaction between treatment and temperature.

3.4 DISCUSSION

In germination studies, evaluating the viability percentage of the seed samples in use, before the commencement of the experiment is important consideration (**CROSTI et al., 2006**). In this study, viability percentage of most of the seed samples used was found to be > 70% (**Table 3.1**) and was considered satisfactory for running the subsequent experiments. The findings from this (smoke-germination) experiment support the hypothesis that plant-derived smoke and fire-mediated changes in temperature significantly influence the germination and seedling growth of the common species of the Tall Grassland. Furthermore, in the long-term this is likely to alter grassland composition.

Smoke treatments at higher temperatures generally enhanced GR, FGP and seedling growth of *Hyparrhenia hirta*, *Themeda triandra* and *Tristachya leucothrix* (**Figure 3.1 and 3.2**). However, smoke solutions tested at different constant temperatures did not show significant effects on GR and FGP of *Aristida junciformis* and *Eragrostis curvula*, with some exceptions at higher temperatures. This finding agrees with that of **ZACHARIAS et al. (1988)**, where percent germination for *Aristida junciformis* remained unaffected in response to fire, though it is unknown whether *Aristida junciformis* failed to respond to the heat or smoke component of fire. **ADKINS et al. (2003)** reported that smoke failed to alter the natural germination of *A. ramose*. According to these authors, *A. ramose* responded to smoke-water only at higher temperatures. In this study, *Aristida junciformis* also responded to high temperature of 35°C in the presence of smoke-water. Other species of *Aristida*, such as *Aristida vegans* failed to respond to smoke-water treatments (**CLARKE and FRENCH, 2005**). Germination rate in *Eragrostis curvula* was not affected by smoke-water or butenolide treatments but there was an improvement in FGP and shoot and root length at higher temperatures. **CLARKE and FRENCH (2005)** found that germination percentage in *Eragrostis curvula* was increased by heat treatment but only in the presence of smoke. The other species of *Eragrostis* such as *Eragrostis leptostachya* and *Eragrostis benthamii* (**CLARKE and FRENCH, 2005**) are responsive to smoke.

Table 3.2: General analysis of variance (ANOVA) with main effects and their interactions for six mesic grassland species. Treatments at constant temperatures were control, smoke-water (1:500 v/v) and butenolide (10^{-8} M).

Source	df[a]	Sum of squares	Mean squares	*F*-value	*P*-level[b]
Germination rate (% d^{-1})					
Species (S)	5	6073.2	1214.6	459.0	< 0.001
Temperature (T)	4	4142.9	1035.7	391.4	< 0.001
Treatment (TR)	2	332.1	166.0	62.7	< 0.001
S X T	20	1643.5	82.1	31.0	< 0.001
S X TR	10	103.2	10.3	3.9	< 0.001
T X TR	8	155.1	19.3	7.3	< 0.001
S X T X TR	40	227.4	5.6	2.1	< 0.001
Residual	270	714.4	2.6		
Total	359	13392.1			
Final germination (%)					
S	5	30.5689	6.1137	419.2	< 0.001
T	4	8.4896	2.1224	145.5	< 0.001
TR	2	1.0308	0.5154	35.3	< 0.001
S X T	20	3.9652	0.1982	13.6	< 0.001
S X TR	10	1.2429	0.1242	8.5	< 0.001
T X TR	8	0.1476	0.0184	1.2	0.262
S X T X TR	40	1.1692	0.0292	2.0	< 0.001
Residual	270	3.9374	0.0145		
Total	359	50.5517			

Interestingly, all the growth parameters of *Themeda triandra* studied were significantly enhanced by smoke-water and butenolide treatment at nearly all the temperatures tested. This species is suggested to have evolved under fire regimes **(EVERSON and TAINTON, 1984; O'CONNOR, 1997)**. In the absence of fire such species are not capable of maintaining their population size and they are unable to

grow vigorously **(EVERSON and TAINTON, 1984; MORGAN and LUNT, 1999)**. Many authors have associated such a reaction of *Themeda triandra* to post-fire nutrient enrichment, greater access to light and water, competitive release and lowered productivity **(O'CONNOR, 1997; MORGAN and LUNT, 1999; FYNN, 2004; FYNN et al., 2005)**. The results of this study clearly show that in addition to the other factors, plant-derived smoke can enhance the germination and seedling growth of *Themeda triandra.* Studies have showed that smoke-water was effective in breaking the dormancy and stimulating the germination and seedling vigour of *Themeda triandra* **(GROVES et al., 1982; BAXTER et al., 1994; READ and BELLAIRS, 1999)**. Such fire dependent species also responded positively to aerosol smoke treatment **(READ and BELLAIRS, 1999)**. For *Tristachya leucothrix*, a perennial obligate seed reproducing species, response to smoke-water and butenolide treatment was not that different from that of *Themeda triandra*. In nearly all the variables considered, *Tristachya leucothrix* positively responded to the smoke solutions.

At higher temperatures, GR and FGP of *Hyparrhenia hirta* (a species with low viable seeds) were significantly enhanced when treated with smoke-water and butenolide. Smoke-water showed a pronounced effect on shoot and root growth of *Hyparrhenia hirta* seedlings. This indicates that *Hyparrhenia hirta* can establish quicker than other less-smoke-responsive species. Smoke solution treatment of *Panicum maximum* seeds showed an increasing trend of GR and FGP with only significant effects at higher temperatures. The result of this experiment to an extent is similar to the findings of **ADKINS et al. (2000)**, in which smoke treatment enhanced the germination rate but there was no significant change in FGP.

In this study, butenolide did not show consistent effects, which may be attributed to the level of concentration used **(LIGHT, 2006)**. The seeds of all grass species tested were treated with only one level of butenolide concentration, 10^{-8} M. It may show consistent results similar to smoke-water if tested with different concentrations. The effectiveness of the smoke solutions used in this experiment and the interactive effects with temperature and light is however questionable **(KEELEY and FOTHERINGHAM, 2000)**. For example, **CLARKE et al. (2000)** in a similar study on species from grassy woodlands and forests found that certain species that have been previously reported to respond to smoke treatments failed to show any significant germination due to concentration effects. In general, the results from this

study showed that there was a high variability in both germination and seedling growth responses of grasses. Species such as *Themeda triandra*, *Tristachya leucothrix* and *Hyparrhenia hirta* can be considered as more responsive to smoke than the rest of the species investigated (**Figure 3.3**).
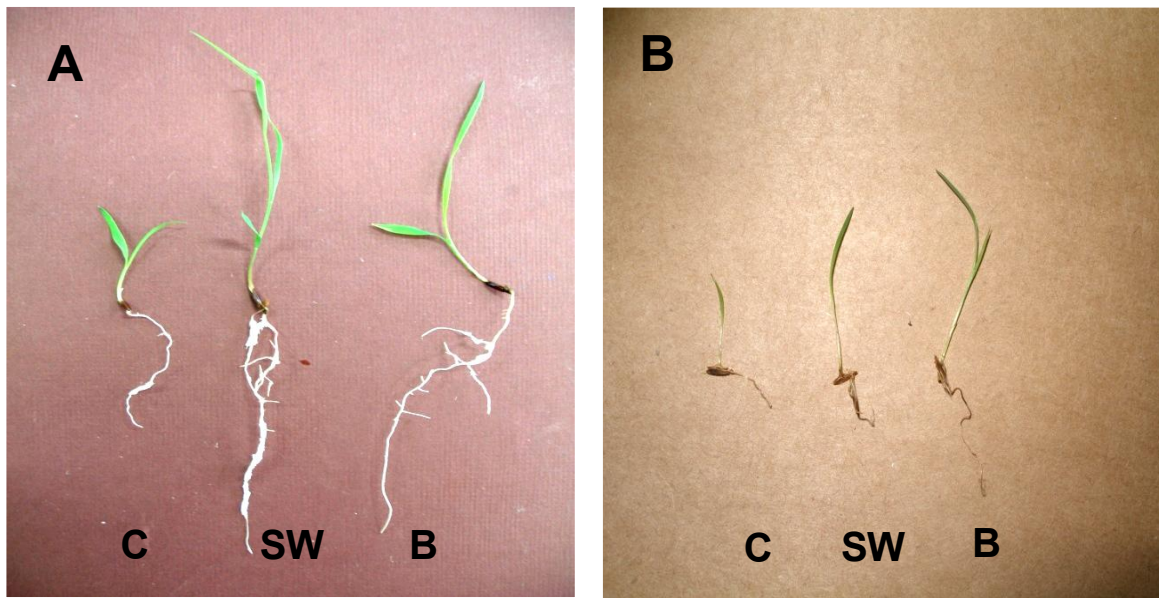
Figure 3.3: Effect of smoke-water (SW 1:500 v/v), butenolide (B 10^{-8}M) and Control (C) on seedling growth of *Themeda triandra* Frossk (A) and *Hyparrhenia hirta* (B). The photos were taken after incubating the seeds for 16 days at 30 °C under 16:8 h light:dark conditions.

In conclusion, under conditions of high fire frequency and consequent exposure to smoke, species such as *Themeda triandra*, *Tristachya leucothrix* and *Hyparrhenia hirta* should show a competitive advantage over those less or non-smoke-responsive species such as *Aristida junciformis*, *Eragrostis curvula* and *Panicum maximum*. In the long run changes in the frequency of fire and the subsequent exposure to smoke is likely to influence the germination and seedling growth of grass species differently, which can affect the community composition. Recent advances in smoke research have shown that smoke can induce genes to elicit a faster germination rate and greater seedling vigour **(SOÓS et al., 2009a,**

2009b, respectively**)**. This has opened new avenues to study genomics of smoke-responsive grasses.

3.5 MANAGEMENT IMPLICATIONS AND CONCLUSION

It is the view of several scientists that smoke biotechnology can be a useful tool for grassland regeneration and restoration of degraded lands. Smoke stimulation can also be used as a strategy for depleting the soil seed bank in weed control programmes **(ADKINS and PETERS, 2001; KĘPCZYŃSKI et. al., 2006; DAWS et al., 2007; DAWS et al., 2008)**.Thus, an understanding of the seed germination ecology of grasses in response to fire (smoke) can assist in predicting their potential distribution and developing effective management strategies. Findings from this experiment imply that smoke, due to its selective stimulation characteristics may be a useful tool in grassland management. For instance, *Themeda triandra* and *Tristachya leucothrix*, both perennial obligate seed reproducing species **(O'CONNOR, 1991)**, among the most economically important native grass species in South Africa are prone to extinction in areas left unburnt. On the other hand, less desirable and weedy species such as *Aristida junciformis* can increase in less frequently burnt situations. The results from this study demonstrate that part of this species turnover may originate from species responses to fire (smoke) and the interactive effect of smoke with temperature on germination and seedling growth of the species. Thus, regular burning of the mesic grassland could therefore be a useful device to restore degraded grasslands, where spontaneous colonization of smoke responsive grass species is very limited. Furthermore, knowledge of the seed smoke interaction would assist in weed and invasive species management programmes. However, large-scale and well organised screening of the germination characteristics of the mesic grassland flora and detailed field studies on this subject is needed to quantify the net effect of smoke on grassland dynamics.

CHAPTER FOUR

The role of smoke-water and butenolide in overcoming stress due to high temperature and low osmotic potential

"I feel that the greatest reward for doing is the opportunity to do more"

Jonas Salk (1914–1995)

4.1 INTRODUCTION

Since the discovery of smoke as germination cue **(DE LANGE and BOUCHER, 1990)**, the application of plant-derived smoke solutions for agricultural and horticultural purposes have shown promising results (see **Chapter 2 section 2.8.1**). Experiments conducted on tomato, okra, maize, rice, celery and other crops have showed some positive effects of plant-derived smoke in enhancing seed germination and seedling growth **(SPARG et al., 2006, VAN STADEN et al., 2006, KULKARNI et al., 2007)**. Similarly, seeds of grassland species (Poaceae) from various vegetation types worldwide have shown positive germination and vigour responses to smoke treatments (See **Chapter 3, Section 3.4**; **Appendix 1**). However, understanding of the mode of action as to how smoke enhances seed germination and accelerates seedling growth is still incomplete. There is a general suggestion that smoke solutions may widen the temperature range over which germination can occur and override effects of stress **(MERRITT et al., 2005, JAIN et al., 2006)**. In this study, attempts were made to characterise the role of smoke-water (1:500 v/v)

and smoke-derived butenolide (10^{-8} M) with certain economically important traits such as imbibition (water uptake by seeds) and tolerance to stress conditions arising from high temperature and low osmotic potential. For this purpose *Eragrostis tef*, a species with fairly good tolerance to east African severe drought and high temperature has been chosen **(KETEMA, 1997)**. An additional purpose of this study was to examine the possibility of using smoke solutions in maximisation of *Eragrostis tef* grain yield.

Eragrostis tef belongs to the family *Poaceae*, sub-family *Chloridoideae*, tribe *Chlorideae* and genus *Eragrostis*. It is a C_4 annual graminaceous species classified as intermediate between tropical and temperate grasses **(GIBBS-RUSSELL et al., 1990)**. Traditionally, it is classified as a major cereal crop and source of a staple food in the Horn of Africa countries, mainly Eritrea and Ethiopia, feeding around 100 million people **(NATIONAL RESEARCH COUNCIL, 1996, KASSIER, 2002)**. *Eragrostis tef* is the source of the fermented sour pancake recognised as *Enjera* in Eritrea, Ethiopia, eastern Sudan, Kenya and parts of Somalia. In South Africa, *Eragrostis tef* is primarily used as a forage crop and makes a good pasture. It is also increasingly used for rehabilitation work to establish a rapid vegetation cover, stabilize soil and allow other perennial plants to emerge and dominate.

Eragrostis tef produces many tiny seeds per head. Owing to very small seeds (1000 seeds = 265 mg approximately), seedbed preparation is crucial for uniform stand establishment of seedlings **(DU PLOOY, 1957, SKERMAN and RIVEROS, 1990, STALLKNECHT et al., 1993)**. The wind or rain exhumes small seedlings in the soil before they are established **(NATIONAL RESEARCH COUNCIL, 1996)**. This suggests that initially seeds of *Eragrostis tef* must germinate rapidly and grow vigorously to improve survival rates. Generally, during early developmental stages of the seedlings there are several environmental factors such as temperature fluctuation, soil structure and water stress that affect seedling growth, plant establishment and crop yield **(ORZOLEK, 1991, PRASAD et al., 2006)**. In these kind of situations, *Eragrostis tef* undergoes heat and water stress due to late onset of rain affecting germination and stand establishment, which results in low productivity **(KETEMA, 1997)**. This study was therefore conducted to evaluate the effect of smoke-water and a smoke-isolated butenolide compound on seed germination and seedling growth of a selected grass species i.e. *Eragrostis tef* under high

58

temperatures and low osmotic potentials and thereby, characterise the role of plant-derived smoke in overcoming stresses.

4.2 MATERIALS AND METHODS

4.2.1 Seed source and viability testing

One-year-old *Eragrostis tef* seeds (cultivar SA Brown) were purchased from McDonalds Seeds (Pietermaritzburg, South Africa). Seeds were stored at room temperature for two weeks until they were used. Prior to the germination experiment, viability of the seeds was determined using 1% aqueous solution of tetrazolium salt (2,3,5-triphenyl tetrazolium chloride). Three replications of 25 filled seeds were soaked in distilled water for 18 h, subsequently, the lemma and palea were removed and the caryopsis was bisected longitudinally with a razor blade to expose the main structures of the embryo, and half of the caryopsis was immersed in the tetrazolium solution. After 3 h of dark incubation at 25°C, caryopses with completely red stained embryos were scored as viable **(GRABE, 1970)**.

4.2.2 Smoke-water and smoke-compound (butenolide) solutions

A number of studies have described the procedure of preparing smoke-water extract using different plant material **(DE LANGE and BOUCHER, 1990, BROWN, 1993b, JÄGER et al., 1996, BOUCHER and MEETS, 2004, VAN STADEN et al., 2004)**. In this study, dry *Themeda triandra* Forssk (Poaceae) leaf material (5 kg) was burnt in a 20-L metal drum and smoke generated from it was passed through a glass column containing 500 mL of tap water for 45 min **(BAXTER et al., 1994)**. This smoke-extract was filtered through Whatman No. 1 filter paper and was used as the stock solution. The test solution was prepared by diluting 1 mL of smoke-extract with 500 mL of distilled water (1: 500 v/v). A pure butenolide (3-methyl-2*H*-furo[2,3-c]pyran-2-one) used in this experiment was isolated from plant-derived smoke-water as described by **VAN STADEN et al. (2004)**. The concentration of the compound tested was 10^{-8} M.

4.2.3 Seed imbibitions

Seeds of *Eragrostis tef* (500 mg) were allowed to imbibe water or test solutions for 48 h at 25 °C. At different intervals, the seeds were blotted dry, weighed and returned to the wet filter paper. The percentage imbibition was calculated as: Imbibition (%) = (weight of seeds after imbibition) initial weight of seeds)/initial weight of seeds x 100 **(GOVENDER et al., 2008)**.

4.2.4 Polyethylene glycol (PEG) solutions

Polyethylene glycol (PEG) is a polyether compound with many applications in natural sciences. PEG is a flexible, thick and water-soluble polymer which has no specific interactions with biological chemicals. These properties make PEG one of the most useful compounds for applying osmotic pressure in physiological experiments, in particular when using the osmotic stress technique **(KAUFMANN, 1969; MURILLO-AMADOR et al., 2002)**. Many studies have used PEG for creating water-stress conditions during seed germination and seedling growth **(KAUFMANN, 1969, NAYLOR, 1992, ADAMS, 1999, TOBE et al., 2000, MURILLO-AMADOR et al., 2002)**. In this experiment, different osmotic potential concentrations (MPa) were prepared by adding PEG 6000 (Merck, Darmstadt, Germany) to distilled water or test solutions **(MICHEL and KAUFMANN, 1973)**.

4.2.5 Germination and seedling growth experiments

Seeds of *Eragrostis tef* were placed in 90 mm Petri dishes on two layers of filter paper (Whatman No. 1). Fifty seeds were replicated four times for each treatment. The germination was recorded daily. Radicle protrusion of 1 mm was taken as the criterion for seed germination. To determine the effect of temperature, seeds were incubated at 20, 25, 30, 35, 40 and 30/15 °C (16:8 h light:dark) with treatment solutions or distilled water (control) (3.5 mL). Seeds were also tested under constant dark and light conditions at 25 °C. The germination under constant dark conditions was recorded using a green 'safe light' (0.5 µmol m^{-2} s^{-1}) **(DREWES et al., 1995)**. Germinated seeds incubated at different temperatures and light conditions were retained for 16 days in the same Petri dishes for seedling development. In the case

of water-stress experiments, the seeds were incubated at 25 °C with 16:8 h light:dark conditions. The osmotic potential of the PEG 6000 solutions were 0, -0.05, -0.15, -0.30 and -0.49 MPa. This experiment was continued for 7 days. The filter paper was remoistened when needed with distilled water or appropriate test solutions during the course of all the experiments. The seedling vigour index was calculated as VI = seedling length (mm) X percentage germination **(DHINDWAL et al., 1991)**.

4.2.6 Statistical analysis

Percentage germination data were arcsine transformed before analysis. Germination and seedling growth data were subjected to one-way analysis of variance (ANOVA) and Tukey's post hoc test was used for pair-wise comparison at a 5 % level of significance ($P < 0.05$). Statistical analysis was conducted using MINITAB® statistical package, release 12.1 (Minitab Inc., State College, PA, USA).

4.3 RESULTS

Seed viability, as determined by tetrazolium analysis was nearly 100%. In the germination experiment, in comparison with the control, smoke-water treatment significantly improved percentage germination of *Eragrostis tef* seeds at 25 °C (**Figure 4.1**). Although smoke-water and butenolide solution produced a trend of increasing percentage germination at 20, 30, 35 and 40 °C, these results were not statistically different from the control. These treatments not only improved the percentage germination of *Eragrostis tef* seeds, but also significantly increased seedling size at 25 °C (**Table 4.1**). **Figure 4.2** shows the increase in seedling lengths (tip of shoot to tip of root) in seeds treated with butenolide and smoke-water at 30 °C.

Alternating temperatures (30/15 °C) produced the longest seedlings in comparison with all the other temperatures tested. Seedlings grown with smoke-water or butenolide at these temperatures and under dark conditions at 25 °C were significantly longer than the control (**Table 4.1**). Smoke treatments had a positive influence on vigour index of *Eragrostis tef* seedlings. At 25, 30, 35, 40 and 30/15 °C, the vigour indices were significantly greater for smoke-water and butenolide treatments than the control (**Figure 4.3**). The vigour index values of smoke-water

61

treatment under constant light and dark conditions were significantly greater than the butenolide and control (**Figure 4.3**). Smoke-water and butenolide increased percentage imbibition from 0 to -0.49 MPa osmotic potential (**Figure 4.4**). Smoke-water-treated seeds of *Eragrostis tef* showed significantly higher percentage germination than the control at osmotic potential of -0.15, -0.30 and -0.49 MPa under 25 °C and 16:8 h light:dark conditions (**Figure 4.5**). Whereas the seeds incubated with butenolide solution at -0.49 MPa osmotic potential showed significantly higher percentage germination than the control (**Figure 4.5**).
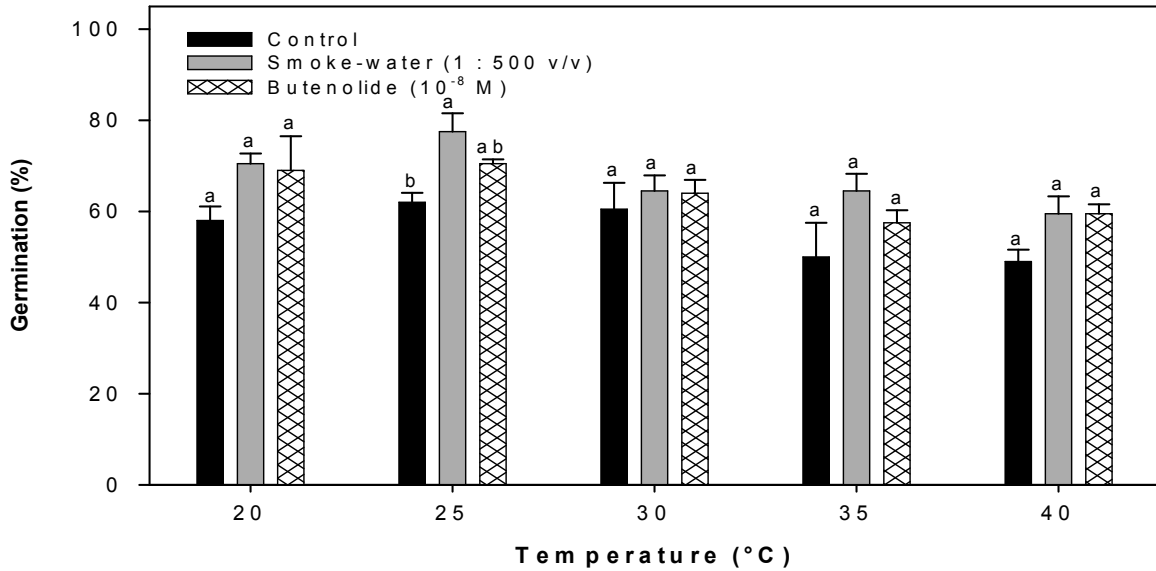
Figure 4.1: Effect of smoke treatments (±S.E.) on seed germination of *Eragrostis tef* at different temperatures under 16:8 h light:dark conditions. Bars with different letters indicate significant differences within treatments (*P* < 0.05).

Table 4.1: Effect of smoke treatments on seedling growth of *Eragrostis tef* after incubating for 16 days at different temperature and light conditions. Mean value (±S.E.) in the row with a different letter is significantly different ($P < 0.05$).

Temperature and light conditions	Seedling length (mm)		
	Control	Smoke-water	Butenolide (10^{-8} M)
20 °C	29.7 ± 1.6 a	32.7 ± 2.0 a	34.2 ± 2.9 a
25 °C	25.6 ± 4.5 b	39.6 ± 2.9 a	41.9 ± 3.3 a
30 °C	29.1 ± 2.8 b	40.6 ± 0.8 a	46.6 ± 1.4 a
35 °C	34.9 ± 1.2 b	49.4 ± 0.7 a	52.7 ± 1.5 a
40 °C	23.0 ± 4.0 b	45.6 ± 3.6 a	47.5 ± 3.9 a
30/15 °C	35.2 ± 2.8 b	52.3 ± 0.3 a	50.9 ± 2.9 a
Constant light (25 °C)	33.0 ± 0.9 b	47.6 ± 1.8 a	35.4 ± 2.9 b
Constant dark (25 °C)	45.5 ± 1.0 c	70.1 ± 0.6 a	55.6 ± 0.3 b
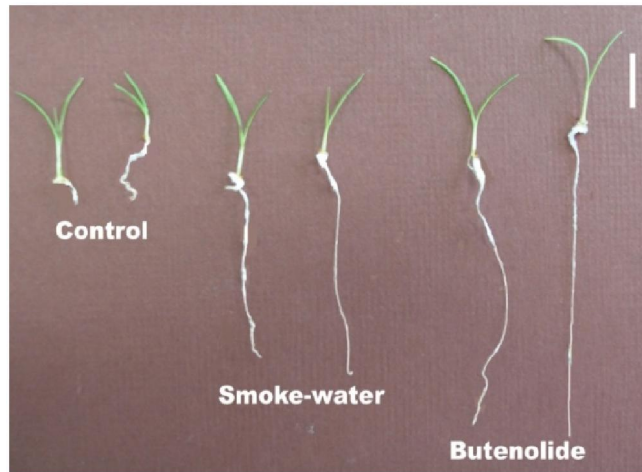
Figure 4.2: Effect of smoke-water (1:500 v/v) and butenolide (10^{-8} M) on seedling growth of *Eragrostis tef* after incubating for 16 days at 30 °C under 16 :8 h light:dark conditions. Bar indicates 10 mm.

Beyond -0.05 MPa the percentage germination of the control seeds began to decline, such that at -0.49 MPa no germination was recorded for the control seeds. In contrast, germination of smoke-water- and butenolide-treated seeds only declined beyond -0.15 MPa. At an osmotic potential of 0 to -30 MPa seedlings of *Eragrostis tef* treated with smoke-water were significantly longer than control seedlings (**Table 4.2; Figure 4.7**). Although butenolide treatment produced longer seedlings, these values were not significantly different from the control. However, butenolide at -0.49 MPa osmotic potential significantly enhanced seedling growth relative to the control. From 0 to -30 MPa osmotic potential smoke-water-treated seedlings exhibited a significantly increased vigour index compared with the control (**Figure 4.7**). At these osmotic potentials, butenolide also increased the vigour index but the values were not significantly different from the control. However, butenolide at -0.49 MPa osmotic potential had a significantly higher vigour index than control and smoke-water treatments.
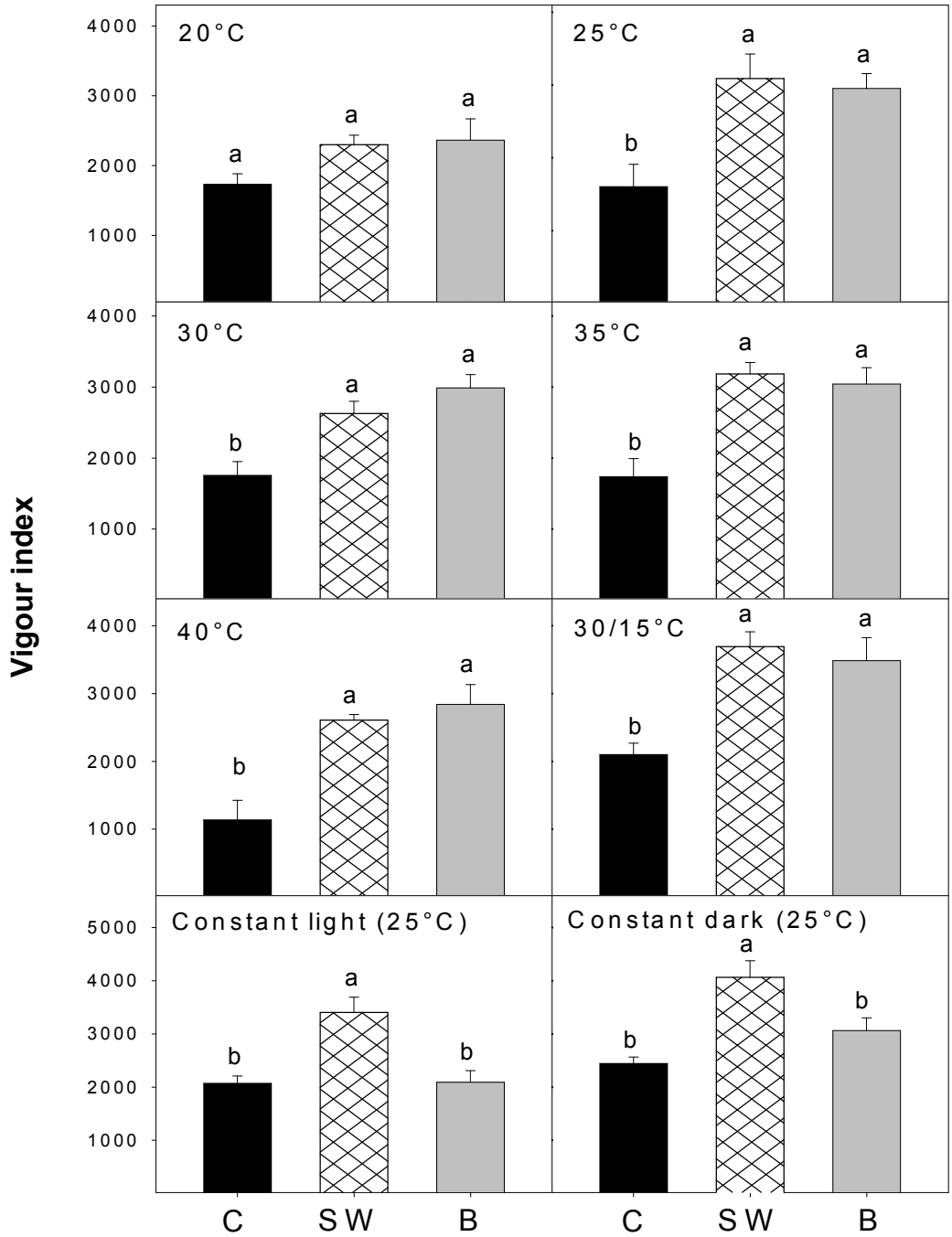
Figure 4.3: Effect of distilled water (C), smoke-water (SW 1:500 v/v) and butenolide (B 10^{-8}M) on seedling vigour index (±S.E.) of *Eragrostis tef* after incubating for 16 days at different temperatures (16:8 h light:dark) and light conditions. Bars with different letters indicate significant differences within treatments(*P*<0.05).

Table 4.2: Effect of smoke treatments on seedling growth of *Eragrostis tef* after incubating for 7 days at different osmotic potentials at 25 °C and 16:8 h light:dark conditions. Mean value (±S.E.) in the row with a different letter is significantly different ($P < 0.05$).

Osmotic potential (MPa)	Seedling length (mm)		
	Control	Smoke-water (1 : 500 v/v)	Butenolide (10^{-8} M)
0	17.4 ± 0.7 b	22.1 ± 0.6 a	18.2 ± 0.5 b
−0.05	12.6 ± 0.7 b	16.8 ± 0.9 a	14.0 ± 0.3 ab
−0.15	10.0 ± 0.9 b	15.2 ± 1.2 a	12.2 ± 0.2 ab
−0.30	7.6 ± 1.4 b	10.0 ± 1.2 a	7.9 ± 0.7 ab
−0.49	0 b	0.7 ± 0.2 b	4.2 ± 0.7 a

Figure 4.4: Effect of smoke-water (1:500 v/v) and butenolide (10^{-8}M) solution on seedling growth of *Eragrostis tef* after incubating for 7 days at -0.15 MPa osmotic potential at 25 °C and 16:8 h light:dark conditions. Bar indicates 1mm.
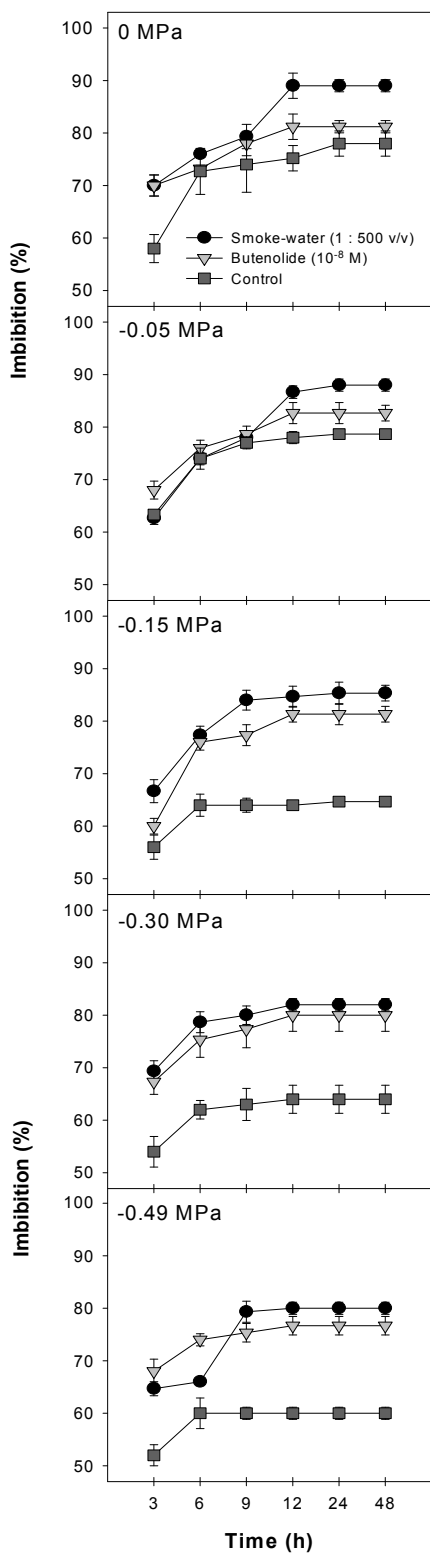
Figure 4.5: Imbibition curves for *Eragrostis tef* seeds treated with smoke-water (SW 1:500) and butenolide (B 10^{-8}M) solutions at various osmotic potentials using PEG 6000 as the osmoticum. Distilled water was used to prepare the solutions for control seeds and all treatments were incubated at 25 °C. Bars indicate standard error.
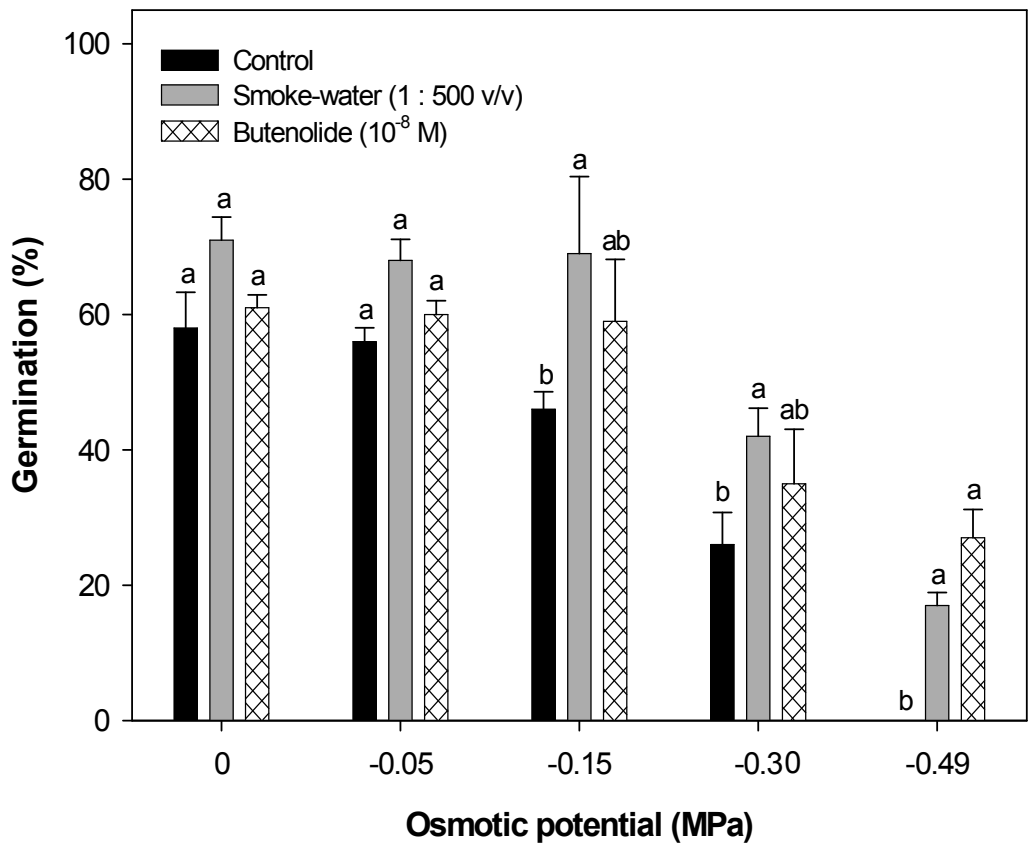
Figure 4.6: Effect of smoke treatments on seed germination (±S.E.) of *Eragrostis tef* at different osmotic potentials at 25 °C and 16:8 h light:dark conditions. Bars with different letters indicate significant differences within treatments (*P* < 0.05).
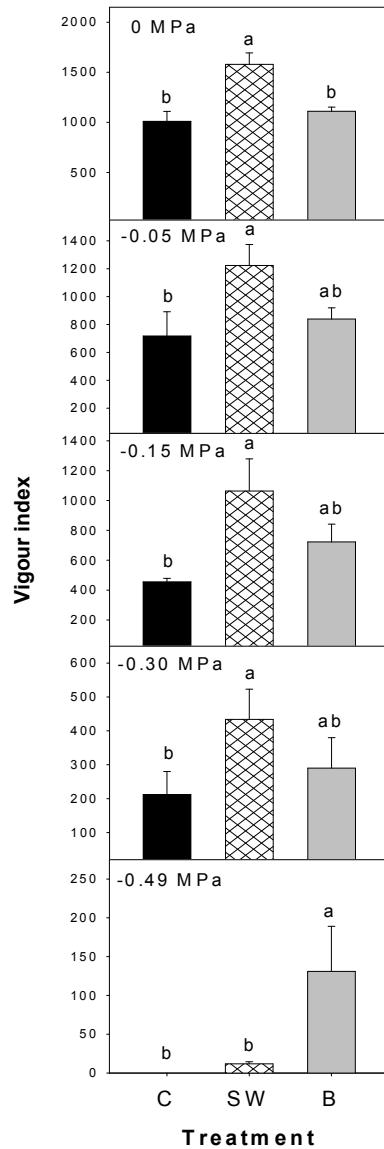
Figure 4.7: Effect of smoke-water (SW 1 : 500 v/v) and butenolide (B 10^{-8} M) on seedling vigour index (±S.E.) of *Eragrostis tef* after incubating for 7 days at different osmotic potential at 25 °C and 16:8 h light:dark conditions. Control = C. Bars with different letters indicate significant differences within treatments (*P* < 0.05).

4.4 DISCUSSION

There is not much information available on seed germination of *Eragrostis tef* with respect to temperature and moisture requirements. It is suggested that a temperature range of 10–27 °C is suitable for cultivating this crop **(KETEMA, 1997)**. In this study, untreated seeds obtained maximum germination of ≤ 62 % at constant temperatures of 20, 25, 30 and alternating temperatures of 30/15 °C with no germination at 15 °C. The percentage germination at these temperatures was low suggesting that *Eragrostis tef* seeds may require specific temperatures or other conditions for optimum germination that requires further investigation. However, the seeds that were treated with smoke-water and butenolide solution showed enhanced percentage germination across the temperature range between 20–40 °C (**Figure 4.1**). Importantly, even though non-significant, smoke-water and butenolilde treatments were effective in improving the percentage germination of *Eragrostis tef* seeds at higher temperatures. This phenomenon has been reported by **MERRITT et al. (2005)** for a number of horticultural crops. Consequently, not only at normal temperatures but particularly at 35 and 40 °C these treatments helped in improving seedling length and the vigour indices of *Eragrostis tef.* In another study, tomato seeds which were treated with smoke-water and butenolide grew into phenotypically normal seedlings at non-optimum temperatures **(JAIN et al., 2006)**. This indicates that smoke-water or butenolide treatments can overcome the detrimental effects of temperature fluctuations on seed germination and seedling growth thereby minimizing losses. At all the temperature and light conditions examined, *Eragrostis tef* seeds treated with smoke-water produced the longest seedlings with increased vigour indices under constant dark conditions at 25 °C (**Table 4. 1; Figures 4.2 and 4.3**).

Rice seedlings showed a similar response when treated with smoke-water under constant dark conditions **(KULKARNI et al., 2006)**. This phenomenon is important from an agronomical and practical point of view, as smoke-water-treated seeds could be sown at a greater depth (restricting light), which may result in taller seedlings with better stand establishment in the soil. In *Eragrostis tef*, good stand establishment is a major problem **(TEFERA et al., 1992, KASSIER, 2002)**. The first process of seed germination is the imbibition of water (i.e. uptake of water), which

relies on the water potential of soils. A dry soil delays the process of imbibition thereby affecting the initiation of seed germination **(BOYD and VAN ACKER, 2004)**. Water stress is another crucial problem for seed germination of *Eragrostis tef* on vertisol soils that suffer from crusting. Seedbed packing is therefore practiced by farmers to cope with water-stress conditions during late onset of rain **(KETEMA, 1997)**. Subjecting the seeds of *Eragrostis tef* at different water potentials (0 to -0.49 MPa) with smoke-water and butenolide clearly showed an enhanced percentage imbibition (**Figure 4.4**). Under conditions of low osmotic potential, where water is limiting, smoke-water and butenolide may actually facilitate water uptake, i.e. imbibition, which allows the commencement of metabolic activities that culminate in radicle emergence **(BEWLEY, 1997)**. In the control seeds it is likely that water uptake was impeded and hence germination could not reach completion. **VAN STADEN et al. (1995)** suggested that smoke may activate the enzymes which are responsible for the mobilization of stored reserves in seeds or change the membrane permeability so that the translocation of regulators becomes easy. Recently, **JAIN and VAN STADEN (2007)** showed that butenolide-primed seeds of tomato had a higher percentage of nuclei at the 4C stage than water-primed seeds. This resulted in faster emergence of the radicle with significantly higher vigour indices under various stress conditions such as salinity, osmoticum or temperature. In this study, lowering osmotic potential decreased percentage germination of control seeds of *Eragrostis tef* with no germination at -0.49 MPa. On the other hand, seeds which were germinated with smoke-water and butenolide solution from high to low osmotic potential exhibited a higher percentage germination than the control (**Figure 4.5**). Smoke treatments promoted some germination even at the lowest osmotic potential (-0.49 MPa). Subsequently, smoke-water effectively improved seedling length and vigour indices at different water potentials (**Table 4.2; Figures 4.6 and 4.7**). These results suggest that smoke-water can play a significant role in germination and seedling growth of *Eragrostis tef* under water-stress conditions. Although treatment with butenolide enhanced germination and seedling growth, in most of the cases these results were not significantly different from the control. This could be attributed to the concentration of butenolide used in this study. Most likely, the seeds of *Eragrostis tef* may require higher concentrations of butenolide than that tested. However, at this stage, the application of smoke-water would be a convenient, low

cost method until further progress is made in synthesizing or isolating butenolide on a large scale.

4.5 CONCLUSIONS

The results of this study clearly show that smoke-water and butenolide treatments can effectively enhance seed germination and seedling growth of *Eragrostis tef* under higher temperatures and low osmotic potentials, which are normally detrimental to the crop. This study clearly shows that smoke solutions widen the temperature range over which germination of *Eragrostis tef* can occur and override stresses arising from moisture deficit. The two major conclusions which can be drawn from findings of this study are the following: Firstly, smoke solutions are able to overcome stresses arising from sub-optimal temperatures and moisture in certain smoke-responsive species which has further ecological implications for grassland dynamics. In other words, smoke enhances seed germination in certain smoke-responsive species perhaps due to its effects on facilitating water uptake by seeds. However, further comparative studies on the role of plant-derived smoke on water uptake (imbibition) by smoke-responsive and non-smoke responsive species is needed.

Secondly, smoke treatment is a possible option for resource-limited farmers in Eritrea, Ethiopia and other neighbouring countries where *Eragrostis tef* is a staple crop. However, further *in situ* studies on the role and application of plant-derived smoke on *Eragrostis tef* germination, seedling growth, flowering and seed production are needed to better understand and quantify the net contribution of smoke biotechnology to Tef farming in the specified regions. Further studies are also needed in establishing out the most viable method of smoke application on Tef lands as either smoke aerosol or otherwise smoke solutions.

CHAPTER FIVE

Does fire (burning) affect the soil smoke chemistry? Germination activity of smoke residue in soils following a fire

"Each soil has had its own history. Like a river, a mountain, a forest, or any natural thing, its present condition is due to the influences of many things and events of the past."

Charles E. Kellogg (1902-1980)

5.1 INTRODUCTION

It has been recognised that fire as a disturbance factor affects both the physiochemical properties of the soil and the atmospheric chemistry **(RAISON, 1979; ANDREAE, 1991)**. The role of the physical (e.g. light, temperature) and chemical (e.g. nutrients) factors associated with fire in influencing plant species recruitment is also well known **(ROSS and HARPER, 1972; RAISON, 1979; JUTILA and GRACE, 2002)**. Since the 1990's however, the role of smoke and aqueous smoke solutions as a new factor in regulating seed germination has fascinated plant biologists and the subject has been extensively examined **(VAN STADEN et al., 2000)**. To date, plant-derived smoke has been shown to stimulate the germination of many fire-dependant and fire-independent species. In total, over 1200 species from 80 genera representing a wide diversity of both fire and non-fire prone environments have showed marked germination increases in response to plant-derived smoke **(BROWN and BOTHA, 2004; DIXON et al., 2009)**. The effects of plant-derived

smoke may extend beyond the germination stimulation of certain smoke-responsive species. Smoke also stimulates somatic embryogenesis **(SENARATNA et al., 1999)**, root initiation **(TAYLOR and VAN STADEN, 1996)** and flowering **(KEELEY, 1993)**. It also acts in enhancing seedling vigour and accelerating plant growth **(SPARG et al., 2006; Chapter 2, Section 2.5; Table 2.1)**.

Over the last decade, following the preliminary reports on smoke-stimulated seed germination, researchers have attempted to isolate and characterize the chemical(s) in plant-derived smoke **(CHIWOCHA et al., 2009; LIGHT et al., 2009)**. The identity of a highly active germination triggering compound in plant-derived smoke **(VAN STADEN et al., 2004)** and/or cellulose-derived smoke **(FLEMATTI et al., 2004)** has been identified as 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one **(FLEMATTI et al., 2004; VAN STADEN et al., 2004)**. This smoke-derived compound (butenolide) is a simple organic compound and has been shown to increase both the level and the rate of seed germination, widen the environmental range over which germination can occur and have a positive effect on seedling vigour **(LIGHT et al., 2009)**. Butenolide is formed during pyrolysis reactions and may have an effect during fires, in the immediate post-fire environment, considerable periods after the fire, or perhaps more importantly, in plant communities long distances away from the active fire site. Though research has answered several relevant questions behind the discovery, knowledge on the effects of fire on altering the soil-smoke chemistry and the persistence of the active germination compound in the soil, in the context of its water-soluble nature, is still lacking. The spatial and temporal dimensions of fire and smoke influence on the chemistry of the actively burned sites and the sites surroundings the burn is also unknown.

Smoke is a highly complex system of particulate matter (ash, soot), droplets and gases emitted when a material undergoes combustion or pyrolysis. Vegetation fires produce huge clouds of smoke which can waft into neighbouring unburnt areas carried by wind **(ANDREAE, 1991; KEELEY and FOTHERINGHAM, 1997)**. In the event of rain, such clouds of smoke emissions in air would dissolve into the moisture, essentially producing a smoke solution. Smoke may also leach into the soil either in a gaseous or liquid form stimulating the soil seed bank **(JÄGER et al., 1996; HOLMES and RICHARDSON, 1999; READ et al., 2000; CROSTI et al., 2006)**. Hence, knowledge of the effect of fire on altering the soil-smoke chemistry and the

subsequent influence on the germinable soil seed bank is important from conservation, restoration and weed control perspectives, particularly with regard to the fire-prone grasslands of South Africa, where the use of fire for managing the health of the grassland is a common practice **(SCOTT, 1971)**. Despite the wide use of fire (smoke) however, its effects on soil seed bank germination and the subsequent impact on grassland composition remains unknown.

The objectives of the present study were: (1) to investigate if grassland burning affects the soil-smoke content; (2) to determine if this soil property differs along depths in the soil profile; (3) if the activity of smoke along the soil core differs with presence or absence of rain; (4) if the soil-smoke activity differs with time since the day of burn (persistence). In this study a derived index of relative smoke concentration was used to index the biological activity of the burnt or otherwise unburnt soil extracts to germinate seeds of light-sensitive lettuce (*Lactuca sativa*) Grand Rapids achenes (hereafter referred to as seeds). Grand Rapid lettuce seeds are particularly responsive to smoke extracts and are effectively used as a rapid bioassay to evaluate the biological activity of various extracts **(DREWES et al., 1995)**.

5.2 MATERIALS AND METHODS

5.2.1 Site description, burning and the soil samples

The sites used for collecting burnt soils are located at Ukulinga, the research farm of the University of KwaZulu-Natal, Pietermaritzburg (29° 24' E, 30° 24' S). The plots are situated on top of a small plateau ranging in altitude from 847 m to 838 m and the sites were not burned for the last two years. Soils at the site were classified as Westleigh forms **(SOIL CLASSIFICATION WORKING GROUP, 1991)**. The vegetation of the area is classified as southern Tall Grassveld **(ACOCKS, 1988)**. The sward varied between 40 to 60 ± 6.33 SE cm in height (average of 20 random samples) and is dominated by herbaceous species mainly *Themeda triandra* Forssk, *Heteropogon contortus* Beauv. ex Roemer & J. A. Schultes and *Tristachya leucothrix* Trin. ex Nees.

On 29 May 2008, a head fire was applied on the grassland site under the following weather conditions; air temperature 30 °C; wind speed 6 km h^{-1} and relative humidity 52%. To keep the fire within the outlined limits, plants surrounding the plot (edge effect of 2 m) were cut short and soaked with water before burning.

Figure 5.1: A) Typical mesic grassland in South Africa on fire, B) Simulated rain (water) is being applied on earmarked treatment plots.

Burning commenced at 12.30 pm and was sustained for only a few seconds duration at any given point. This was expected to be sufficient to deposit a strong

smoke cue to the soil. Soil samples were subsequently collected from four depths i.e. 0-2, 2-4, 4-6 and 6-8 cm using a 3 cm diameter auger from plots under three different conditions (see **Table 5.1**). To determine if the presence or absence of rain following a fire affected the vertical distribution of smoke activity along the soil profile, approximately 8 x 10^7 mm^3 of water (simulated rain) was sprayed over 1 m^2 on earmarked plots and soil core sampling was done 10 min after the water had completely drained. Besides the artificially sprayed (simulated rain), no natural rain event was recorded during the eight week period. To assess the effect of burning on soil-smoke activity and to examine the persistence of the influence through time, 96 soil samples (6 treatments x 4 depths x 4 replications) were collected for 8 successive weeks i.e. 0 (immediately after burn), 2, 4 and 8 weeks after burn. Hence, a total of 312 soil samples were collected and tested for germination activity.

5.2.2 Fuel load and fuel moisture

Prior to burning, available fuel loads were estimated by collecting dead and live plant material from 5 quadrats (1 x 5 m) along an established 50 m transect. Following collection, fuel samples were oven dried at 120 °C for 72 h to obtain an estimate of available fuel load and fuel moisture content. Fuel load and fuel moisture content were calculated as: Fuel moisture content (wet basis) = (total mass of wet biomass–dry mass)/total mass of wet biomass x 100 **(ANDERSON and KOTHMANN, 1982)**. Dry biomass weights per square meter were converted to tonnes per hectare.

5.2.3 Soil extraction methods

Soil samples were individually stored in clear plastic bags at 5 °C. Each soil sample was crushed using a mortar and pestle and sieved to remove stones and organic material. The soil samples were extracted with three volumes (100 mL and 50 mL x 2) of distilled (purified) dichloromethane. Dichloromethane was used as an extracting solvent, because it is known that the smoke-derived butenolide can be extracted with this solvent **(VAN STADEN et al., 2004)**. Preliminary experiments comparing dichloromethane and water for preparing the soil extracts showed that the dichloromethane extraction was a suitable method. The dichloromethane was evaporated and the residue was redissolved in 10 mL of distilled-water. This extract

was diluted giving three concentrations (1:1, 1:10 and 1:100 v/v). These extracts were then used to initiate germination of the Grand Rapids lettuce seeds.

Table 5.1: Soil core samples collected before and after burning mesic grassland sites in South Africa.

Treatments	Abbreviated as	Conditions under which soil cores were collected
Unburnt, no water	UBNW	Soil cores collected from an unburnt plot two days before burning
Unburnt, watered	UBW	Soil cores collected from an unburnt plot two days before burning and sprayed with water
Adjacent, no water	ANW	Soil cores collected from an unburnt adjacent plot after burning (5 m away)
Adjacent, watered	AW	Soil cores collected from an unburnt plot after burning but sprayed with water (5 m away)
Burnt, no water	BNW	Soil cores collected from actively burnt plot on the day of the burn
Burnt, watered	BW	Soil cores collected from actively burnt plot on the day of the burn and sprayed with water

5.2.4 Seed source and the lettuce seed bioassay

Mature achenes (seeds) of *Lactuca sativa* L. cv. Grand Rapids (Peto Seeds, Saticoy, USA) were used in this study. Four replicates of 25 seeds each were placed on two layers of Whatman No. 1 filter paper in 65 mm plastic Petri dishes moistened with 2.2 mL of distilled water (control) or the test solutions. The seeds were kept in light-proof boxes and incubated in the dark at 25 ($\pm$) 0.5 °C for 24 h whereafter germination was recorded. All manipulations were conducted under a green safe

light (0.5 µmol m^{-2} s^{-1}) (**DREWES et al., 1995**) and germination was determined as the emergence of the radicle through the seed coat. Since germination of Grand Rapids lettuce seeds can be inhibited by high concentrations of smoke extracts (**DREWES et al., 1995; JÄGER et al., 1996**) a dilution series of each extract was routinely tested. To verify the effect of smoke on germination, the Grand Rapids lettuce seeds were also subjected to smoke-water (1:500 v/v) and butenolide (10^{-8}M) solutions. A solvent blank extract (dichloromethane extract prepared in the manner of the soil extracts without soil sample) was also tested.

5.2.5 Statistical analysis

Arcsine transformed data obtained from the experiments were subjected to one-way analysis of variance (ANOVA) and Tukey's multiple range test at a significance level of 5%. GenStat (version 11.1, Rothamsted Research Harpenden, UK) statistical package was used to analyse the data of these experiments.

5.3 RESULTS

5.3.1 Fuel load and fuel moisture content

The fuel load of the unburnt areas was estimated to be 1.24 ± 0.18 t ha-1. The moisture content of the fuel constituted 19.23 ± 0.91% of the biomass. The soil moisture content of the site was 10.33 ± 0.87%. Such a fuel load for the given moisture content was sufficient to produce hot fires and smoke. All the dead and live grass cover was instantly consumed.

5.3.2 Germination activity of the soil extracts

The results of smoke-water and butenolide solutions clearly show a 3-fold significant increase in percentage germination (> 80%) of Grand Rapids lettuce seeds compared to controls (**Figure 5.2**). Results of the germination activity of soil extracts are given in **Figure 5.3(a-d)**. At 0 time, soil extracts of BNW (see **Table 5.1** for the abbreviations used) and BW plots from 0-2 and 2-4 cm depths tested with different

concentrations, showed significantly higher percentage germination than the soil extracts from other plots (**Figure 5.3a**).
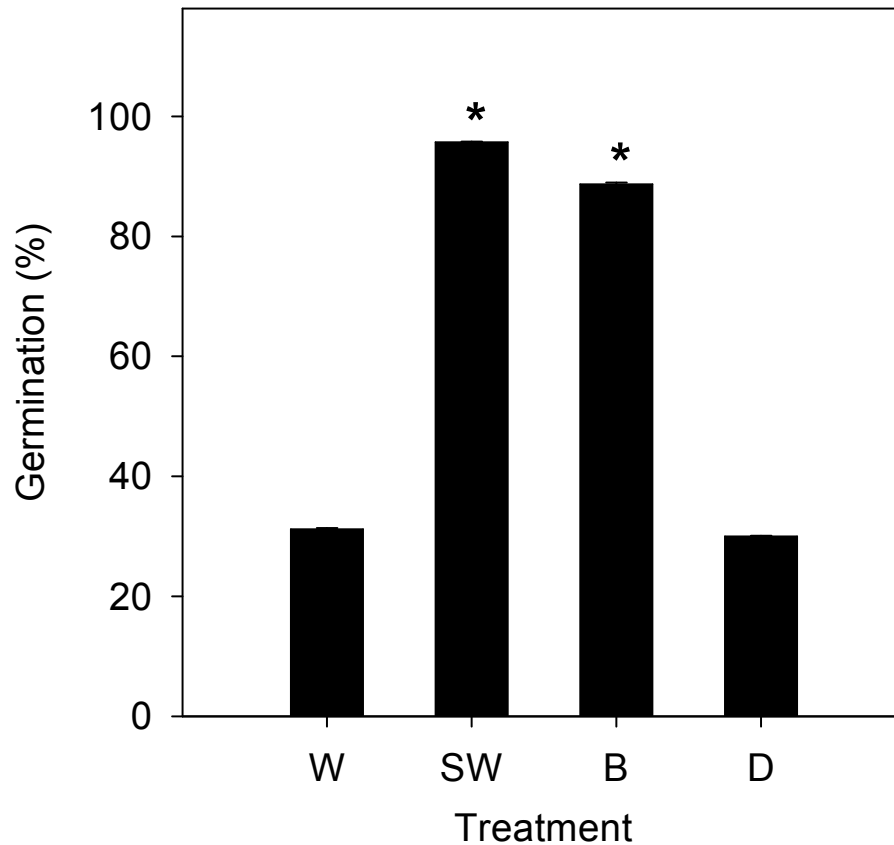
Figure 5.2: Germination of Grand Rapids lettuce seeds imbibed in Smoke-Water (SW1:500 v/v), Butenolide (B 10^{-8} M), Dichloromethane (D) and Distilled-water (W) after 24 h incubation at 25°C in the dark. Asterisks denote a significant (P < 0.05) difference from distilled-water and dichloromethane control. Error bars indicate SE.

Soil extracts of the same plots from a depth of 6-8 cm tested with higher concentrations (1:1 and 1:10), showed a significant increase in percentage germination compared to other plots (**Figure 5.3a**). After 2 weeks, most of the concentrations of extract prepared from soils of 0-2, 2-4 and 4-6 cm depths of BNW and BW plots, exhibited significantly greater germination than the soil extracts from other plots (**Figure 5.3b**). At a depth of 6-8 cm, only the highest concentration (1:1) significantly increased percentage germination in comparison to other concentrations tested (**Figure 5.3b**). In the soil samples collected after 4 weeks from 0-2 and 2-4 cm depths of BNW plots, the concentration of 1:10 of these soil extracts showed a significant increase in percentage germination compared to the other plots examined. At 0-2 cm depth of the BW plot, there was a significant increase in percentage germination for concentration 1:1. On the other hand, for a 2-4 cm depth this increase in percentage germination was achieved in both 1:1 and 1:10 concentrations (**Figure 5.3c**). Soil extracts from depths of 4-6 and 6-8 cm of BNW and BW plots with a concentration of 1:10 showed significantly higher percentage germination than the low or high concentrations. The tested soil extracts from other plots were also significantly different to a 1:10 concentration of BNW and BW plots (**Figure 5.3c**). After 8 weeks, a high concentration (1:1) of soil extracts from depths 0-2 and 2-4 cm of BNW and BW plots showed greater percentage germination than other treatments or plots. No significant germination activity was observed in extracts from unburnt or adjacent plots. All the concentrations of soil extract at depths of 4-6 and 6-8 cm of ANW and AW significantly decreased percentage germination of the Grand Rapids lettuce seeds. **Table 5.2** of ANOVA shows that treatment, concentration, depth and time (weeks) and their interactions were highly significant ($P < 0.001$).
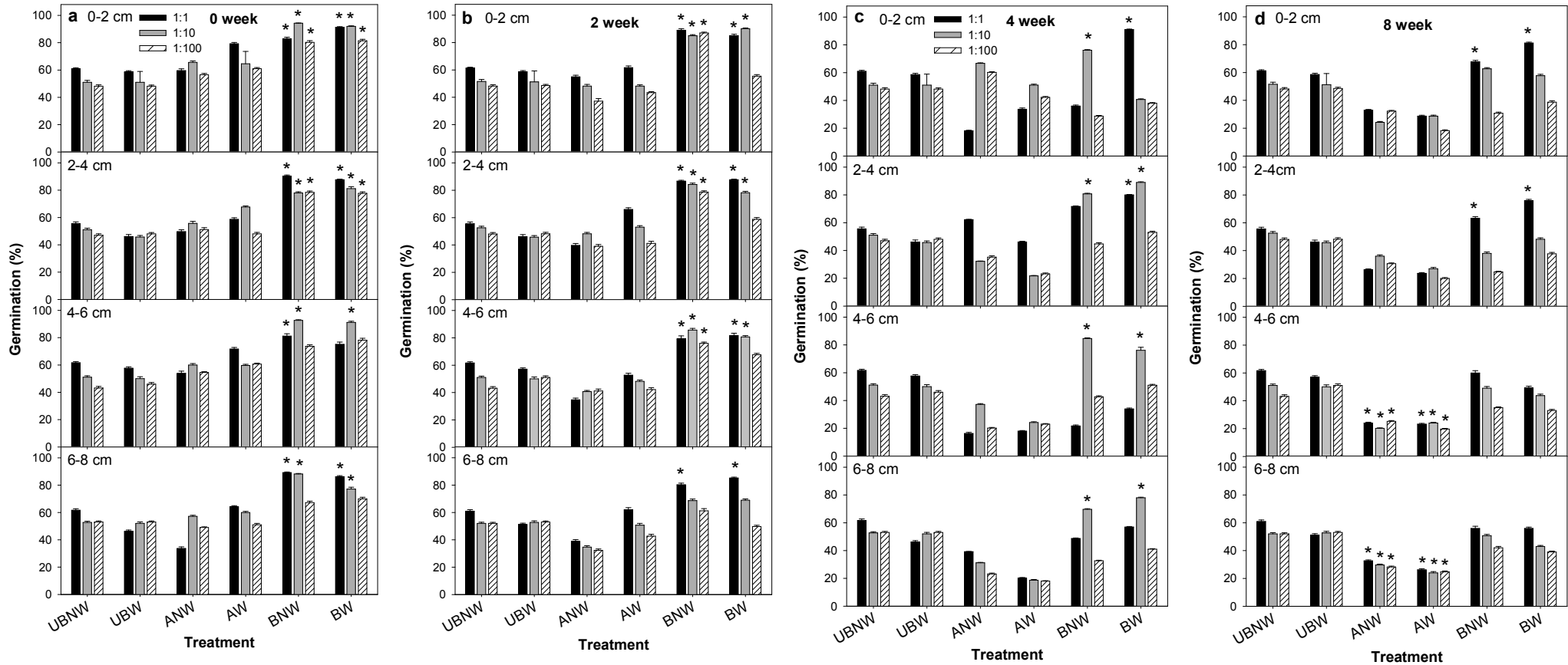
Figure 5.3: The effect of various soil extracts at three dilution levels on the germination of Grand Rapids lettuce seeds 24 h in the dark at 25 °C. a) in the 0 week after burn; b) in the 2nd week after burn; c) in the 4th week after burn; and d) in the 8th week after burn. Asterisks denote a significant ($P < 0.05$) difference from the other plots. Error bars indicate SE.

Table 5.2: General analysis of variance (ANOVA) with main effects and their interactions for the 3 burning treatments, 3 concentration levels, 4 soil depths and 4 time periods (weeks).

Source of variation	Degrees of freedom	Sum of squares	Mean square	Variance ratio	F probability
Treatment	5	73.96604	14.79321	424.27	< 0.001
Concentration	2	14.37701	7.18851	206.17	< 0.001
Depth	3	3.52999	1.17666	33.75	< 0.001
Week	3	47.75627	15.91876	456.55	< 0.001
Treatment*Concentration	10	8.86450	0.88645	25.42	< 0.001
Treatment*Depth	15	2.93393	0.19560	5.61	< 0.001
Concentration*Depth	6	0.99939	0.16656	4.78	< 0.001
Treatment*Week	15	31.53703	2.10247	60.30	< 0.001
Concentration*Week	6	2.68842	0.44807	12.85	< 0.001
Depth*Week	9	1.79077	0.19897	5.71	< 0.001
Treatment*Concentration*Depth	30	6.31627	0.21054	6.04	< 0.001
Treatment*Concentration*Week	30	7.18249	0.23942	6.87	< 0.001
Treatment*Depth*Week	45	4.82040	0.10712	3.07	< 0.001
Concentration*Depth*Week	18	1.76411	0.09801	2.81	< 0.001
Treatment*Concentration*Depth*Week	90	10.74055	0.11934	3.42	< 0.001
Residual	3168	110.45953	0.03487		
Total	3455	329.72670			

5.4 DISCUSSION AND CONCLUSIONS

Studies conducted on various vegetation types worldwide suggest that post-fire increases in germination and massive seedling emergence of certain species is due to direct heat **(TROLLOPE, 1984; KEELEY and KEELEY, 1987; ZACHARIAS et al., 1988; READ et al., 2000; WILLS and READ, 2002)**, increases in nutrient levels **(HARPER 1977; KINLOCH and FRIEDEL 2005)**, better access to light **(KEELEY and KEELEY, 1987; HULBERT 1988)**, smoke-induced germination **(BROWN, 1993a; DIXON et al., 1995; PIERCE et al., 1995; BROWN and VAN STADEN, 1997; SNYMAN, 2005)** and the combined effect of the heat shock and smoke **(BLANK and YOUNG, 1998; GILMOUR et al., 2000; WILLS and READ, 2002)**. There are relatively few similar studies on South African grasslands and information on how burning (smoke) influences the germination of the soil seed bank is scarce

(ZACHARIAS et al., 1988). The lettuce seed bioassay can be used to determine the effect of smoke on germination thereby giving an indication of germination potential of smoke-derived compounds present in the soil.

It was found that the soil-smoke activity as detected by the ability of the various soil extracts to stimulate germination of the Grand Rapids lettuce seeds, significantly differed among treatments, dilution levels, soil depth and time since burn. The highest variation was accounted for by weeks followed by treatments (**Table 5. 2**). In comparison to the water control, the Grand Rapids lettuce seeds exhibited greater germination percentages when treated with burnt soil extracts (at 0-4 cm depths) throughout the 8-week study period. This enhanced germination of Grand Rapid lettuce seeds in response to burning (smoke) is in agreement with previous studies on smoke-induced germination of various species **(KEELEY et al., 1985; DIXON et al., 1995; BROWN and VAN STADEN, 1997; READ et al., 2000)**. These results imply that the smoke produced from burning biomass penetrates and incorporates itself into the soil and would play a significant role in enhancing germination of certain smoke-responsive species stored in the soil seed bank **(BROWN, 1993a; DIXON et al., 1995; KEELEY and FOTHERINGHAM, 1998; READ et al., 2000; WILLS and READ, 2002)**.

The stimulatory role of fire (smoke) on the germination of the soil seed bank may be related to soil depth **(PEART, 1984; READ and BELLAIRS, 1999; READ et al., 2000; ENRIGHT and KINTRUP, 2001; STEVENS et al., 2007)**, dilution level **(PRESTON and BALDWIN, 1999; STEVENS et al., 2007)** and time since burning **(PEART, 1984; PRESTON and BALDWIN, 1999; READ and BELLAIRS, 1999; READ et al., 2000)**. In this study, water treatment did not affect the vertical distribution of smoke activity along the core. This could probably be because of the amount of simulated rain applied and perhaps the high water holding capacity of the Westleigh soils at the site **(SOIL CLASSIFICATION WORKING GROUP, 1991)** with more clayish topsoil not allowing the smoke activity to drain deeper. Recently, **STEVENS et al. (2007)** have demonstrated that the germination compound in plant-derived smoke can be leached through the soil profile, using an experimental system with a column of silica sand. Evidently, smoke activity in the burnt plots was higher in the topsoil than in the lower soil profiles. The smoke activity declined with time,

maybe due to the water-soluble nature of the germination compound in smoke, only allowing it to persist in the soil for a limited period of time **(DE LANGE and BOUCHER, 1990; BROWN and VAN STADEN, 1997; PRESTON and BALDWIN, 1999; READ et al., 2000)**. However, unlike the findings in this present study, a similar study conducted by **PRESTON and BALDWIN (1999)** suggested that the germination compound in smoke can remain in the soil up to seven years. Similarly, **ROCHE et al. (1998)** observed that the germination cue from burning plant material can be retained in the soil well beyond the time of burning or the application of smoke solutions.

Interestingly, in the zero week after burning, germination activity in the adjacent areas was significantly higher than in the unburnt control. However, towards the 8th week, germination significantly decreased (inhibited) in the adjacent plots. It has been reported that although, smoke in general provides a positive stimulus for germination it also contains inhibitory compounds that may block the action of the stimulator, preventing germination of seeds **(DREWES et al., 1995; PRESTON and BALDWIN, 1999; DAWS et al., 2007; LIGHT et al., 2010)**. Thus findings from this study suggest that the germination triggering signal in smoke was probably transported to the unburnt adjacent plots, causing positive and negative effects on germination. **PRESTON and BALDWIN (1999)** have found evidence that the germination compound in smoke can be transported by wind and water into adjacent unburnt areas (some 40 m away from a burnt site). These authors also demonstrated the existence of negative, germination-inhibiting factors in burning plant material. Hence, wind and water transport of the smoke cue, may not essentially stimulate germination in adjacent unburnt areas, because the litter-derived factors may not be able to override the positive effects of the smoke cue **(PRESTON and BALDWIN, 1999)**. In many cases, the negative role of smoke on germination depended on its concentration and some species-specific inhibitors in smoke **(ADKINS and PETERS, 2001; DAWS et al., 2007; LIGHT et al., 2010)**.

One of the most striking results obtained from this study is that unburnt soil extracts exhibited greater germination percentage compared to the water control. This probably suggests that other organic factors (besides smoke) may be present in the soil due to the degradation of soil organic matter and such chemicals may play a

role in promoting germination **(DIXON et al., 1995; KEELEY and FOTHERINGHAM, 1997)**. In other words, smoke (burning) may only play a supplementary role in promoting germination of species in the post-fire environment **(DIXON et al., 1995)**. It is well known that ethylene promotes germination in seeds from a wide range of species, including Grand Rapids lettuce seeds **(JÄGER et al., 1996)**. Therefore, seed banks of certain species may have to react additively to a number of cues in the habitat.

The marked positive germination responses that are observed following a fire are the result of the sum total of effects of smoke and non-smoke factors in the habitat. From previous studies on smoke-stimulated germination, certain grass species which dominate South African mesic grasslands (e.g. *Heteropogon contortus*, *Themeda triandra* and *Tristachya leucothrix*) have shown a positive germination and vigour response to smoke **(BAXTER et al., 1994;** see also **Chapter 3)**, though they are generally unable to survive fire **(ZACHARIAS et al., 1988)**. Hence, considering that the soil seed bank consists of viable seeds, at a moderate depth and that they are initially unaffected by the heat of the fire, smoke (burning) should give a competitive advantage to the smoke-responsive species over those less responsive species, and such a competitive advantage would likely impact on the composition of the grassland. However, detailed field studies on the reaction of the soil seed bank **(READ et al., 2000; ADKINS and PETERS, 2001; KĘPCZYŃSKI et al., 2006)** to fire and smoke are needed to gain a better insight into the effect of fire on issues relating soil, smoke and seed germination.

CHAPTER SIX

Growth responses of grass seedlings to smoke-water and smoke-derived butenolide in the presence or absence of NPK nutrients

"I think science has enjoyed an extraordinary success because it has such a limited and narrow realm in which to focus its efforts"

Ken Jenkins (1940~)

6.1 INTRODUCTION

Grassland communities are the major components of the vegetation in the mesic and semi-arid regions of South Africa. Fire **(SCOTT, 1971; TAINTON, 1999)**, rainfall **(O'CONNOR, 1994; SNYMAN, 2003; SNYMAN, 2009)**, soil nutrients and ungulate grazing **(O'CONNOR, 1985; O'CONNOR et al., 2001)** are some of the most important variables which influence the structure and function of the grasslands **(ANDERSON and KOTHMANN, 1982; O'CONNOR, 1985; SIMS, 1988; SNYMAN, 2009)**. Fire is generally regarded as one of the major natural environmental entities that play a significant role in determining the composition of grasslands **(SCOTT, 1971; TAINTON, 1984; TAINTON, 1999)**. As stated earlier (in **Chapter 3**), post-fire grasslands are generally marked by fast vegetative re-growth with concurrent increases in dry matter production **(OJIMA et al., 1994; BLAIR, 1997)**. A regular versus irregular fire regime often cause contrasting shifts in species composition **(FYNN, 2004)**. Declining fire frequency and intensity initiate a general trend where native grass species of high grazing value give way to economically less desirable growth forms leading to the expansion of woody *Acacia* species **(EVERSON and**

TAINTON, 1984) accompanied by a significant decline in basal cover **(EVERSON and TAINTON, 1984; FYNN, 2004; SNYMAN, 1998; FYNN et al., 2005)**. In other words, the short native species require frequent exposure to fire to remain competitive **(EVERSON and TAINTON, 1984; MORGAN and LUNT, 1999)**. Though much is known of how trends in species composition alter in response to fire and fire-related factors **(EVERSON and TAINTON, 1984; GIBSON and HULBERT, 1987; FYNN, 2004)**, understanding of the underlying mechanisms responsible for these responses is still incomplete. Although widely debated, previous studies on grassland fire suggest that the strong fire-species relationship syndrome is the result of fire-induced temporary increases on light availability **(KNAPP and SEASTEDT, 1986; GHEBREHIWOT et al., 2006)**; lowered productivity **(O'CONNOR, 1997; FYNN, 2004)**; competitive release **(O'CONNOR, 1997; MORGAN and LUNT, 1999; FYNN, 2004)** nutrient enrichments **(FYNN et al., 2005)** and smoke **(BAXTER et al., 1994)**. Hence, quantifying the role of these factors in single or in combination is crucial for understanding the dynamics of such fire-climax ecosystems **(TAINTON, 1999)**. More importantly, an understanding of the constraints on seedling establishment is essential for the ecology and successful management of grasslands.

In the mesic and semi-arid grasslands of South Africa (**Figure 1.1**), the existence of competitive displacement trends among species in response to fire-induced nutrient gradients such as nitrogen (N), potassium (P) and phosphorus (K) has been reported **(SAMUEL and HART, 1992; FYNN et al., 2003; FYNN et al., 2005)**. Fire causes severe losses of large amounts of carbon (C), nitrogen (N) and sulfur (S) present in the herbage through volatilization and erosion of ash, which subsequently results in a significant loss of organic matter content from the soil surface **(HAYES and SEASTEDT, 1989; KAUFFMAN et al., 1993; COOK, 1994; OJIMA et al., 1994; BLAIR, 1997; TURNER et al., 1997; FYNN et al. 2003)**. As a result N becomes more deficient to plants due to a decrease in the rate of its mineralization. Post-fire semi-arid grasslands are also typified by rapid leaching of potassium (K) and phosphorus (P), and as a consequence such elements become less available to plants thereby limiting growth **(ALLEN, 1964)**. **CHAPMAN (1967)** reported that controlled burning of lowland heath vegetation eliminated 95% N, 26% P and 21% K from the burnt site. This indicates that the species which dominate

post-fire habitats thrive more on nutrient-poor soils. Therefore, soil nutrient gradients between burnt and unburnt grasslands may play a significant role in competitive displacement of the species involved **(GRIME, 1979; TILMAN, 1988; GHEBREHIWOT et al., 2006)**.

On the other hand, more recent studies on the role of fire and fire-related factors on germination and regeneration ecology of grasslands indicate that plant-derived smoke influences both the germination and grassland species growth differently **(VAN STADEN et al., 2000; GHEBREHIWOT et al., 2009)**. Further, certain native grass species that dominate frequently burnt South African semi-arid grasslands are significantly more responsive to smoke treatments than others. Such a difference among species would have an inevitable impact on species composition **(BAXTER et al., 1994;** see also **Chapter 3)**. Currently, plant-derived smoke treatments, whether in the form of aerosol or liquid have shown stimulating effects on seed germination and seedling growth **(BROWN and VAN STADEN, 1997; VAN STADEN et al., 2000; BROWN and BOTHA, 2004; SPARG et al., 2005; KULKARNI et al., 2007; COMMANDER et al., 2009; NELSON et al., 2009; DAYAMBA et al., 2010)**. Smoke treatment has also shown positive effects on germination and seedling vigour of different grass species along gradients of temperature and water potential (see **Chapter 4**). A compound isolated from burnt cellulose **(FLEMATTI et al., 2004)** and plant-derived smoke **(VAN STADEN et al., 2004)** known as butenolide (3-methyl-2*H*-furo[2,3-*c*]pyran-2-one) is responsible for enhancing seed germination of many plant species. There is a general suggestion that butenolide (recently termed as KAR_1) behaves in a manner similar to that of other plant hormones **(SENARATNA et al., 1999; VAN STADEN et al., 2000; GARDNER et al., 2001; CHIWOCHA et al., 2009; NELSON et al., 2009)**. Several studies on smoke have shown that aqueous smoke solutions exhibit hormone-like responses in many species and interact with gibberellins, cytokinins, abscisic acid and ethylene in photoblastic and thermodormant seeds **(VAN STADEN et al., 2000; JAIN et al., 2008; NELSON et al., 2009)**. Hence, the effect of plant-derived smoke as a new factor influencing plant species growth **(VAN STADEN et al., 2000; Chapter 3)**, and its interaction with major soil macronutrients (NPK) in influencing seedling growth has become a crucial subject that needs to be understood **(TAINTON and MENTIS, 1984; MATERECHERA et al., 1998)**.

In southern Africa, annual burning is being commonly practiced around large-scale commercial and semi-subsistence grassland farming. South African grass species are poorly represented in smoke-stimulation research and there is very little or no information on the response of species to plant-derived smoke and its interaction with soil nutrients **(ZACHARIAS et al., 1988; BAXTER et al., 1994)**. This study sought to gain greater insight into the role of plant-derived smoke and its interactive effect with major soil nutrients (NPK) in influencing seedling growth. The specific objective was to determine the effect of plant-derived smoke-water and a smoke-isolated butenolide compound, at different concentrations, on various parameters of seedling growth of three grass species from mesic and semi-arid grasslands in South Africa.

6.2 MATERIALS AND METHODS

6.2.1 Seed collection and germination

Grass species *Eragrostis curvula* Nees (Weeping lovegrass), *Themeda triandra* Forssk. (Red grass) and *Panicum maximum* Jacq. (Guinea grass) were selected on the basis of their response to fire frequency **(EVERSON and TAINTON, 1984; FYNN, 2004)**. In addition to this, these species were also selected on the basis of the contrasting germination and seedling vigour response they showed to smoke treatments (see **Chapter 3**), with *Themeda triandra* representing highly smoke-responsive species, *Panicum maximum* representing intermediate and *Eragrostis curvula* representing less smoke responsive species. The seeds of these species were collected in 2008 from the wild vegetation around Pietermaritzburg (30°24'S, 29°40'E), KwaZulu-Natal, South Africa. The seeds were cleaned and stored at ambient laboratory conditions (25 °C) for two months until they were used. Subsequently, these seeds were germinated in 90-mm Petri dishes on two layers of Whatman filter No. 1 wetted with 4 mL distilled water. Petri dishes were incubated in a plant growth chamber set at 25 °C with 16/8 h day/night photoperiod (102 µmol m-2 s-1). After one week, the seedlings of all the three species which were obtained from germinated seeds were transplanted in pots for growth experiments.

6.2.2 Nutrient, smoke-water and butenolide solutions

For the preparation of smoke extract, dry *Themeda triandra* (Poaceae) leaf material (5 kg) was burnt in a 20-L metal drum and the smoke generated from it was passed through a glass column containing 500 mL of tap water for 45 min **(BAXTER et al., 1994; CLARKE and FRENCH, 2005)**. The three different concentrations of smoke-water used in this study were prepared by diluting 1 mL of smoke-extract with 250, 500, and 1000 mL distilled water (1:250, 1:500, and 1:1000 v/v). A pure butenolide (3-methyl-2*H*-furo[2,3-*c*]pyran-2-one) used in this experiment was isolated from plant-derived smoke-water as described by **VAN STADEN et al. (2004)**. The concentrations of the butenolide tested were 10^{-7}, 10^{-8}, and 10^{-9}M. In this trial half-strength (50%) Hoagland's nutrient solution [1] (HS) **(HOAGLAND and SNYDER, 1933)** was used for seedling growth. The effects of three macronutrients N, P, and K with or without different concentrations of test solutions (smoke-water and butenolide) were studied by eliminating each one of these from half-strength HS. Each treatment was then represented as -N, -P, and -K.

6.2.3 Plant growth experiments

The pot experiments were conducted in a greenhouse which was maintained at an air temperature of 25 °C and relative humidity between 25 and 50%. The seedlings were grown in 20 cm pots filled with sterile quartz sand moistened with HS, -N, -P, and -K (controls) solutions. A single pot per treatment containing 15 seedlings was used. One hundred milliliters of these solutions were added to each pot once weekly. Subsequently (after 3 days), once weekly a similar volume of treatment solutions and distilled water (control) were added to these pots. Since smoke solutions were prepared using distilled water, the control pots were supplied with distilled water. The pots were arranged randomly in the greenhouse with a midday photosynthetic photon flux density of 405 ± 7.5 µmol m^{-2} s^{-1} at 22 ± 2.5 °C. The seedlings of *Panicum maximum*, *Themeda triandra Eragrostis curvula* were harvested

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[1] Hoagland's nutrient solution is a method of growing plants feeding the necessary nutrients without soil. The method was developed by **HOAGLAND and ARNON (1933)**.

respectively at 45, 46 and 88 days after transplanting and various seedling growth parameters were recorded. A single seedling was considered as a replicate per treatment. Root volume was determined according to the method of **BURDETT (1979)**. A seedling vigour index formula as reported by **ABDUL-BAKI and ANDERSON (1973)** was modified and calculated as: SVI = [mean shoot length (mm) + mean root length (mm)] X mean seedling weight (g).

6.2.4 Statistical analysis

Data obtained from the pot experiments for different seedling growth parameters were subjected to one-way analysis of variance (ANOVA) and means were separated using Tukey's test at a 5% level of significance ($P<0.05$). General ANOVA was conducted to determine the main effects and their interactions **(GENSTAT, 2008)**.

6.3 RESULTS

Nutrient deficient treatments showed inhibitory effects on seedling growth of grass species studied. The only exception was for seedlings of *Themeda triandra* which showed a significant increase in shoot and root length, weight and root volume in the absence of K (**Table 6.1**). Most of the growth parameters examined for *Eragrostis curvula* and *Panicum maximum* seedlings without N, P or K showed significantly lower values than the control (**Table 6.1**).

   Themeda triandra seedlings did not significantly show an increase or decrease most of the growth parameters when grown with all nutrients and treated with different concentrations of smoke-water and butenolide solutions (**Figure 6. 1A and Table 6. 2**). However, the seedling vigour index was significantly greater than the control at smoke-water concentration of 1:250 (**Figure 6. 2A**). Smoke-water and butenolide treatments under deficiency of N, P or K promoted seedling growth parameters and in many cases these parameters were significantly different from the control (**Figure 6.1A and Table 6.1**). Interestingly, smoke-water and butenolide-treated seedlings at all dilutions showed significantly higher vigour indices than untreated seedlings when there was no supply of N (**Figure 6. 2A**). With the

92

elimination of either P or K, some dilutions of these treatments significantly increased the seedling vigour index (**Figure 6. 2A**). The seedlings of *Eragrostis curvula* grown with all nutrients and treated with smoke-water (1:250 and 1:1000) and butenolide solution (10^{-8}M) showed a significant decrease in shoot length compared to the control (**Figure 6.1B**). In treated seedlings, the number of leaves was less with a significant decrease in number of roots (**Table 6. 2**).

Table 6.1: Effect of nutrient solution (50% Hoagland's) with or without N, P or K on seedling growth parameters of grass species grown under greenhouse conditions.

Treatment	Shoot length (mm)	Root length (mm)	Leaves (no)	Roots (no)	Seedling weight (g)	Root volume (cm^3)
Themeda triandra						
Control (HS)	127 ± 7.0 b	93 ± 4.8 b	7.8 ± 1.1 a	3.6 ± 0.5 a	0.270 ± 0.030 b	0.325 ± 0.038 b
No nitrogen (-N)	109 ± 1.7 c	90 ± 3.4 b	4.9 ± 0.1 b	3.0 ± 0.1 a	0.122 ± 0.004 c	0.270 ± 0.005 c
No phosphorus (-P)	106 ± 3.5 c	111 ± 3.7 a	4.8 ± 0.2 b	3.0 ± 0.1 a	0.259 ± 0.023 b	0.403 ± 0.021 b
No potassium (-K)	161 ± 3.9 a	112 ± 5.1 a	5.1 ± 0.1 b	3.1 ± 0.2 a	0.338 ± 0.020 a	0.520 ± 0.031 a
Eragrostis curvula						
HS	216 ± 17 a	160 ± 20 ab	7.3 ± 0.8 a	7.4 ± 0.5 a	0.307 ± 0.03 a	0.392 ± 0.04 ab
-N	82 ± 9.7 b	139 ± 15 b	4.0 ± 0.1 b	4.4 ± 0.3 b	0.303 ± 0.11 a	0.615 ± 0.24 a
-P	27 ± 2.9 c	159 ± 41 ab	3.0 ± 0.2 b	2.5 ± 0.3 b	0.057 ± 0.01 b	0.105 ± 0.02 b
-K	59 ± 14.5 b	219 ± 30 a	3.4 ± 0.2 b	2.9 ± 0.3 b	0.134 ± 0.05 b	0.379 ± 0.14 ab
Panicum maximum						
HS	307 ± 22.6 a	208 ± 19.8 a	5.0 ± 0.2 b	4.4 ± 0.2 a	1.399 ± 0.199 a	1.214 ± 0.187 a
-N	52 ± 1.75 c	83 ± 6.5 d	2.1 ± 0.1 d	2.0 ± 0.1 d	0.032 ± 0.004 c	0.050 ± 0.010 c
-P	74 ± 2.1 c	120 ± 9.6 c	3.1 ± 0.1 c	2.8 ± 0.1 c	0.053 ± 0.006 c	0.080 ± 0.004 c
-K	181 ± 6.4 b	172 ± 6.7 b	6.0 ± 0.1 a	3.8 ± 0.1 b	0.481 ± 0.033 b	0.730 ± 0.035 b

Mean values (±SE) of each species in column with different letters are significantly different by Tukey's test ($P < 0.05$).

However, the seedling weight, root volume and vigour index was significantly increased at a smoke-water concentration of 1:1000 (**Table 6. 2 and Figure 6. 2B**). Exclusion of N from the nutrient solution significantly suppressed shoot length, seedling weight, root volume and vigour index at all tested concentrations of smoke-water and butenolide (**Figure 6. 1B, Table 6. 2 and Figure 6. 2B**). Some

concentrations of these treatments significantly increased shoot length, other seedling growth parameters and vigour indices in the absence of either P or K (**Figure 6. 1B, Table 6. 2 and Figure 6. 2B**). Smoke-water- and butenolide-treated seedlings of *Panicum maximum* grown with all concentrations and nutrients showed a significant decrease in shoot length, number of roots, seedling weight and vigour index compared to non-treated seedlings (**Figure 6. 1C, Table 6. 2 and Figure 6. 2C**). The removal of N, P or K generally enhanced most of the seedling growth parameters with smoke-water and butenolide solutions and at some concentrations these results were significantly different from control (**Figure 6. 1C, Table 6. 2 and Figure 6. 2C**).

Figure 6.1: Effect of different concentrations of smoke-water (SW) and butenolide (B) on shoot and root growth of grass species in the absence of N, P or K under greenhouse conditions. Bar (±SE) with asterisk symbol is significantly different to the respective control by Tukey's test (*P*<0.05).

Table 6.2: Effect of different concentrations of smoke-water (SW) and butenolide (B) in absence of N, P or K on seedling growth parameters of grass species under greenhouse conditions. wt. = weight; vol. = volume.

Treatment	*Themeda triandra*				*Eragrostis curvula*				*Panicum maximum*			
	Leaves (no)	Roots (no)	Seedling wt. (g)	Root vol. (cm^3)	Leaves (no)	Roots (no)	Seedling wt. (g)	Root vol. (cm^3)	Leaves (no)	Roots (no)	Seedling wt. (g)	Root vol. (cm^3)
HS	7.8 ± 1.1 ab	3.6 ± 0.5 a	0.27 ± 0.03 ab	0.32 ± 0.03	7.3 ± 0.8 a	7.4 ± 0.5 a	0.30 ± 0.03	0.39 ± 0.0 d	5.0 ± 0.2 ab	4.4 ± 0.2 a	1.39 ± 0.19 a	1.21 ± 0.18 a
SW (1:250)	9.2 ± 0.7 a	3.8 ± 0.2 a	0.41 ± 0.05 a	0.50 ± 0.07 a	5.4 ± 0.3 b	6.0 ± 0.4 b	0.20 ± 0.02 d	0.49 ± 0.0 cd	4.6 ± 0.2 bc	3.6 ± 0.1 b	0.62 ± 0.08 c	0.81 ± 0.13 ab
SW (1:500)	9.0 ± 0.7 a	3.3 ± 0.3 a	0.32 ± 0.06 ab	0.34 ± 0.06	7.2 ± 0.6 a	6.2 ± 0.5 b	0.27 ± 0.02 cd	0.59 ± 0.0	4.7 ± 0.2	3.7 ± 0.1 b	0.61 ± 0.06 c	0.67 ± 0.10 b
SW	8.0 ± 1.0 ab	3.8 ± 0.3 a	0.31 ± 0.05 ab	0.40 ± 0.06	5.1 ± 0.4 b	4.7 ± 0.3 b	0.45 ± 0.11 a	0.90 ± 0.1 a	5.2 ± 0.1 a	3.8 ± 0.2 b	0.78 ± 0.09 bc	0.68 ± 0.08 b
B (10^{-7}M)	6.1 ± 0.5 b	3.3 ± 0.3 a	0.25 ± 0.03 b	0.24 ± 0.04 b	6.8 ± 0.9 ab	6.1 ± 0.4 b	0.40 ± 0.03 ab	0.77 ± 0.0 ab	4.8 ± 0.2	3.6 ± 0.1 b	0.90 ± 0.09 bc	0.97 ± 0.14 ab
B (10^{-8}M)	6.4 ± 0.7 b	3.0 ± 0.2 a	0.22 ± 0.02 b	0.25 ± 0.05 b	6.1 ± 0.5 ab	5.3 ± 0.4 bc	0.27 ± 0.02 cd	0.51 ± 0.0 cd	4.4 ± 0.1 c	3.7 ± 0.1 b	0.75 ± 0.09 bc	0.77 ± 0.11 b
B (10^{-9}M)	6.5 ± 0.2 b	3.6 ± 0.3 a	0.28 ± 0.04 ab	0.34 ± 0.06	6.6 ± 0.6 ab	5.6 ± 0.3 bc	0.33 ± 0.04 bc	0.69 ± 0.0	5.2 ± 0.2 a	3.8 ± 0.1 b	0.99 ± 0.12 b	0.92 ± 0.11 ab
-N (Control)	4.9 ± 0.1 a	3.0 ± 0.1	0.12 ± 0.00 b	0.27 ± 0.00 b	4.0 ± 0.1 bc	4.4 ± 0.3 a	0.30 ± 0.11 a	0.61 ± 0.2 a	2.1 ± 0.1 c	2.0 ± 0.1 c	0.03 ± 0.00 d	0.05 ± 0.01 c
SW (1:250)	5.0 ± 0.2 a	2.8 ± 0.2	0.17 ± 0.01 ab	0.30 ± 0.03 b	4.0 ± 0.1 bc	3.1 ± 0.1 c	0.02 ± 0.00 b	0.05 ± 0.01 b	3.7 ± 0.1 a	2.6 ± 0.1	0.03 ± 0.00 cd	0.08 ±0.00 bc
SW (1:500)	4.8 ± 0.2 a	2.8 ± 0.1 b	0.20 ± 0.01 a	0.31 ± 0.03 b	3.6 ± 0.2 c	3.8 ± 0.2	0.03 ± 0.00 b	0.09 ± 0.01 b	4.0 ± 0.1 a	2.4 ± 0.1	0.03 ± 0.00 cd	0.08 ± 0.00 bc
SW	4.9 ± 0.1 a	3.0 ± 0.2	0.18 ± 0.02 a	0.46 ± 0.06 a	4.5 ± 0.2 ab	4.5 ± 0.3 a	0.03 ± 0.03 b	0.05 ± 0.01 b	3.8 ± 0.2 a	2.8 ± 0.1 a	0.05 ± 0.00 bc	0.12 ± 0.01 b
B (10^{-7}M)	4.8 ± 0.2 a	3.2 ± 0.3 a	0.20 ± 0.02 a	0.32 ± 0.04 b	4.7 ± 0.1 a	3.7 ± 0.2 bc	0.05 ± 0.01 b	0.09 ± 0.01 b	3.4 ± 0.1 b	2.7 ± 0.2 a	0.08 ± 0.01 a	0.20 ± 0.02 a
B (10^{-8}M)	5.2 ± 0.1 a	2.8 ± 0.3	0.21 ± 0.01 a	0.29 ± 0.03 b	4.1 ± 0.3	3.3 ± 0.3 c	0.04 ± 0.01 b	0.12 ± 0.03 b	3.0 ± 0.1 b	2.8 ± 0.1 a	0.08 ± 0.01 a	0.18 ± 0.02 a
B (10^{-9}M)	5.0 ± 0.1 a	2.8 ± 0.3	0.16 ± 0.02 ab	0.26 ± 0.04 b	4.1 ± 0.2	4.1 ± 0.3 ab	0.12 ± 0.05 b	0.15 ± 0.02 b	3.1 ± 0.1 b	2.8 ± 0.1 a	0.08 ± 0.00 ab	0.18 ± 0.01 a
-P (Control)	4.8 ± 0.2 d	3.0 ± 0.1	0.25 ± 0.02 c	0.40 ± 0.02	3.0 ± 0.2 c	2.5 ± 0.3 c	0.05 ± 0.01 c	0.10 ± 0.02 c	3.1 ± 0.1 b	2.8 ± 0.1 b	0.05 ± 0.00 d	0.08 ± 0.00 d
SW (1:250)	7.2 ± 0.6 a	3.6 ± 0.3	0.32 ± 0.03	0.42 ± 0.08	3.7 ± 0.2 bc	3.5 ± 0.2 b	0.02 ± 0.00 c	0.11 ± 0.02 c	4.3 ± 0.2 a	3.0 ± 0.1 b	0.12 ± 0.02	0.34 ±0.04 ab
SW (1:500)	6.5 ± 0.3 ab	3.6 ± 0.2	0.40 ± 0.04 a	0.67 ± 0.06 a	4.8 ± 0.2 ab	4.4 ± 0.3 b	0.12 ± 0.01 b	0.29 ± 0.03 b	4.0 ± 0.2 a	3.6 ± 0.1 a	0.24 ± 0.06 a	0.43 ± 0.09 a
SW	5.5 ± 0.2	2.7 ± 0.3 c	0.21 ± 0.03 c	0.35 ± 0.06 c	5.2 ± 0.6 a	6.1 ± 0.5 a	0.19 ± 0.03 a	0.43 ± 0.05 a	4.1 ± 0.2 a	3.0 ± 0.2 b	0.11 ± 0.01 cd	0.16 ± 0.02 cd
B (10^{-7}M)	5.4 ± 0.2 cd	3.8 ± 0.1	0.31 ± 0.03	0.36 ± 0.05	3.7 ± 0.1 bc	3.3 ± 0.2 c	0.03 ± 0.00 c	0.09 ± 0.01 c	4.4 ± 0.2 a	3.0 ± 0.2 b	0.23 ± 0.03 ab	0.37 ± 0.04 ab
B (10^{-8}M)	6.0 ± 0.4 bc	4.0 ± 0.3 a	0.28 ± 0.03 bc	0.41 ± 0.05	3.2 ± 0.2 c	3.8 ± 0.2 bc	0.05 ± 0.00 c	0.12 ± 0.01 c	4.4 ± 0.1 a	3.0 ± 0.1 b	0.23 ± 0.01 ab	0.30 ± 0.03
B (10^{-9}M)	5.0 ± 0.2 cd	3.9 ± 0.3 a	0.38 ± 0.04 ab	0.55 ± 0.06	4.5 ± 0.2 ab	4.4 ± 0.2 b	0.10 ± 0.02 b	0.21 ± 0.03 b	4.2 ± 0.2 a	2.6 ± 0.1 b	0.20 ± 0.04	0.24 ± 0.04 bc
-K (Control)	5.1 ± 0.1 c	3.1 ± 0.2	0.33 ± 0.02 bc	0.52 ± 0.03	3.4 ± 0.2 c	2.9 ± 0.3 b	0.13 ± 0.05	0.37 ± 0.14	6.0 ± 0.1 a	3.8 ± 0.1 b	0.48 ± 0.03 b	0.73 ± 0.03 ab
SW (1:250)	5.6 ± 0.2 bc	2.4 ± 0.2 d	0.29 ± 0.03 c	0.31 ± 0.04 b	5.3 ± 0.4 b	5.8 ± 0.4 a	0.12 ± 0.02 c	0.33 ± 0.04	5.2 ± 0.2 b	3.8 ± 0.1 b	0.64 ± 0.11 ab	0.81 ±0.13 ab
SW (1:500)	5.8 ± 0.4	3.3 ± 0.2	0.28 ± 0.03 c	0.44 ± 0.06	5.0 ± 0.5 b	5.1 ± 0.3 a	0.12 ± 0.01 bc	0.31 ± 0.03	4.9 ± 0.2 bc	4.7 ± 0.2 a	0.85 ± 0.16 a	1.01 ± 0.15 a
SW	6.7 ± 0.6 ab	3.7 ± 0.3 b	0.35 ± 0.06 bc	0.60 ± 0.10 a	3.1 ± 0.1 c	3.4 ± 0.2 b	0.13 ± 0.04	0.22 ± 0.04 b	5.1 ± 0.2 b	4.9 ± 0.3 a	0.84 ± 0.10 a	1.04 ± 0.10 a
B (10^{-7}M)	5.2 ± 0.2 c	3.7 ± 0.2 b	0.44 ± 0.04 ab	0.55 ± 0.06 a	6.8 ± 0.8 a	5.5 ± 0.6 a	0.21 ± 0.03 a	0.48 ± 0.05 a	5.1 ± 0.3 b	4.6 ± 0.2 a	0.50 ± 0.08 b	0.57 ± 0.08 b
B (10^{-8}M)	6.9 ± 0.4 a	4.4 ± 0.3 a	0.51 ± 0.05 a	0.60 ± 0.07 a	5.3 ± 0.2 b	5.3 ± 0.1 a	0.21 ± 0.01	0.44 ± 0.03 a	4.4 ± 0.2 cd	3.4 ± 0.2 b	0.68 ± 0.10 ab	0.72 ± 0.14 ab
B (10^{-9}M)	6.6 ± 0.5 ab	3.0 ± 0.0	0.43 ± 0.05 ab	0.50 ± 0.07	5.1 ± 0.2 b	5.2 ± 0.3 a	0.21 ± 0.03 ab	0.48 ± 0.05 a	4.0 ± 0.1 d	3.8 ± 0.2 b	0.83 ± 0.05 a	0.89 ± 0.068

Mean values (±SE) of each species and nutrient condition in column with different letters are significantly different by Tukey's test ($P < 0.05$).
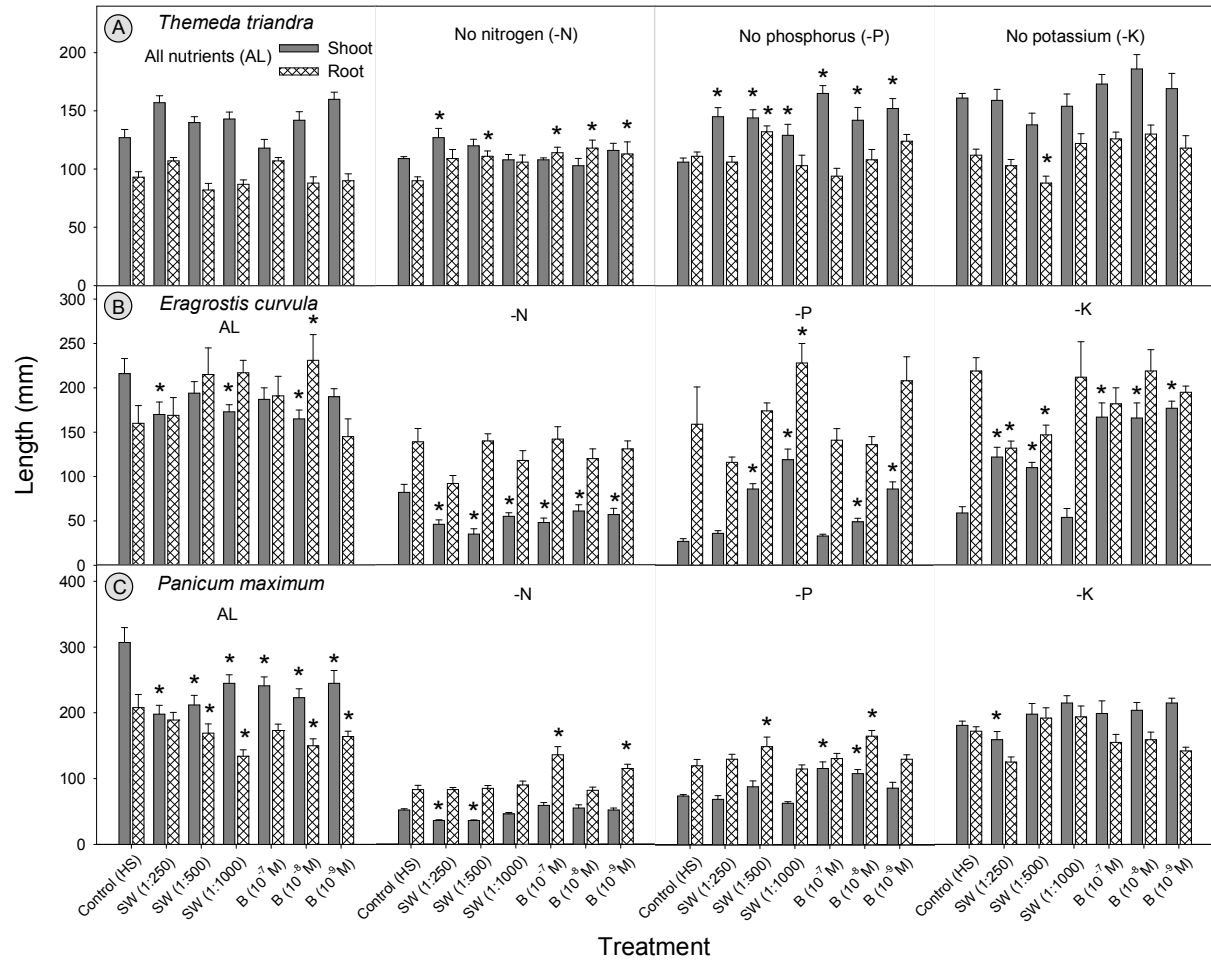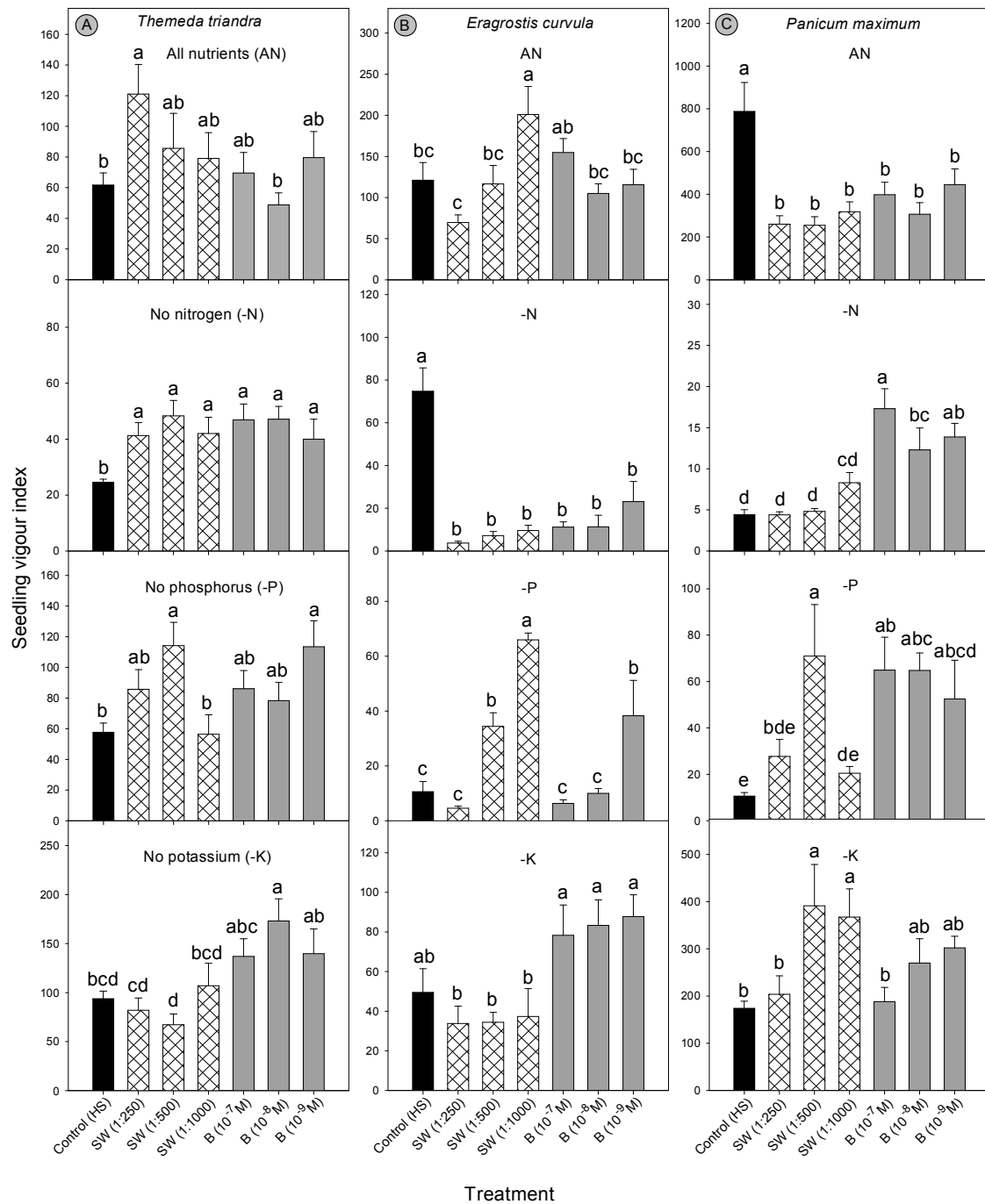
Figure 6.2: Effect of different concentrations of smoke-water (SW) and butenolide (B) on seedling vigour index of grass species in the absence of N, P or K under greenhouse conditions. Bar (±SE) with different letters is significantly different by Tukey's test ($P < 0.05$).

6.4 DISCUSSION AND CONCLUSIONS

Fire is one of the main driving forces in shaping the structure and composition of South African semi-arid grasslands **(HALL, 1984)**. Recent changes in the grasslands may be attributed to either under-use or over-use practices of fire **(TAINTON and MENTIS, 1984)**. These studies therefore continue to receive more attention on the species composition, cover and yield of grassland **(TAINTON, 1984)**. A significant effect of fire in semi-arid grassland is reduction in plant cover, which may change soil properties **(DEBANO et al., 1970; DEBANO et al., 1976; EMMERICH and COX, 1992; SNYMAN, 2004)**. It is suggested that there are high losses of C, N and S from the biomass during burning **(MATERECHERA et al., 1998)**. Nutrient cycling, soil fertility and grassland dynamics after burning is however, not well understood **(TAINTON and MENTIS, 1984; MATERECHERA et al., 1998)**.

Seedling establishment is a critical process determining the successful recovery, growth and long-term persistence of grass populations. Under conditions of repeated disturbance by fire or grazing, due to the inter-relationship among nutrients, soil moisture and fire-related cues, seedlings of some grass species may respond quickly, while others species take longer or even may not respond **(O'CONNOR, 1985; O'CONNOR, 1994)**. Therefore, it is possible that the interaction of smoke with major nutrients (NPK) can play an important role in fire-climax species. To test this hypothesis, this study was undertaken on three grass species as described earlier. The seedling growth parameters of *Themeda triandra* studied was generally better in the absence of K. This finding suggests that this grass may not perform well in soils with high levels of K. When these seedlings were grown without N or P and treated with smoke-water and butenolide solutions they exhibited longer shoots and roots than control seedlings. In the absence of K, smoke treatments improved shoot or root lengths but not significantly. Under these conditions the other growth parameters such as number of leaves and roots, seedling weight, root volume and vigour index were significantly enhanced. Interestingly, all smoke treatments and concentrations without N yielded the best seedling vigour index (**Figure 6. 2A**).

Table 6.3: General ANOVA with main effects and their interactions for seedling vigour index of grass species under greenhouse conditions.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	*F*-value	*P*-value
Species (S)	2	3607633	1803816	159.72	< 0.001
Treatment (T)	6	206734	34456	3.05	0.006
Nutrient (N)	3	4983543	1661181	147.09	< 0.001
S * T	12	717321	59777	5.29	< 0.001
S * N	6	6197397	1032899	91.46	< 0.001
T * N	18	1325730	73652	6.52	< 0.001
S * T * N	36	2754750	76521	6.78	< 0.001
Residual	1176	13281057	11293		
Total	1259	33074164			

Asterisk symbol denotes interactions.

These findings clearly indicate that seedlings of *Themeda triandra* can perform well after burns under nutrient-deficient soil conditions **(GHEBREHIWOT et al., 2006; FYNN and NAIKEN, 2009)**. This could be an advantage for many fire-climax grasses to compete with other species such as *Eragrostis curvula* which requires a high nutrient level. **EVERSON and TAINTON (1984)** reported that irrespective of soil nutrients, *Themeda triandra* requires regular burning to sustain its dominance in grasslands. The productivity of *Themeda triandra* declined when there was a long interval between fires **(MORGAN and LUNT, 1999)**.

Eragrostis curvula seedlings responded better when supplied with all nutrients. The elimination of N, P or K negatively affected the growth indicating the necessity of soil nutrients. The shoot growth and number of roots were significantly suppressed when they were grown with all nutrients and treated with smoke solutions. When N was removed, all the smoke treatments significantly inhibited shoot growth, seedling weight, root volume and seedling vigour. These results clearly indicate that deficiency of N in post-burn soils will not promote growth of *E. curvula* seedlings. Studies have shown that addition of N to the grassland resulted in the replacement of *Themeda triandra* by *Eragrostis curvula* **(FYNN and O'CONNOR, 2005)**, which indicates that N is important for the growth of *Eragrostis curvula*. Lower

concentrations of smoke-water (1:1000) and butenolide (10^{-9}M) stimulated most growth parameters in the absence of P. Whereas with no supply of K, some treatment solutions significantly improved shoot growth, number of leaves and roots but overall seedling vigour was not affected. The presence of both P and K (-N treatment) in the soils after fire therefore can be responsible for suppressing the growth of *Eragrostis curvula* seedlings (**Figure 6. 2B**). In this study without K, the butenolide treatment significantly improved seedling vigour of *Eragrostis curvula* over smoke-water. This can be attributed to pure butenolide which can effectively promote the growth of seedlings in the absence of K. On the other hand, smoke-water contains several other compounds and a newly discovered inhibitor **(LIGHT et al., 2010)**, which may interact with micro- or macronutrients in the soils, generating suppressive effects. However, this needs further investigation.

Panicum maximum seedlings grew better in presence of all nutrients. This result suggests that *Panicum maximum* can compete with other species in fertile soils. The performance of *Panicum maximum* was superior to *Themeda triandra* and *Eragrostis curvula* in high-fertility treatments **(FYNN, 2004; GHEBREHIWOT et al., 2006)**. In contrast, when these seedlings were exposed to smoke-water and butenolide solutions they showed inhibitory effects on most of the seedling growth parameters examined. However, the response of *Panicum maximum* seedlings to smoke treatments was better in the absence of N, P or K. These findings suggest that although *Panicum maximum* requires nutrients for growth under normal conditions, it can also show better performance after fires with less macronutrients in soils.

Overall results of this study confirm that *Themeda triandra* can be more competitive when there is frequent burning and less availability of soil nutrients **(FYNN, 2004; FYNN et al., 2005)**. On the other hand, deficiency of N after fires can suppress the growth of *Eragrostis curvula* whereas *Panicum maximum* can still show some competence ability after fires with low soil nutrients. The interaction between species, treatment and nutrient for seedling vigour index was highly significant ($P < 0.001$) (**Table 6. 3**). Clearly, though nutrients had an overriding effect of seedling growth of the species tested, the interaction of the smoke solutions with macronutrients also appears to be important. It is ecological significant that the application of smoke-water, smoke-isolated karrikinolides and inhibitors present in

smoke in relation to soil fertility can be a useful tool in studying the post-fire dynamics of grasses or vegetation under field conditions. However, the effects of temperature, moisture, light, cutting, mowing and grazing cannot be completely ruled out when studying the composition of post-fire grasslands.

CHAPTER SEVEN

Smoke and heat: influence on seedling emergence from mesic grassland soil seed bank in South Africa

"I conceive that the land belongs to a vast family of which many are dead, few are living, and countless numbers are still unborn"

~Author Unknown

7.1 INTRODUCTION

Formerly *Themeda triandra* Forssk. dominated grasslands, probably the most economically important grasslands in South Africa covered the greater part of the country **(BAYER, 1955)**. By contrast, recent reports show that large areas of the originally *Themeda* dominated grasslands are now invaded by less economically desirable species and few of the formerly *Themeda* grasslands remain in pristine condition **(ADAMS, 1996; GRANGER and BREDENKAMP 1996; WISEMAN et al., 2002)**. Decline in populations of *Themeda triandra* in response to the time since the last fire has also been reported in many temperate grasslands elsewhere **(EVERSON et al., 1985; MCDOUGALL, 1989; EVERSON, 1994; MORGAN and LUNT, 1999)**. This has enormous economic and ecological implications and as a consequence management strategies aimed at maximising effective germination, emergence and vigour of the indigenous grass species for the purpose of restoring degraded[2] grasslands are of paramount importance **(ACOCKS, 1988; EVERSON, 1994; ADAMS, 1996; WOLFSON, 1999; WISEMAN et al., 2002)**. Restoration of

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[2] For the purpose of this study, grassland degradation is defined as the compositional change, which further decreases the ability of the grassland to produce palatable species to maintain livestock production **(TAINTON, 1999)**.

such degraded grasslands involves the re-introduction of locally extinct desirable species **(MCDOUGALL, 1989; ADAMS, 1996; WISEMAN et al., 2002; SNYMAN, 2003)**, and/or proliferation of desirable species that have declined in abundance. In whichever way, recruitment through germination followed by vigorous establishment of the indigenous grass species for genetic material in grassland restoration programmes is crucial **(SINDEL and GROVES, 1990; EVERSON, 1994; ADAMS, 1996; O'CONNOR, 1997; SNYMAN, 2003)**.

Development of vegetation from seed requires a seed bank, germination and emergence followed by seedling survival. Seed banks are thought to be biodiversity reservoirs reflecting past and present ecological conditions, offering potential for regeneration of degraded or invaded ecosystems **(THOMPSON and GRIME, 1983)**. Presently, various fire-related agents either single or in combination have been shown to promote germination and subsequent emergence of soil-stored seeds from a range of species in several vegetation types worldwide **(DIXON et al., 1995; ENRIGHT et al., 1997; ROCHE et al., 1997; BLANK and YOUNG, 1998; READ et al., 2000; WILLS and READ, 2002; CROSTI et al., 2006)**. Furthermore, different techniques of smoke treatment of soil-stored seeds have been used for obtaining maximum germination efficiencies in successful propagation of rare and threatened species **(BROWN, 1993a; DIXON et al., 1995; GRANT and KOCH, 1997; READ et al., 2000; VAN STADEN et al., 2000)**. Germination stimulation in response to fire could be due to the effects of heat **(JEFFERY et al., 1988; VAN DE VENTER and ESTERHUIZEN, 1988)**, smoke and/or some combinations of the two **(BLANK and YOUNG, 1998; READ et al., 2000; CLARKE and FRENCH, 2005; THOMAS et al., 2007)**. It is generally suggested that heat and smoke are complementary triggers of soil-stored seeds **(GRANT and KOCH, 1997)**, where smoke stimulated seeds tend to be killed by the lethal heat from fires **(ZACHARIAS et al., 1988)** and that heat-stimulated seeds are generally unaffected by smoke. This notion is generally questionable and the relative significance of heat or smoke as germination cues is still undetermined **(BASKIN and BASKIN, 1998; KEELY and FOTHERINGHAM, 2000; WILLS and READ, 2002; FIGUEROA et al., 2009; MOREIRA et al., 2010)**. For example, recent reports on germination behaviour of species from the Mediterranean basin suggest that smoke has a limited role as a post-fire germination cue **(FIGUEROA et al., 2009; MOREIRA et al., 2010)**. By contrast, many other

studies (e.g. **GONZALEZ-RABANAL and CASAL, 1995; HERRÁNZ et al., 1998; HERRÁNZ et al., 1999; REYES and TRABAUD, 2009)** demonstrate that heat indeed enhances germination in many of the species tested. Hence, disentangling the role of heat and smoke as germination cues **(FIGUEROA et al., 2009; REYES and TRABAUD, 2009; MOREIRA et al., 2010)** is of great significance. In view of this, relatively little *in situ* research work has been done on understanding the influence of fire (heat) and smoke on soil seed bank germination and emergence of the indigenous vegetation in South Africa **(ZACHARIAS et al., 1988; EVERSON, 1994)**. Furthermore, no studies have documented how the size and composition of the soil seed bank varies with grassland management practices such as burning, grazing and mowing. Consequently, our knowledge of the reaction of the soil seed bank to heat-shock and smoke is still limited.

Previous laboratory-based germination studies (see **Chapter 3**) clearly showed that certain perennial native grass species that dominate the mesic grasslands of South Africa such as *Themeda triandra* are responsive to smoke treatments. Subsequent laboratory-based studies on fire-stimulated germination of Grand Rapids lettuce seeds (see **Chapter 5**) suggested that burning influences the germination activity of the soil. In this study, in order to gain greater insight into the germination effect of fire on soil seed bank and disentangle the role of heat and/or smoke, soil seed bank samples taken from classic mesic grassland site in South Africa were treated with smoke-water solution, heat and heat + smoke-water combined. The aim of this study was to assess the possibility of using smoke techniques for large-scale restoration of native grasslands, which are generally seen to be decreasing in extent. Differences in the number of viable seeds (germinants or emergents) among species would suggest varying abilities of grass species to recover after disturbance by fire. The questions posed were:

1. Do fire-associated cues of high temperature (heat) and smoke affect the germination/emergence level? If yes, do they increase or decrease the germination level?
2. Do fire-associated cues of high temperature (heat) and smoke affect time to germination?

3. Do fire-loving grass species e.g. *Themeda triandra* have relatively a greater number of germinants from heat, smoke or smoke + heat treated soil seed bank samples?

4. Do heat and smoke-water treatment affect the initial seedling growth?

7.2 MATERIALS AND METHODS

7.2.1 Study site

The site used for collecting soil core samples is located at Ukulinga, the research farm of the University of KwaZulu-Natal, Pietermaritzburg (29° 24' E, 30° 24' S). The mean annual precipitation in the locality is 790 mm and about 79% of this rain falls during summer (October–April). Mean monthly maximum and minimum temperatures range from 26.4 °C in February to 8.8 °C in July, respectively. The plots are situated on top of a small plateau ranging in altitude from 847 m to 838 m. The grassland site was left unburnt and ungrazed for at least the past five years. Soils at the site were classified as Westleigh forms **(SOIL CLASSIFICATION WORKING GROUP, 1991)**. The vegetation of the area is classified as southern Tall Grassland **(ACOCKS, 1988)**. The sward varied between 40 to 60 ± 6.33 SE cm in height (average of 20 random samples) and is dominated by herbaceous species mainly *Themeda triandra* Forssk, *Heteropogon contortus* Beauv. ex Roemer & J. A. Schultes and *Tristachya leucothrix* Trin. ex Nees. Other species of common occurrence in the site include *Hyparrhenia hirta* (L.) Staph, *Aristida junciformis* Trin. & Rupr and *Cymbopogon validus* Stapf ex Burtt Davy. In the absence of burning (fire), indigenous trees such as *Acacia karroo* Hayne., *Acacia nilotica* Wiltd. and *Celtis africana* Burm., exotic trees such as *Acacia mearnsii* DeWild. and *Melia azedarach* L. are common species.

7.2.2 Soil collection and the treatments applied

A soil seed bank is defined as seeds at or beneath the soil surface that are capable of germination **(SAGAR and MORTIMER, 1976)**. Soil seed bank sampling was undertaken on the 10th of March 2010, after most key grass species that dominate

the grassland had finished fruiting and released their seeds. Three linear transects (100 m long each) were established in parallel direction and soil seed bank samples were taken at 5 m intervals. The transects were positioned so that they included a range of within site variation. Soil core samples were randomly taken from a 20 x 20 cm area and 0-5 cm depth cores along the previously established transects using a sharp spade (with 20 cm wide blade). Attention was given to the superficial stratum of the soil seed bank (0-5 cm) as this horizon holds most of the viable seeds **(EVERSON, 1994)**. Surface litter was included as this layer is potentially an important seed source **(WILLS and READ, 2002)**.

Immediately after collection, all soil samples were transported to the laboratory, air-dried and stored under laboratory conditions for one week until used. Following this, all the soil samples were mixed well and sieved (3.36 mm mesh) to remove gravel and plant material that might reproduce vegetatively. The soil samples were divided into two equal groups (A and B) by weight. Soil sample group A was dry heated in a drying oven at 100 °C for 15 min. From previous studies this temperature range was suggested to provide maximum germination **(ENRIGHT et al., 1997; WILLS and READ, 2002)** in many species without killing the seeds. Soil sample group A (heated soil) was further sub-divided into A1 and A2, where, A1, soil subsamples were potted in nursery trays and treated with smoke-water (1:500 v/v) (Preparation of the smoke-water solution used in the study is described in **section 4.2.2**), while A2 trays remained only preheated. Soil samples in group B (unheated soil) were also further sub-divided in to B1 and B2, where the B1 group soil subsamples were potted in nursery trays and further treated with smoke-water (1:500 v/v), while the B2 group formed a control (no heat or no smoke). This procedure produced four (4) treatments (**Table 7.1**) with each treatment being duplicated eight (8) times giving a total of 32 potted soil core samples. Before potting the soil samples coarse litter was manually removed from each sample. Each soil core sample (n = 8) was then spread into nursery trays lined with perforated cloth to allow drainage of excess water through holes at the bottom of the trays.

Table 7.1: The heat and smoke-water treatments applied on soil seed bank samples collected from a classic mesic grassland site in South Africa.

Treatments	Abbreviated as	Procedures of treatment application
Control	C	Soil seed bank samples not heated and not smoke-water treated
Heat	H	Soil samples heated at 100 °C for 15 min
Smoke-water	SW	Soil samples treated with smoke-water (1:500 v/v) once in a week
Smoke-water+Heat	SW+H	Soil samples heated at 100 °C for 15 min and treated with smoke-water (1:500 v/v) once in a week

7.2.3 Seedling emergence trial

Seedling emergence from the mesic grassland soil seed bank was quantified using the germination method from all soil samples exposed to four treatments (**Table 7.1**). The seedling emergence trial was commenced on 17 March, 2010 after soils had dried for one week. Dry storage methods had been shown to enhance germination of many species from the soil seed bank **(THOMPSON and GRIME, 1983)**. Each soil seed bank sample was then spread on nursery trays (35 cm x 30 cm x 5 cm deep) which were placed on 1 meter high greenhouse benches. This provided a soil depth of 2 cm which was expected to provide sufficient surface area for germination. The study was carried out in a greenhouse which was maintained at an air temperature of 25 °C, relative humidity between 25-50 % and a midday photosynthetic photon flux density of 405 ± 7.5 µmol m^{-2} s^{-1} at 22 ± 2.5 °C. The trays were randomly placed in the greenhouse and were rotated (at weekly intervals) to minimize positional effects. Seeds in the soil samples were allowed to germinate under moist conditions manually watered to field capacity every day. Smoke-water treatment consisted of drenching the trays with smoke-water solution (1:500 v/v) once per week. This was done so as to abtain maximum germination of seeds from the soil seed bank. Seedling emergence was monitored once per week (for 90 days) and newly germinated seeds in a tray were recorded, and these seedlings were identified to the

species level (where possible) or to the nearest possible genus. After the 90 days of experimentation, there were almost no new germinating seeds in all soil samples, hence the experiment was terminated and the total number of germinated seeds for each species per tray was determined for each treatment. In situations where identification of young seedlings at species level was difficult, seedlings were transplanted into separate terracotta pots until flowering then identified **(**Nomenclature follows **ARNOLD and DE WET, 1993)**. It was particularly difficult identifying *Berkheya* (*Asteraceae*) and *Sporobolus* (*Poaceae*) to species level, hence hereafter they are named as *Berkheya sp.* and *Sporobolus sp.*

7.2.4 Plant measurements and observations

During the course of the 90 days, seedling emergence was determined by weekly counting the number of newly emerged seedlings in trays with 8 replications. Seedling emergence data were analyzed using the methods described by **BILBRO and WANJURA (1982)**. According to this method, speed of emergence (SOE), Mean emergence date (MED), emergence rate index (ERI) were calculated directly from the emergence counts as follows:

$$\text{SOE} = \frac{N1 + N2 + ... + Nn}{t1 + t2 + ... + tn} \quad\text{...[1]}$$

$$\text{MED} = \frac{N1t1 + N2t2 + ... + Nntn}{t1 + t2 + ... + tn} \quad\text{...[2]}$$

$$\text{ERI} = \frac{N1 + N2 + ... + Nn}{\text{MED}} \quad\text{...[3]}$$

Where $N_1, N_2, ..., N_n$ are the number of newly emerged seedlings in time $t_1, t_2, ..., t_n$ since the start of seedling emergence trial, respectively. In addition to these variables, to assess the effect of the fire-related treatments on seedling growth, total biomass production (shoot + root) of all the emerged species (i.e. grass, forbs and tree species per tray) was harvested, placed in brown paper bags and, oven dried at 80 °C for 72 hours to obtain dry mass. Root biomass from each tray was carefully

washed on top of a mesh sieve to avoid the loss of fine roots and acquire maximum precision **(CAHILL, 2002)**. Hence, variation in total dry biomass weights were used to index species response to the treatments applied (**Table 7.1**). All emerged seedlings survived, and no single seedling death was recorded during the course of the study period.

7.2.5 Statistical analysis

The data obtained from this study was subjected to one-way analysis of variance (ANOVA) to examine the effect of treatments on seedling emergence. The treatment means were separated using Duncan's multiple range test at 5% level of significance ($P < 0.05$). GenStat version 11.1 statistical package was used to analyze the data. Similarly, the data on seedling growth parameters were subjected to one-way analysis of variance (ANOVA) and means were separated using Duncan's multiple range test at 5% level of significance ($P < 0.05$). General ANOVA was conducted to determine the main effects and their interactions **(GENSTAT, 2008)**.

7.3 RESULTS

7.3.1 Seedling emergence

In total 790 individuals from 11 different plant families representing 37 graminoid (*Poaceae*) and non-graminoid species of flowering plants were identified from the soil seed bank samples treated with 4 different fire-related cues (**Table 7.3**). Out of the 37 species identified, 17 species were grasses, 19 species were forbs and 1 was a tree species. Seedlings of perennial species were better represented rather than annuals, holding 75.2% and 24.8% of the germinable soil seed bank respectively. The most abundant plant families that emerged were *Poaceae* (17 species) followed by *Asteraceae* (11 species). Of the total plant species identified, grasses (*Poaceae*) contributed 17.85% of the germinable seed bank, while non-grass (forbs, sedges and trees) species contributed > 80%. The most abundant grass species emerged was *Themeda triandra* (*Poaceae*) and the most common forb species was *Centella asiatica* (*Mackinlayaceae*) holding 5.95% and 14.3% of the germinable soil seed bank respectively (**Table 7.3**). Smoke-water (SW) and/or smoke-water + heat

(H+SW) treatment of soil seed bank samples resulted in a statistically significant ($P <$ 0.001) mean number of seedlings emerging compared to the control (**Table 7.2 and Figure 7.1**).

Table 7.2: One-way analysis of variance for seedling emergence in response to the four treatments applied.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	788.62	262.88	9.20	< 0.001
Residual	28	800.25	28.58		
Total	31	1588.88			
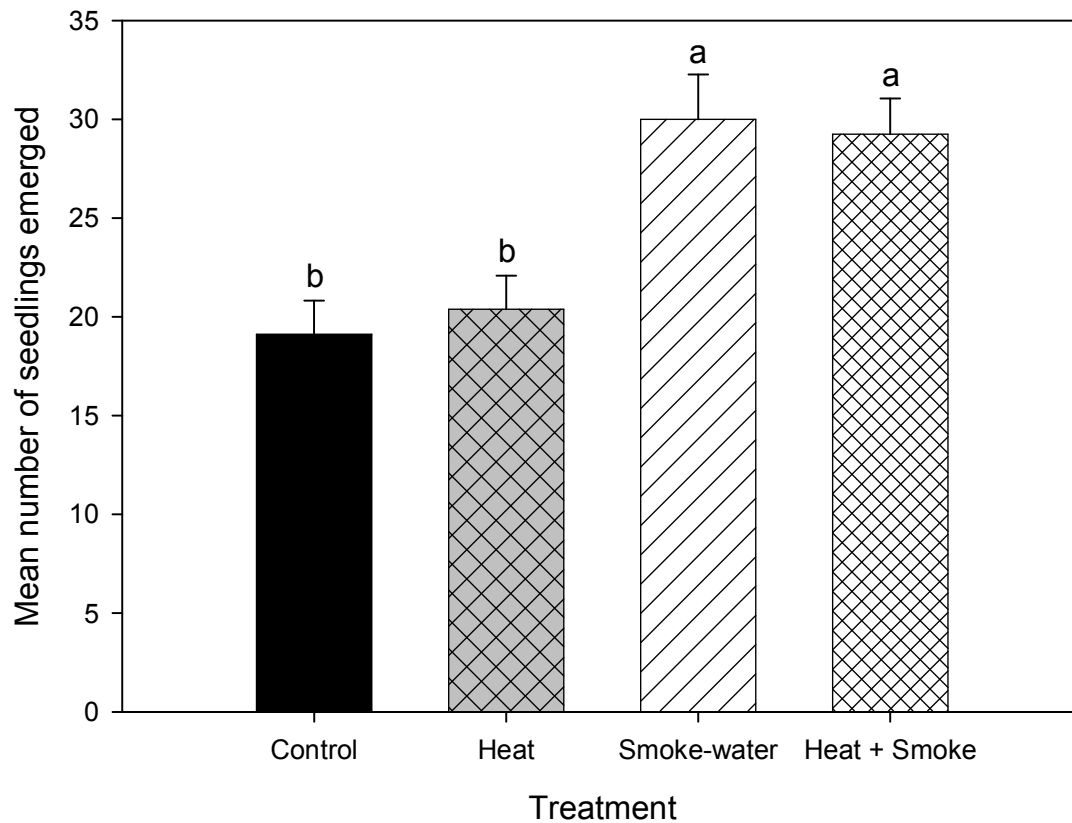
Figure 7.1: Mean seedling emergence (±SE) of the germinable soil seed bank samples (0-5 cm) in response to four different fire-related treatments. Shared alphabet letters indicate no significant difference between treatments according to Duncan's multiple range test at 5% level of significance.
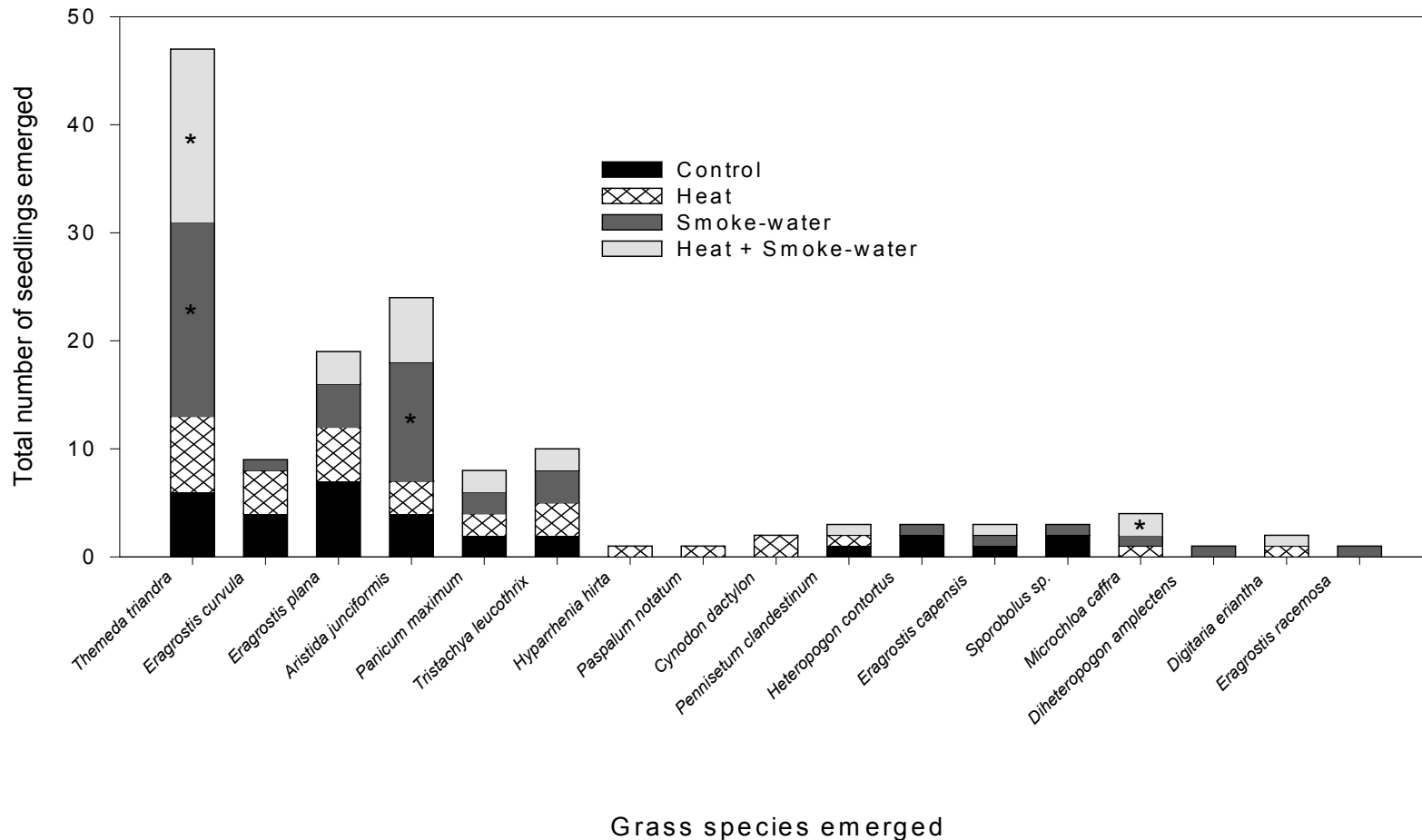
Figure 7.2: Total number of grass seedlings (*Poaceae*) emerged from the germinable soil seed bank of a mesic grassland site in South Africa in response to four different fire-related treatments. Asterisks denote a significant ($P < 0.05$) difference from other treatments.
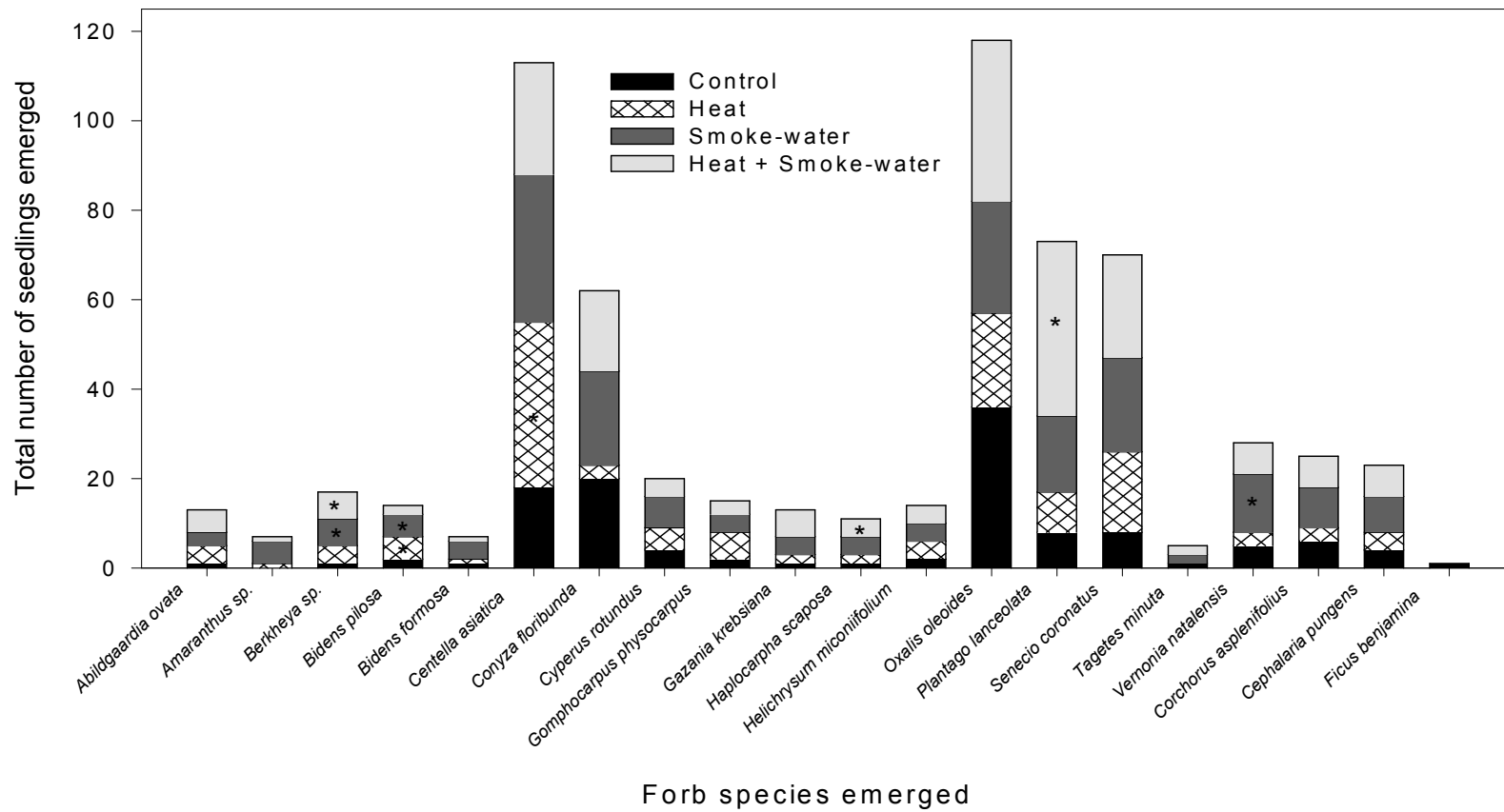
Figure 7.3: Total number of forb seedlings emerged from the germinable soil seed bank of a mesic grassland site in South Africa in response to four different fire-related treatments. Asterisks denote a significant ($P < 0.05$) difference from other treatments.

These increases were manifest by 1.5-fold greater emergence of grass, and ≥ 1.5-fold increases in forbs species emergence compared to the control. However, heat treatment by itself alone had no statistically significant difference from the control. Compared to the control (19.1± 1.7), the mean number of seedling emergence was significantly increased by smoke-water (30.0 ± 2.27) and the combined effect of smoke-water + heat (29.3 ± 1.83) (**Table 7.4**). Comparison of the germinable soil seed bank of heated and unheated (control) soil samples showed that heat alone had no significant effect on mean number of seedling emergence, though certain forb species have shown marked increases in emergence.

Three grass species and six forb species showed significant differences in their emergence in response to the fire-related treatments applied. Five species of grasses (*Aristida junciformis, Eragrostis plana, Panicum maximum, Themeda triandra* and *Tristachya leucothrix*) were common to all treatment combinations. However, seedling emergence of six other species was restricted to a specific treatment (3 in heat, 2 in smoke-water and 1 in control). In the control treatment, no emergence was recorded for seven grass species i.e., *Cynodon dactylon*, *Digitaria eriantha, Diheteropogon amplectens, Eragrostis racemosa, Hyparrhenia hirta, Microchloa caffra* and *Paspalum notatum*. However, these seven species have shown some emergence from heat or smoke-water treated soil samples, though not statistically significant. Importantly, the native fire-climax species *Themeda triandra* showed 3-fold increases in emergence in response to smoke-water or smoke-water + heat combined treatments. Of the 17 species of grasses indentified, *Themeda triandra* represented 33.35 % of the total emergence from the germinable soil seed bank. *Aristida junciformis* showed increased emergence in response to smoke-water treatment while *Microchloa caffra* responded positively to the combined effect of smoke-water + heat. Considering all the treatments applied, the species that emerged with the highest abundance were annual and perennial forbs such as *Centella asiatica*, *Oxalis oreoides*, *Conyza floribunda, Plantago lanceolata* and *Senecio coronatus* (**Figure 7.2**). Dry heat treatment of the soil samples significantly increased seedling emergence in *Centella asiatica* and *Bidens pilosa* while smoke-water treatment resulted in a significantly higher emergence in *Berkheya sp.*, *Bidens pilosa* and *Vernonia natalensis*.

Table 7.3: Total number of seedlings emerged per treatment from the soil seed bank collected from a mesic grassland in South Africa after heat and smoke-water treatment (heat, smoke-water and heat + smoke-water).

Species	Family	Lifeform	Cont	Heat	SW	H+SW	Total	Frequency %
				Treatment				
Abildgaardia ovata	Cyperaceae	Perennial	1	4	3	5	13	1.65
Amaranthus sp.	Amaranthaceae	Perennial	0	1	5	1	7	0.89
Aristida junciformis	Poaceae	Perennial	4	3	11	6	24	**3.04**
Berkheya sp.	Asteraceae	Perennial	1	4	6	6	17	2.15
Bidens pilosa	Asteraceae	Annual	2	5	5	2	14	1.77
Bidens formosa	Asteraceae	Annual	1	1	4	1	7	0.89
Centella asiatica	Mackinlayaceae	Annual	18	37	33	25	113	**14.30**
Cephalaria pungens	Dipsacaceae	Perennial	4	4	8	7	23	2.91
Conyza floribunda	Asteraceae	Annual	20	3	21	18	62	**7.85**
Corchorus asplenifolius	Tiliaceae	Perennial	6	3	9	7	25	**3.16**
Cynodon dactylon	Poaceae	Perennial	0	2	0	0	2	0.25
Cyperus rotundus	Cyperaceae	Perennial	4	5	7	4	20	2.53
Digitaria eriantha	Poaceae	Perennial	0	1	0	1	2	0.25
Diheteropogon amplectens	Poaceae	Perennial	0	0	1	0	1	0.13
Eragrostis curvula	Poaceae	Perennial	4	4	1	0	9	1.14
Eragrostis plana	Poaceae	Perennial	7	5	4	3	19	2.41
Eragrostis racemosa	Poaceae	Perennial	0	0	1	0	1	0.13
Eragrostis capensis	Poaceae	Perennial	1	0	1	1	3	0.38
Gazania krebsiana	Asteraceae	Perennial	1	2	4	6	13	1.65
Gomphocarpus physocarpus	Apocynaceae	Perennial	2	6	4	3	15	1.90
Haplocarpha scaposa	Asteraceae	Perennial	1	2	4	4	11	1.39
Helichrysum miconiifolium	Asteraceae	Perennial	2	4	4	4	14	1.77
Heteropogon contortus	Poaceae	Perennial	2	0	1	0	3	0.38
Hyparrhenia hirta	Poaceae	Perennial	0	1	0	0	1	0.13
Microchloa caffra	Poaceae	Perennial	0	1	1	2	4	0.51
Oxalis oleoides	Oxalidaceae	Perennial	36	21	25	36	118	**14.94**
Panicum maximum	Poaceae	Perennial	2	2	2	2	8	1.01
Paspalum notatum	Poaceae	Perennial	0	1	0	0	1	0.13
Pennisetum clandestinum	Poaceae	Perennial	1	1	0	1	3	0.38
Plantago lanceolata	Plantaginaceae	Perennial	8	9	17	39	73	**9.24**
Senecio coronatus	Asteraceae	Perennial	8	18	21	23	70	**8.86**
Sporobolus sp.	Poaceae	Perennial	2	0	1	0	3	0.38
Tagetes minuta	Asteraceae	Perennial	1	0	2	2	5	0.63
Themeda triandra	Poaceae	Perennial	6	7	18	16	47	**5.95**
Tristachya leucothrix	Poaceae	Perennial	2	3	3	2	10	1.27
Vernonia natalensis	Asteraceae	Perennial	5	3	13	7	28	**3.54**
Ficus benjamina Tree sp.	Moraceae	Perennial	1	0	0	0	1	0.13

Table 7.4: Seedling emergence indicators from mesic grassland soil seed bank samples under four different fire-related treatments in a greenhouse trial. The indicators considered were: MNSE: mean number of seedling emerged; SOE: speed of emergence (plants per week); MED: mean emergence date (week); ERI: emergence rate index (per week). Growth indicators FW and DW represent fresh and dry weights respectively.

Treatment	Seedling emergence indicators				Growth indicators	
	MNSE (no)	SOE (Plants per week)	MED (weeks)	ERI (Per week)	FW (g)	DW (g)
C	19.1 ± 1.7 b	0.108 ± 0.01 a	2.52 ± 0.19 ab	7.70 ± 0.59 c	6.39 ± 0.27 c	0.96 ± 0.22 b
H	20.4 ± 1.6 b	1.104 ± 0.01 a	2.32 ± 0.15 b	8.75 ± 0.34 bc	10.79 ± 0.95 b	1.23 ± 0.23 b
SW	30.0 ± 2.27 a	0.122 ± 0.01 a	3.18 ± 0.26 a	9.64 ± 0.64 ab	14.55 ± 0.82 a	4.41 ± 0.88 a
SW+H	29.3 ± 1.83 a	0.112 ± 0.01 a	2.74 ± 0.24 ab	10.88 ± 0.54 a	18.20 ± 0.83 a	4.89 ± 0.42 a
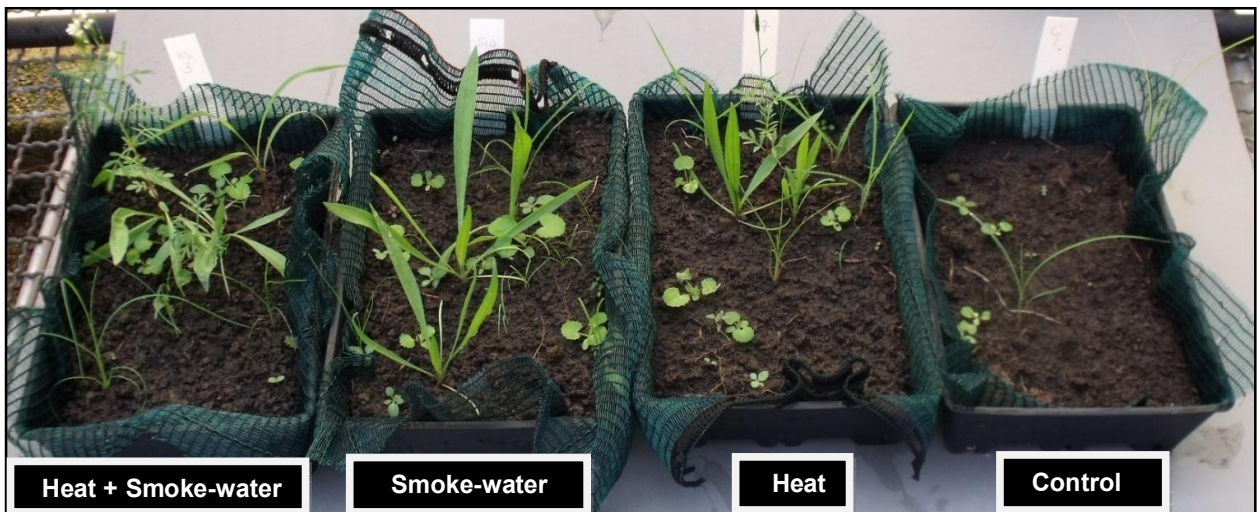
Figure 7.4: Emergence and establishment of seedlings over the 90 day trial in response to the four, fire-related treatments applied on the mesic grassland soil seed bank samples.

Seedling emergence indicators (**Table 7.4**) showed that, smoke-water treatment by itself and/or in combination with heat (SW+H) significantly increased the rate of seedling emergence from the topsoil (0-5 cm) of the seed bank (**Table 7.4**). Smoke-water treatment resulted in significant increases in mean emergence date (MED, plants per week). Assessments of seedling emergence of the first week of the trial showed that 75% of the total (*n* = 68) seedlings emerged were either from SW or SW+H treated soil samples. Emergence rate index (ERI, per week) was significantly increased due to SW+H and SW treatment (**Table 7.4**). However, speed of emergence (SOE, plants per week) did not show significant differences in response to the treatments applied.

7.3.2 Seedling growth indicators

The growth indicators (**Table 7.4**) showed that soil seed bank samples treated with smoke-water (1:500 v/v) and those samples treated with smoke-water combined with short-lived dry heat produced significantly higher biomass as fresh weight (**FW**) and/or dry weights (**DW**) (**Table 7.4; Figure 7.4**) compared to the untreated control. In comparison to the control, smoke-water (SW) and SW+H treatment of the mesic grassland soil seed bank samples resulted in 4 to 5-fold greater dry biomass weight (**DW**) production respectively (**Table 7.4**).

7.4 DISCUSSION

Common to nearly all post-fire grasslands is the flush of vigorous seedling emergence from canopy and/or soil-stored seed banks. The number of seedlings emerging from a post-fire soil seed bank differs depending on many factors related to the number of seeds that an individual species produces (**O'CONNOR, 1991; EVERSON, 1994**), fire behaviour (as stimulatory or lethal) (**ZACHARIAS et al., 1988; SNYMAN, 2005; 2006**), soil depth and topography (**ASHTON et al., 1998**), season (**THOMPSON and GRIME, 1979; EVERSON, 1994; SNYMAN, 2005**), past history of land use (**BAKKER et al., 1997; SOLOMON et al., 2006**) and mainly due to the nature of the soil seed bank as persistent or transient (**THOMPSON and GRIME, 1983**). In persistent seed banks, the seeds remain viable in the soil for more than one season. By contrast, in transient seed banks seeds germinate within the

116

year of initial dispersal, depleting the contents of the soil seed bank. Hence, it is generally suggested that plant communities with a persistent seed bank are more likely to be successful in re-establishment programmes after disturbance **(GRIME, 1979; GRIME et al., 1988; BAZZAZ, 1996; VON BLANCKENHAGEN and POSCHLOD, 2005)**. High numbers of emerging grass and forb seedlings indicate that the soil seed bank can be a key factor in regeneration. By contrast, a low number of grass and forb emergents suggests that the soil seed bank is characteristic of a transient seed bank and is relatively less useful for restoration purposes **(MAJOR and PYOTT, 1966)**. Not many previous studies in South Africa have characterised the type of the soil seed bank and insufficient data are available on how the amount and composition of the soil seed bank varies with fire cues (heat and smoke) and across the landscape **(ZACHARIAS et al., 1988; EVERSON, 1994; O'CONNOR, 1997)**. Locally calibrated estimates of seed bank size and composition could assist resource managers in determining possible understory responses to management activities. In addition to this, knowledge of these seed bank characteristics could be used to predict which species could become conservation concerns or for deciding whether to seed native species based on their abundance in seed banks **(GLASS, 1989; ADAMS, 1996; BEKKER et al., 1997; BLANK and YOUNG, 1998; SNYMAN, 2005; VON BLANCKENHAGEN and POSCHLOD, 2005)**. This study was undertaken neither to determine the absolute seed density nor to characterise the type of the soil seed bank. The aim was simply to determine if seedling emergence of some common plant seeds of South African Tall Grassland is positively influenced by the products of vegetation fires and to isolate factors responsible for elevated germination/emergence, thereby results can be linked to short-term post-fire seedling germination and recruitment scenario in the field.

The results of the study show that there was a considerable variation in the mean number of seedlings that emerged from the germinable soil seed bank in response to the fire-related treatments applied. The hypothesis that plant-derived smoke by itself **(BROWN, 1993a; KEELEY and FOTHERINGHAM, 1997; VAN STADEN et al., 2000)**, and/or in combination with moderate heat-shock **(KEITH, 1997; MORRIS, 2000; READ et al., 2000; THOMAS et al., 2007)** influences seed germination and subsequent seedling emergence was strongly supported; ≥ 1.5-fold higher mean seedling emergence was achieved when mesic grassland topsoil (0-5

cm) samples were treated with smoke-water solution (1:500 v/v) alone or in combination with moderate heat (100 °C for 15 min) (**Table 7.4**). Noticeably, smoke-water alone and/or when combined with heat significantly promoted both the total and mean number of seedling emergence compared to the control. Mean seedling emergence date was also fastest in SW and/or SW+H treated soil (**Table 7.4**). However, heat treatment by itself did not significantly increase or decrease the total or mean number of seedling emergence, though certain forb species e.g. *Centella asiatica* and *Bidens pilosa* have showed marked increases in emergence in response to heat exposure. In contrast with many studies from the Mediterranean regions **(THANOS and GEORGHIOU, 1988; THANOS et al., 1992; HARRANZ et al., 1998; KEELEY, 1994; IZHAKI et al., 2000; LUNA et al., 2007; MOREIRA et al., 2010)** and Australia **(WILLS and READ, 2002)** where heat plays a major role in regulation of the post-fire emergence, smoke is perhaps the major fire-related factor in promoting seed germination and seedling emergence in the mesic Tall Grassland of South Africa. The interaction of heat-shock and smoke also appears to be equally important **(KEITH, 1997; MORRIS, 2000; READ et al., 2000; THOMAS et al., 2007)** in stimulating germination/emergence of the species forming the mesic grassland soil seed bank **(THOMAS et al., 2007; MOREIRA et al., 2010)**. These results suggest that increased germination and seedling emergence in the field, following moderate intensity fire is perhaps the result of smoke-induced stimulation of the soil seed bank and its interaction with heat **(BAXTER and VAN STADEN, 1994; BLANK and YOUNG 1998; READ et al., 2000)**. Smoke treatments of the soil seed bank have previously been shown to result in multi-fold increase in seedling emergence. For instance, in a native forest community in New South Wales Australia, **READ et al. (2000)** have shown that smoke treatment of the soil seed bank resulted in 4.3-fold increases in total seedling emergents. Similarly, aerosol smoke treatment of Jarrah forest soils around bauxite mines Western Australia resulted in 48-fold increases in total germinants **(ROCHE et al., 1997)**.

Smoke treatment alone or in combination with heat has also significantly increased the rate of seedling emergence (MED and ERI) from the germinable topsoil seed bank. Perennial grasses and forbs showed positive responses to these treatments, emerging significantly faster from SW and/or SW+H treated samples. This enhanced rate of germination and seedling emergence following smoke-

involved treatment has important implications for a speedy restoration and rehabilitation of grasslands **(BELLAIRS and BELL, 1993)**. Early establishment of many species during rehabilitation is important, as later recruitment of new species in later seasons is often poor due to competition with established plants **(BELLAIRS and BELL, 1993; EVERSON, 1994; VAN ZYL, 1998)**.

The species richness, as measured by the total number of species emerged (37) was relatively high compared to other studies. For instance, in south-eastern Australian sand heathland, **WILLS and READ (2002)** recorded only 20 species. From species-rich Chilean Matorral soil seed bank samples **FIGUEROA et al. (2009)** recorded only a total of 23 taxa. This suggests that the soil seed bank of the mesic Tall Grassland in South Africa contains a diverse range of plant species making it a valuable seed source for rehabilitation purposes **(ADAMS, 1996; SNYMAN, 2003)**. In this study, the highest mean seedling density obtained was from smoke-water-treated soil samples (30.0 ± 2.27 per tray), which is equivalent to 750 ± 56.57 seedlings m^{-2}. These results compare well with other South African soil seed bank studies of 172 seedlings m^{-2} in a semi-arid *Themeda* grassland **(SNYMAN, 2004)**, 200 seedlings m^{-2} from the mesic grassland in KwaZulu-Natal **(ADAMS, 1996)** and 350 seedlings m^{-2} from *Themeda* grassland in the semi-arid savanna **(O'CONNOR, 1997)**.

Themeda triandra and *Aristida junciformis* are fairly well represented in the germinable soil seed bank contributing 5.59 and 3.04% respectively. However, most perennial C_4 grasses which dominate the mesic Tall Grassland (> 80% relative frequency) **(FYNN et al., 2005)** are poorly represented in the buried seed bank (< 18%). The *Eragrostis* species i.e. *Eragrostis curvula* and *Eragrostis plana* were represented by >1% in the germinable soil seed bank. Seedling emergence for these species was not significantly affected by the treatments applied. Both species produce small seeds with out any enclosing structures. These seeds are most likely to be found close to the top soil which is only few millimetres deep. This makes the species more susceptible to effects of fire than other species capable of protecting themselves from fire or heat treatment **(CLARKE and FRENCH, 2005)**. Species such as *Heteropogon contortus* and *Hyparrhenia hirta* were represented by less than 3 individual emergents. Such a poor representation of most perennial and annual grass species in the soil seed bank suggests that the contribution of the seed bank in

the re-establishment of a particular grassland species might not be satisfactory **(EVERSON, 1994)**. One of the reasons for such a low representation of grasses could be due to low viability of the seeds **(EVERSON, 1994; O'CONNOR, 1997),** or other dormancy involving factors **(EVERSON, 1994; CZARNECKA, 2004)**. For instance, **EVERSON (1994)** reported that the viability of *Themeda triandra* seeds in the Montane grasslands of the Drakensberg significantly decreased within a few months of the trial. Other factors which contribute to the low representation of grass species in the soil seed bank could perhaps be the natural low seed rain and high levels of insect and rodent predation which removes 70% of the annual seed budget **(EVERSON, 1994; O'CONNOR, 1997)**. Another important factor, especially in thick and heavy litter stands, is that seeds may also become trapped in litter so that low numbers of seeds reach the soil seed bank **(EVERSON, 1994)**. Nevertheless, the results of this study show that SW treatment alone and/or combined with moderate heat can effectively (as much as 3-fold) increase the number of *Themeda triandra* emergents from the germinable soil seed bank. Hence, smoke techniques can be implemented to improve recovery of disturbed lands. Moreover, these findings clearly show that the application of smoke techniques together with some grassland seeding **(ADAMS, 1996)** can be useful practices in encouraging maximum seed germination and emergence of smoke-responsive species (e.g. *Themeda triandra*) from heavily overgrazed and disturbed commercial lands in South Africa **(BAXTER et al., 1994; ROCHE et al., 1994; DIXON et al., 1995; SNYMAN, 2006)**.

Another point of particular significance was that after 90 days of growth, SW and SW+H treated seedlings produced significantly greater plant mass than their controls. This elevated plant mass production in response to the fire-related treatments has been repeatedly shown for a range of agricultural and horticultural crops **(THOMAS and VAN STADEN, 1995; DREWES et al., 1995; KULKARNI et al., 2010)**. Though the mechanisms responsible for the elevated growth rate are not fully understood, there is a general suggestion that the active compound (s) in plant-derived smoke perhaps influence enzyme systems which control growth rate **(TAYLOR and VAN STADEN, 1996; BLANK and YOUNG, 1998; LIGHT et al., 2010)**.

Findings from this greenhouse experiment can be extrapolated to natural processes occurring at population and community levels in that species that

responded to a smoke and/or heat stimulus are obviously more likely to germinate from soil seed bank following a fire in the field. Furthermore, the phenomenon of smoke-induced enhanced growth appears to be ecologically significant in that faster growth rate of certain smoke-responsive species will enable them to deposit relatively more root and shoot biomass making them efficient competitors for limiting resources than the less smoke-responsive species **(CHAPIN, 1980; TILMAN, 1988)**. In situations of high fire and smoke frequency, such smoke-responsive species are expected to have a competitive and survivorship advantage over those plants with relatively lower growth rates. By contrast, in the absence of fire for longer periods, such species may die out if their requirements for germination and establishment are not fully met. In other words, species that do not require a heat or smoke stimulus for germination are expected to increase in abundance in prolonged absence of fire **(AULD et al., 2000; WILLS and READ, 2002)**.

Findings from this study suggest that germination/emergence of some native perennials grasses (e.g. *Themeda triandra*) from the soil seed banks can be enhanced by using smoke techniques. Management techniques for accomplishing this could include grassland seeding, reducing grazing pressure especially during periods of seed production, prescribed burning timed to maintain seed production and stimulate seedling emergence, or actively planting or seeding species of high conservation interest.

7.5 CONCLUSIONS

The significant variations in the number of seedlings that emerged during the course of the trial could be explained due to the effect of the four fire-related treatments applied on the soil seed bank samples. From the standpoint of seedling emergence and total biomass production, burning (exposure to heat and smoke) should provide conditions conducive to maximum total emergence and biomass production of smoke-responsive species such as *Themeda triandra* from burnt mesic grassland sites rather than unburnt sites. Therefore, smoke treatment of the mesic grassland soil seed bank can play a significant role to improve the recovery of degraded sites and rehabilitation programmes. However, future research prospects need to quantify the net contribution of broad-scale surface application of smoke compounds in

effectively enhancing germination, improving seedling densities and species richness of such fire-prone vegetation communities in South Africa.

CHAPTER EIGHT

General discussion and conclusions

"He who chooses the beginning of a road chooses the place it leads to. It is the means that determine the end"

Harry Emerson (1878-1969)

8.1 INTRODUCTION

The primary goal of scientific theory such as the theory of vegetation change (succession) is the understanding and explanation of an observed reality by means of concepts, laws and empirically based generalisations **(GLENN-LEWIN et al., 1992)**. A key factor in achieving this goal is an understanding of the principal variables influencing the abundance and distribution of plant species along the successional landscape. Some of the most important variables influencing the distribution and abundance of species are habitat fertility, productivity, competition and the nature of site disturbances which destabilise the resource base **(GRIME, 1979; BAZZAZ, 1983; TILMAN, 1988; BAZZAZ, 1996)**. In such an ever changing environment, the growth and success of species may be influenced by a myriad of biotic and abiotic factors including temperature, light availability, pH, salinity, soil depth, topography, soil moisture, nutrient availability, perdition, disease and disturbances of many forms (e.g. fire, grazing and mowing) and various interactions among these factors.

It has been long recognised that fire as an ecological disturbance factor influences many attributes of the post-fire environment **(SOUSA, 1984)**. It affects the

soil nutrient status **(OJIMA et al., 1994; FYNN et al., 2003)**, light availability, soil moisture **(KNAPP and SEASTEDT, 1986; SNYMAN, 2003)**, soil and atmospheric chemistry **(RAISON, 1979; ANDREAE, 1991)** and the soil temperature **(SAVAGE and VERMUELEN, 1983; AULD and BRADSTOCK, 1996)**. These fire-related variables directly or indirectly influence seed germination and seedling growth of species differently even when species are exposed to equal access to resources.

It has been well understood for years that seed germination as an important trait leading to a successful population growth of species can be affected by the interactions between disturbance regimes such as fire and species biology **(HARPER, 1977; PICKETT and WHITE, 1985)**. Recently however, the intriguing role of products of vegetation combustion (smoke, heat, ash, charred wood and some nitrogenous compounds) in stimulating seed germination and influencing plant growth has received considerable global attention. As a result, stimulation of germination from the soil seed bank using fire-related cues of smoke and heat has become a well-known phenomenon in fire-prone vegetations world-wide and identifying the component of fire causing elevated germination has been a common research aim **(ROCHE et al., 1997; ROKICH et al., 2002; FIGUEROA et al., 2009; MOREIRA et al., 2010)**. In South Africa, there exists limited information on germination requirements of the local flora and how recruitment of grassland species is influenced by fire, fire-related factors. The interaction of these factors among themselves is virtually unknown. There is also limited knowledge on how fire-related cues such as heat-shock and smoke can combine to affect the germination and growth response of seeds from fire-prone vegetation because combinations of multiple levels of both cues have rarely been investigated. In this comprehensive study, various investigations were conducted on different aspects relating to fire, smoke, heat, soil and nutrients, in an attempt to gain a greater understanding of the effect of fire (burning) and fire-related factors on seed germination and seedling growth of some common grassland species. The objective of this final Chapter is to provide a general synthesis of the observed patterns in grassland species seed germination and seedling growth in response to fire-related cues and the interaction among them. It is also the aim of this Chapter to shed some light on further research avenues on the subject of smoke-stimulated seed germination and its imminent significance for vegetation science.

8.2 The effect of plant-derived smoke solutions on mesic grassland seed germination and seedling growth

South Africa hosts one of the world's longstanding (60 years) *in situ* grassland burning (fire) and mowing experiments running at the plateau of Ukulinga (29° 24' E, 30° 24' S), the research farm of the University of KwaZulu-Natal. These studies have provided significant insights into the ecology and management of the indigenous grassland **(FYNN et al., 2003; FYNN, 2004)**. Despite the number of years and the volume of the work done on grassland fire (burning) research however, the role of smoke and other fire-associated factors in influencing the regeneration and recruitment potential of the grassland has never been examined or not to the required extent **(ZACHARIAS et al., 1988; BAXTER et al., 1994)**. This was because of the traditionally held view that in the mesic Tall Grassland, regeneration comes mainly due to vegetative reproduction and the contribution from seed germination to the overall grassland regeneration following an event of fire is negligible **(DANCKWERTS, 1984; ZACHARIAS et al., 1988; EVERSON, 1994)**. Findings from this study are in direct contrast with the previously held view **(DANCKWERTS, 1984; ZACHARIAS et al., 1988; EVERSON, 1994)**. Findings from this study rather support the view that in the mesic grassland of South Africa species turnover in response to fire is partly regulated by the effects of smoke, heat and nutrient availability in the habitat. Though the net contribution of seed germination to the total grassland dynamics still needs to be determined, the preceding germination experiments **(Chapter 3**) undertaken using smoke solutions conform that plant-derived smoke solutions indeed, significantly enhance germination rate and germination percentage in 3 of the 6 species examined. The degree of responsiveness varied from species to species and across different temperatures. Fascinatingly enough, the smoke solutions used could also promote seedling vigour (vegetative growth) in a few of the species examined. This is particularly true for fire-loving species of *Themeda triandra* and *Tristachya leucothrix*, where percentage germination and seedling vigour indices were markedly modified by the smoke treatments applied **(BAXTER et al., 1994)**.

It is generally considered that *Themeda triandra* has evolved under constant fire regime **(O'CONNOR, 1997)**. Agreeing with such a notion, findings of this study demonstrate that conditions of low available nitrogen (N) **(FYNN et al., 2003)** and

high presence of smoke cues in the habitat **(BROWN, 1993a)** created due to frequent burning may favour the germination and initial seedling growth of *Themeda triandra* rather than the other co-existing species which require more available nitrogen e.g. *Eragrostis curvula* **(FYNN and NAIKEN, 2009)** and are relatively less responsive to smoke treatments (see **Chapter 3 and 6**). This explains why the short native species *Themeda triandra* retrieve successfully in post-fire habitats. However, natural low annual seed rain **(O'CONNOR, 1997),** natural poor ability of *Themeda triandra* seeds to retain their viability to the next season **(EVERSON, 1994)**, vulnerability of surface seeds for rodent and insect perdition **(EVERSON, 1994; GRANGER and BREDENKAMP 1996)** and poor resistance of surface seeds to lethal fire (heat) **(ZACHARIAS et al., 1988)** may account for the low population growth of the species. Nevertheless, the results of the laboratory and greenhouse germination and seedling vigour experiments (**Chapters 3, 5, 6 and 7**) undoubtfully throw some light on the ecological significance of plant-derived smoke as a fire-related cue influencing the *in situ* germination and initial seedling growth of South African mesic grassland species differently which can further influence the grassland composition. Furthermore, the strong fire-species relationship syndrome observed in frequently burnt mesic grasslands is partly due to the strong relationship that exists between the effect of fire-induced cues such as smoke and heat on one hand, and the interaction of these factors with nutrients (fertility) influencing seed germination, seedling growth and possibly subsequent growth on the other hand. Hence, successful re-establishment of degraded native *Themeda triandra* grasslands can perhaps be achieved by frequent burning of grasslands which lowers the habitat productivity (low N availability) and deposits high smoke and heat cues in the habitat. This situation establishes a selection of species in favour of plants capable of rapid post-fire regeneration either through seed germination and/or by vegetative means.

8.3 The ecological relevance of smoke and butenolide in the natural environment

The potential role of plant-derived smoke and smoke-isolated butenolide for large–scale use in various fields of environmental applications such as weed control, restoration and rehabilitation programmes has been projected by **LIGHT and VAN**

STADEN (2004). However, the topic has not received enough attention and there are relatively few studies which investigated the effectiveness of smoke and butenolide in the field **(MERRITT et al., 2006; STEVENS et al., 2007)**. Likewise, the use and effectiveness of smoke and butenolide for ecological restoration and weed control purposes in South Africa are nearly untouched subjects.

The experiments and observations of this study demonstrate that there is high variability in germination and seedling growth responses of the grass species to the presence or absence of fire and fire-related factors in the environment. These studies suggest that smoke and butenolide are effective as germination stimulants and can overcome the requirements for light, moisture and optimum temperature (**Chapters 3 and 4**). It has also been demonstrated that smoke solutions interact with soil macronutrients to influence plant growth differently (**Chapter 6**). These findings have ecologically important implications for mesic and semi-arid South African grasslands, where, soil nutrient status **(FYNN et al., 2005),** temperature and moisture **(O'CONNOR, 1997)** determine population dynamics. For instance, in semi-arid regimes of South Africa, **O'CONNOR (1997)** reported that the major cause of decline in populations of *Themeda triandra* was that of mortality of established seedlings due to severe drought and high temperature. This suggests that in the arid and semi-arid regions, finding ways to reduce the ecological impacts of drought should be a major research thrust.

The important role of smoke and butenolide in relaxing the natural germination zone of species by overcoming stresses from low osmotic potential and high temperature therefore, can be used as a remarkable remedy to curb the loss of seedlings of species of high economic and conservation interest. It is important to note that this is likely to have a follow on ecological effects to community structure and function.

8.4 CONCLUSIONS

The major conclusions which can be drawn from the findings of this study are the following: (a) plant-derived smoke solutions influence seed germination, seedling vigour and seedling emergence from the soil seed bank of the mesic Tall Grassland in South Africa, b) these smoke solutions also widen the temperature and moisture

zones over which seed germination and growth can occur. Smoke stimulated-germination can be the result of at least two causes (1) the breaking of dormancy by smoke and smoke plus heating (2) smoke-induced enhancement of water uptake by seeds (facilitating imbibition), c) the role of plant-derived smoke in influencing seed germination and promoting initial seedling growth is not independent of the temperature and nutrient status of the habitat. Evidently, post-fire presence of high smoke cues in the habitat, in relation to increases on soil temperature and changes in nutrient status (NPK) of South African mesic grassland habitat appear to be important factors influencing the composition of fire-prone grassland communities.

8.5 Future research avenues

It is often the case with scientific investigations that more questions than answers are produced. The positive aspects of this legacy being the opening of new avenues for future research efforts in the seasons to come. The following section is intended to provide some of the questions raised during the course of this study and call for future research effors on the subject.

1. Research conducted over the last couple of decades on smoke-induced seed germination no doubt has resulted into the selection of species recognised as smoke-responsive species and non-smoke-responsive species **(DIXON et al., 2009)**. Why do species differ in their response to smoke? and what is the mode of action of the biologically active compound (butenolide) in promoting seed germination and enhancing plant growth? These questions are some of the most relevant issues that need to be addressed. Hence, understanding the physiological differences of responsive and non-smoke responsive seeds is an important first step for understanding of the underlying mechanisms of smoke action on seed germination. A comparative microarray (analysis and gene expression and profiling) studies on smoke-responsive and non-smoke responsive seeds/species at molecular and gene level can be used as a tool for unravelling the underlying mechanisms and genetic controls which might be at work.

2. Understanding of soil seed bank dynamics in relation to fire, grazing and other major biotic and abiotic influences in the habitat is an important first step for the ecology and sound management of grassland ecosystems. Preliminary studies on the role and effect of burning on soil seed bank stimulation have been demonstrated in this study. Results showed that seed germination and seedling emergence from the germinable soil seed bank can be enhanced using smoke treatments. However, to acquire more knowledge on the feasibility of using smoke techniques for large-scale restoration, rehabilitation and weed invasion control projects, systematically designed field studies on how soil seed bank dynamics varies in relation to season, past history of land use, topography and ecological disturbances is needed.

3. It is now well established that plant-derived smoke plays a significant role on seed germination and seedling growth of certain smoke-responsive species which dominate South African grasslands. However, not all species are responsive to smoke or not to the same degree. Hence, the effect and role of plant-derived smoke and butenolide on grassland species tillering, budding and under natural conditions, how species competitive interaction (both competitive effect and competitive response) among species, in the presence/absence of smoke influences grassland dynamics requires a detailed study.

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APPENDICES

Appendix 1

Examples of grass species (Poaceae) which showed a significant germination response to various smoke treatments.

Species	Treatment	Reference
Achnatherum hymenoides	SA	BLANK and YOUNG, 1998
Achnatherum occidentalis	SA	BLANK and YOUNG, 1998
Achnatherum thurberianum	SA	BLANK and YOUNG, 1998
Alopecurus myosuroides	SW	ADKINS and PETERS, 2001
Amphipogon amphipogonoides	SA	ROCHE et al., 1997
Aristida racemosa	SW	ADKINS and PETERS, 2001
Austrodanthonia teuior	SW	CLARKE and FRENCH, 2005
Austrostipa compressa	SW	SMITH et al., 1999
Avena fatua	SW	ADKINS and PETERS, 2001; ADKINS et al.,2003; STEVENS et al., 2007
Avena sterilis	SW	ADKINS and PETERS, 2001
Cenchrus ciliaris	SW	ADKINS et al., 2003
Chloris ventricosa	SW	READ and BELLAIRS, 1999
Dactylis glomerata	SA	PEREZ-FERNANDEZ and RODRIGUEZ-ECHEVERRIA, 2003
Dichnthaium sericeum	SW	READ and BELLAIRS, 1999
Digitaria diffusa	SA	READ and BELLAIRS, 1999
Digitaria ramularis	SA	READ et al., 2000
Eragrostis curvula	SW	CLARKE and FRENCH, 2005
Eragrostis benthamii	SW	CLARKE and FRENCH, 2005
Eragrostis cilianensis	SA	READ et al., 2000
Eragrostis leptostachya	SA	READ et al., 2000; CLARKE and FRENCH, 2005
Eragrostis sororia	SA	READ et al., 2000
Festuca megalura	Charet	KEELEY et al., 1985
Euclasta condylotricha	SW	DAYAMBA et al., 2010
Hesperostipa comata	SA	BLANK and YOUNG, 1998
Hetropogon contortus	SW	ADKINS et al., 2003
Melica ciliata	SA	REYES and TRABAUD, 2009
Hordeum leporinum	SW	STEVENS et al., 2007
Melinis minutiflora	SA	ADKINS et al., 2000
Neurachne alopecuroidea	SA	DIXON et al., 1995
Oryza sativa	SW	DOHERTY and COHN, 2000
Panicum decompositum	SW	READ and BELLAIRS, 1999
Panicum effusum	SW	READ and BELLAIRS, 1999
Panicum maximum	SA	ADKINS et al., 2000
Panicum simile	SW	CLARKE and FRENCH, 2005

Pentaschistis colorata	SA	BROWN and BOTHA, 2004
Phalaris paradoxa	SW	ADKINS et al., 2000
Poa labillardieri	SW	READ and BELLAIRS, 1999
Pseudopentameris macrantha	SA	BROWN and BOTHA, 2004
Sorghum halepense	SW	ADKINS and PETERS, 2001; ADKINS et al.,2003
Stipa compressa	SA	ROCHE et al., 1998
Stipa scabra	SA	READ and BELLAIRS, 1999
Themeda triandra	SW & SA	READ and BELLAIRS, 1999; BAXTER et al., 1994
Zea mays	SA	MODI, 2002; SPARG et al., 2006

Appendix 2

Examples of grass species (Poaceae) which did not show a significant germination response to various smoke treatments.

| Species	Treatment	Reference
Aira elegans	SW	ENRIGHT and KINTRUP, 2001
Andropogon ascinodis	SW	DAYAMBA et al., 2010
Andropogon gayanus	SA	DAYAMBA et al., 2008; DAYAMBA et al., 2010
Aristida ramosa	SW	CLARKE and FRENCH, 2005
Arista vagas	SW	CLARKE and FRENCH, 2005
Austrodanthonia racemosa var. racemosa	SW	CLARKE and FRENCH, 2005
Austipa rudis spp. Rudis setacea	SW	CLARKE and FRENCH, 2005
Avena barbata	Charet	KEELEY et al., 1985
Avena sterilis	SA	REYES and TRABAUD, 2009
Bothriocholoa decipiens	SW	READ and BELLAIRS, 1999
Bothriochloa macra	SW	CLARKE and FRENCH, 2005
Bormus diandrus	SW	ADKINS et al., 2003
Chloris truncata	SW	READ and BELLAIRS, 1999
Cymbopogon refractus	SW	READ and BELLAIRS, 1999
Cymbopogon schoenanthus	SW	DAYAMBA et al., 2010
Danthonia caespitosa	SA	ROCHE et al., 1997
Danthonia eriantha	SW	READ and BELLAIRS, 1999
Danthonia linkii var. fulva	SW	READ and BELLAIRS, 1999
Danthonia pallida	SW	READ and BELLAIRS, 1999
Danthonia racemosa	SW	READ and BELLAIRS, 1999
Dichanthium sericeum	SW	CLARKE and FRENCH, 2005
Dichelachne micrantha	SW	CLARKE and FRENCH, 2005
Digiteria brownii	SW	READ and BELLAIRS, 1999
Digiteria ramularis	SW	CLARKE and FRENCH, 2005
Echinopogon caespitosus	SW	CLARKE and FRENCH, 2005
Ehrharta calycina	SW	SMITH et al., 1999
Entolasia stricta	SW	CLARKE and FRENCH, 2005
Eragrostis elongata	SW	READ and BELLAIRS, 1999
Eriochloa pseudoacrotricha	SW	READ and BELLAIRS, 1999
Euclasta condylotricha	SW	DAYAMBA et al., 2010
Melica imperfecta	Charet	KEELEY and KEELEY, 1987
Microlaena stipoides	SW	READ and BELLAIRS, 1999
Microlaena stipoides var. stipoides	SW	CLARKE and FRENCH, 2005
Neurachne alopecuroidea	SA	WARD et al., 1997
Paspalidium distans	SW	CLARKE and FRENCH, 2005
Sporobolus indicus var. capensis	SW	CLARKE and FRENCH, 2005

Rottboellia exaltata	SW	DAYAMBA et al., 2010
Stipa coronata	Charet	KEELEY et al., 1985
Stipa lepida	Charet	KEELEY and KEELEY, 1987
Stipa scabra	SW	READ and BELLAIRS, 1999
Tetrarrhena laevis	SW	ROCHE et al., 1997
Themeda australis	SW	CLARKE and FRENCH, 2005

Appendix 3

Electro-microscopic photos of the seeds of the six grass species used in this study. Bar indicates 1000 µm.

Aristida junciformis
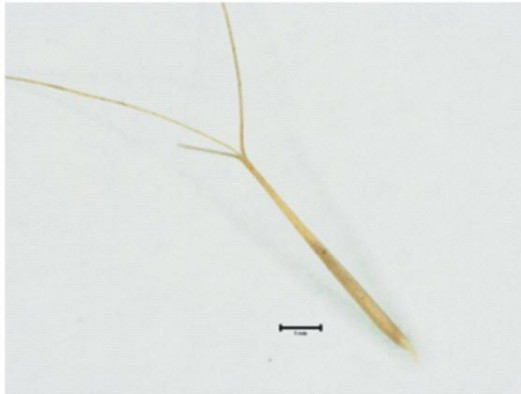
Panicum maximum

Eragrostis curvula

Tristachya leucothrix

Hyparrhenia hirta

Themeda triandra