

***Vachellia sieberiana* var. *woodii* a high-altitude encroacher: the effect  
of fire, frost, simulated grazing and altitude**

**by**

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Nature, in her most dazzling aspects or stupendous parts, is but the background and  
theatre of the tragedy of man.

John Morley

## ABSTRACT

There is increasing evidence that savannas and grasslands throughout the world are experiencing bush encroachment. The replacement of grassy biomes with woody biomes has serious consequences for net primary productivity. The grasslands of South Africa are not exempt from this phenomenon. Despite this, the drivers of the tree:grass dynamics are still robustly debated. In mesic and moist savannas and grasslands, the tree:grass balance appears to be maintained mainly through disturbance such as fire, frost and herbivory or a combination of disturbances. Other factors such as competition for resources may play a modifying role. High altitude grasslands are frequently within a climatic zone that would support trees, yet trees are absent. The answer as to what mechanism excludes trees from these grassy biomes continues to elude researchers. Very often low temperature is cited as a possible mechanism. *Vachellia sieberiana* var. *woodii* is a typical savanna tree which is absent from high altitudes. However, it has been encroaching into the grasslands along the escarpment of the Drakensberg, KwaZulu-Natal, South Africa, over several decades, although is still excluded from the top of the escarpment. I acquired aerial photographs and satellite images covering the Van Reenen's Pass area, north-western KwaZulu-Natal, dating from 1955 to 2015. These images confirmed that *V. sieberiana* was increasing in density along the escarpment, but that no *V. sieberiana* was present on top of the escarpment, despite the successful establishment of other tree species. The photographs and images also suggested that *V. sieberiana* was extending its range into higher altitudes. Because fire, frost and herbivory are generally thought to be the determinants of the structure of grasslands and savannas, I conducted field trials along the altitudinal gradient on Van Reenen's Pass, investigating the effect of these determinants on the establishment of transplanted *V. sieberiana* saplings at three different altitudes. Competition for resources were briefly taken into consideration, although they were not the main thrust of the project: soil nutrients and root gaps. Soil moisture was not a concern as the area is what is defined as mesic. The transplanted saplings were smallest at the high-altitude site and largest at the low-altitude site after two growing seasons in the field. The response of the saplings to the various treatments was not consistent at the three sites. There was no response to the treatments at the high-altitude site; a significant response to fire, frost and simulated grazing at the mid-altitude sites; and a significant response to frost and simulated grazing at the low-altitude site.

There was no interactive effect between treatments. There was no correlation between root gap and tree density, but there was a correlation between soil nutrients and transplanted sapling biomass. Further to the field trials, I investigated the effect of white frost and black frost on the survival rate of *V. sieberiana* saplings in a controlled environment and whether *V. sieberiana* saplings, grown from seed collected from two different altitudes, might exhibit a preadaptation to tolerate frost. I found that *V. sieberiana* saplings were able to coppice readily after 100% topkill as a result of white frost and that they could survive black frost down to -6 °C. While saplings grown from seed collected from the low-altitude site were more robust than those grown from seed from the mid-altitude site, both groups responded similarly to white and to black frost. I also ran a preliminary investigation into the possible strategy employed by *V. sieberiana* to tolerate frost by testing for an increase in soluble sugars in the cell sap after chilling and whether *V. sieberiana* seeds remained viable after freezing. Unlike *Vachellia tortilis*, *V. sieberiana* did not increase soluble sugars within the cell sap in response to stress. It may be that, as a deciduous tree, *V. sieberiana* avoids frost damage through dormancy in the winter months, rather than increasing metabolites within the cell sap. The seeds of *V. sieberiana* are resistant to freezing, maintaining viability at -9 °C, although the germination rate was reduced. While there were significant responses to the various treatments, none of the treatments or combinations of treatments were able to prevent the establishment of *V. sieberiana*. With the dual spectres of global warming and increasing levels of atmospheric CO<sub>2</sub> looming, *V. sieberiana* may become an even more aggressive encroacher. Managers of grassy biomes, therefore, will need to be vigilant in their monitoring of the presence of potential woody encroachers and devise novel methods in mitigating the risks.

## **PREFACE**

The research contained in this thesis was completed by the candidate while based in the Discipline of Grassland Science, School of Life Sciences of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg, South Africa. The research was financially supported by the College of Agriculture, Engineering and Science, the National Research Foundation and N3 Toll Concession.

The contents of this work have not been submitted in any form to another university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.



---

Signed: Dr Michelle J Tedder

Date: 21 February 2019

## DECLARATION 1: PLAGIARISM

I, Jennifer Mary Russell, declare that:

(i) the research reported in this thesis, except where otherwise indicated or acknowledged, is my original work;

(ii) this thesis has not been submitted in full or in part for any degree or examination to any other university;

(iii) this thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons;

(iv) this thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

a) their words have been re-written, but the general information attributed to them has been referenced;

b) where their exact words have been used, their writing has been placed inside quotation marks, and referenced;

(v) where I have used material for which publications followed, I have indicated in detail my role in the work;

(vi) this thesis is primarily a collection of material, prepared by myself, published as journal articles or presented as a poster and oral presentations at conferences. In some cases, additional material has been included;

(vii) this thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the References sections.



Signed: J M Russell

Date: 21 February 2019

## **DECLARATION 2: PUBLICATIONS**

\* Indicates corresponding author

### **Chapter 2**

#### **Publication 1 – submitted to the African Journal of Range & Forage Science**

Russell JM\* and Tedder MJ 2018. Aerial photographs track woody cover changes on Van Reenen's Pass, KwaZulu-Natal, South Africa, over a 60-year period, with particular focus on *Vachellia sieberiana* var. *woodii*.

The research is based on aerial photographs and satellite imagery supplied by the Chief Directorate of National Geo-Spatial Information, Mowbray, South Africa. Dr M Tedder and I conceived the research. I analysed the photographs for changes in woody plant density and wrote the paper. Dr Tedder contributed further with valuable comments to the manuscript and facilitated data analysis.

### **Chapter 3**

#### **Publication 2**

Russell JM\* and Tedder MJ 2018. The effect of fire, frost and simulated grazing on *Vachellia sieberiana* var. *woodii* along an altitudinal gradient in north-western Kwazulu-Natal, South Africa.

This paper is based on field trials undertaken along the Drakensberg escarpment, KwaZulu-Natal, South Africa. Drs David Ward and Michelle Tedder conceived the research, with further contributions supplied by myself. I undertook the research and wrote the paper. Dr M Tedder contributed further with valuable comments to the manuscript and facilitated data analysis.

### **Chapter 4**

#### **Publication 3**

Russell JM\* and Tedder MJ 2018. Frost tolerance in *Vachellia sieberiana* var. *woodii* (formerly *Acacia sieberiana* var. *woodii*)

This paper is based on research undertaken at the University of KwaZulu-Natal, Pietermaritzburg campus. Drs David Ward and Michelle Tedder conceived the research, with further contributions supplied by myself. I undertook the research and wrote the paper. Dr M Tedder contributed further with valuable comments to the manuscript and facilitated data analysis.

A handwritten signature in black ink, appearing to read 'J M Russell', written over a horizontal line.

Signed: J M Russell

Date: 21 February 2019



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## Chapter 4

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## CHAPTER 1: GENERAL INTRODUCTION AND LITERATURE REVIEW

### 1.1 Introduction

Woody plant or bush encroachment has been defined by Van Auken (2000) as an increase in the density and cover of indigenous woody species as opposed to the introduction of woody species into areas where they have not previously existed. It is generally perceived as a form of land degradation, which may become a threat to any ranching activity (Eldridge *et al.* 2011). This may, *inter alia*, take the form of loss of biomass and diversity within the herbaceous layer (Van Vegten 1984; Stuart-Hill and Tainton 1989; Knapp *et al.* 2008), the replacement of palatable woody species with non-palatable species (Gordijn *et al.* 2012); soil erosion (Grellier *et al.* 2012b) and soil hydrology (Jackson *et al.* 2005; Honda and Durigan 2016); and may alter microclimates and soil nutrients (Belsky *et al.* 1989). However, it needs to be noted that not all bush encroachment is necessarily negative. Moderate levels of woody plant cover may maximise biodiversity and ecosystem services (Belsky 1992) and only when woody plant cover exceeds 30% to 60% cover, will ecosystem services begin to decline (Eldridge and Soliveres 2015; Barbosa da Silva *et al.* 2016). Maestre *et al.* (2016) showed that woody plant encroachment could reverse land degradation, improving ecosystem services in Mediterranean grasslands. In Africa, however, overwhelming evidence points to woody encroachment having a negative impact on the environment.

There is no formal consensus on the correct use of the terms “encroachment” and “invasion”. In this study we have used the term “encroachment” to describe an increase in tree density or cover in areas where the trees already exist, albeit in small numbers. The term “invasion” has been used to describe the appearance of trees (indigenous or exotic) in areas such as grasslands where there is no historic record of them being present there in the past.

### 1.2 Tree-grass dynamics

About 30% of the land surface is dominated by C<sub>4</sub> grasses (Grace *et al.* 2006), which arose in the tropics and sub-tropics during the late Miocene and early Pliocene (3 – 8 million years ago) (Cerling *et al.* 1997), where they continue to dominate (Bond 2008). The low atmospheric (CO<sub>2</sub>) concentrations, which predate this period, may have been the

selective force for the C<sub>4</sub> photosynthetic pathway (Beerling and Osborne 2006), but the question remained as to what drove the expansion of these grasses to replace the existing C<sub>3</sub> grasses and forests. Scheiter *et al.* (2012) proposed that fire was one of the driving forces and that the evolution of fire-adapted trees led to the creation of savannas (Scheiter *et al.* 2012).

There is much literature on tree-grass interactions, particularly in the context of savannas (see for example: Skarpe (1992); Scholes and Archer (1997); Sankaran *et al.* (2004); Bond (2008)). The discussions range from competition for resources (a bottom-up approach) to fire and herbivory (a top-down approach). The bottom-up approach, in many instances, is effective in maintaining the tree-grass ratio in arid and semi-arid savannas (Ward *et al.* 2013). In moist savannas the tree-grass interactions are governed by an interplay of bottom-up controls, such as water and soil nutrients, and top-down disturbances, such as fire, herbivory and frost (Sankaran *et al.* 2005; Sankaran *et al.* 2008; Murphy and Bowman 2012).

Midgley *et al.* (2010) make the distinction between recruitment and transition. The term, recruitment, is applied to an increase in a plant population (seedlings), whereas transition refers to the increase (or decrease) in size or number of stems of an already established plant. Midgley and Bond (2001) use the term establishment, to refer to the transition between size classes (see also, *inter alia*, Sankaran *et al.* (2004)).

### **1.2.1 The role of competition (bottom-up approach)**

Casper and Jackson (1997) state that most of the competition between plants comes from below ground, particularly when resources, such as soil moisture and nutrients, are limited (Wilson and Tilman 1995). Competition for light, however, plays only a small role (Casper and Jackson 1997) and, generally, when belowground resources are not limited (Wilson and Tilman 1995). It is important, however, whenever discussing tree-grass interactions, to distinguish between mature trees and saplings, as well as the different species of trees and grasses. Tedder *et al.* (2012), for example, noted that different *Vachellia* species respond differently to nutrient availability and different grass species have different competitive abilities when grown in conjunction with *Vachellia* saplings (see also February and Lewis (2016)).

### *1.2.1.1 Soil moisture*

To explain the balance between trees and grasses in a savanna system, H Walter, in 1939, proposed a two-layer hypothesis to explain the tree-grass dynamic in savannas (Ward 2005). Walter suggested that tree seedlings (or shrubs) and grasses competed for soil moisture within the top layer of soil. Once the trees had established themselves with a root system that went below the reach of the grass roots, the two growth forms were no longer in competition (niche separation). Spatial partitioning, or niche separation, may also be horizontal, where, for example, tree and grass roots may occupy the top 20 cm of soil, but with a lower root biomass for grass under tree canopies than in the open (February *et al* 2013a). There may also be a temporal aspect to resource partitioning, with the deeper rooting trees being able to access deep-level water long after the rainfall event (Casper and Jackson 1997; Kulmatiski and Beard 2013).

In arid and semi-arid savannas, where mean annual precipitation (MAP) is between 150 and 650 mm, water availability may limit the seedling recruitment (Breshears and Barnes 1999; Bond *et al.* 2003; Sankaran *et al.* 2005). In these situations, Walter's two-layer hypothesis may be applicable (Ward *et al.* 2013). Kambatuku *et al.* (2013) demonstrated that tree saplings and grasses utilized different sources of soil moisture, but noted there was a zone of overlap where the roots of saplings and grasses occupied an intermediate layer. Their results supported Walter's two-layer hypothesis, but identify three layers, rather than two (Kambatuku *et al.* 2013). In combination with heavy grazing, Walter's two-layer hypothesis is frequently cited to explain the phenomenon of bush encroachment (Van Vegten 1984; Skarpe 1990). If the grass layer was removed through, for example, heavy grazing, young tree seedlings would no longer compete for moisture in the top layer of soil and the system could move from an open savanna to a closed savanna. However, in shallow soils which support both trees and grass, niche separation is not applicable (Wiegand *et al.* 2000; Ward 2005). Nor does niche separation adequately explain the recruitment of tree saplings as the fine roots of both the tree seedlings and the grasses occupy the same soil layer (Ward 2005; February and Higgins 2010; February *et al.* 2013a). Riginos (2009) also refutes niche separation, saying that grass competition can exert a negative effect on the growth of even mature trees. However, it must be remembered that rooting depth and the ability of trees to compete successfully (or not) with grasses for soil resources varies from species to species (Holdo and Brocato 2015).

In a rainfall manipulation study done in the Kruger National Park, South Africa, February *et al.* (2013b) found that the higher the rainfall the greater the grass biomass on both nutrient-rich and nutrient-poor soils which, in turn, successfully prevented tree recruitment. They suggested that tree recruitment would be more successful during drought years when grass biomass was lower. Knoop and Walker (1985) also found evidence in support of grass suppression of trees in savannas with nutrient-rich soils (see also Sankaran *et al.* (2008)), although not in nutrient-poor soils. Kraaij and Ward (2006), working in semi-arid savanna with sandy and stony soils, made the distinction between the number of rainfall events and overall MAP. Trees were more likely to germinate and establish successfully if rainfall is more frequent or pulsed (see also Hoffmann 1996; Wilson and Witkowski 1998). Conversely, Berry and Kulmatiski (2017) found that, on level clay soils, fewer, but more intense, precipitation events increased woody growth, encouraging woody encroachment. However, in moist savannas, soil moisture is no longer limiting and, therefore, does not constrain sapling recruitment (Sankaran *et al.* 2005).

Competition exerted by trees on grasses has also been demonstrated by McDaniel *et al.* (1982) and Simmons *et al.* (2008). They found that the nitrogen-fixing tree, *Prosopis glandulosa* suppressed grass biomass, presumably through competition for soil moisture. This effect was observed during a period of lower-than-average rainfall. During the years of high rainfall, grass biomass was not affected by the presence of *P. glandulosa* (see also Simmons (2003)).

Greenhouse experiments, replicating mesic conditions, conducted by Ketter and Holdo (2018), indicate that the roots of tree saplings and grasses occupy the same soil layers, with the grass roots sometimes penetrating layers deeper than the sapling roots, utilizing both surface soil moisture and moisture in the deeper soil layers. They noted that, while soil moisture was not limited, grasses were still successful competitors and conclude that saplings and grasses compete for other resources, such as nutrients (Ketter and Holdo 2018).

### **1.2.1.2 Nutrients**

Whether trees and grasses compete directly for nutrients and for which nutrients they compete appears to be a moot point. Cramer *et al.* (2010) found that the presence of grass

induced nitrogen-fixing activity in leguminous tree species (see also Cramer *et al.* (2007)). In addition, they noted that nitrogen applied to the substrate increased the growth rates of non-leguminous savanna trees in the presence of grass, although had no effect on growth rates of leguminous trees (Cramer *et al.* 2010). In subsequent research, Cramer and Bond (2013) found that savanna trees, both leguminous and non-leguminous, do not compete with grasses for nitrogen, but, possibly for other nutrients. Their earlier results of 2007 and 2010, which showed different responses between leguminous and non-leguminous trees, were probably due to certain confounding factors (Cramer and Bond 2013).

In their greenhouse trials, Vadigi and Ward (2012) found that addition of nutrients did little to improve the survival rates of *Acacia* (= *Vachellia*) saplings, which are typical African savanna trees that have the ability to fix nitrogen (see also Vadigi and Ward (2013) and Kraaij and Ward (2006)). However, the addition of moderate amounts of nutrients did improve the competitive ability of the saplings when grown with grasses (Vadigi and Ward 2012; Vadigi and Ward 2013). The addition of high levels of nutrients to saplings when grown with grasses resulted in reduced sapling biomass, due to the increased vigour of the grasses (Vadigi and Ward 2012; Vadigi and Ward 2013). Sankaran *et al.* (2008) also found that soil nitrogen availability tended to depress sapling recruitment as a consequence of increased grass cover. As grass cover responds to increased nutrients, water-usage increases, resulting in the depletion of soil water. This leads to water-stress in young tree saplings if the intervals between rainfall episodes are prolonged (Van Der Waal *et al.* 2009).

Mature savanna trees may have an impact on grass biomass and grass nutrient content. Stuart-Hill and Tainton (1989) in the Eastern Cape, South Africa, found that mature *Acacia* (= *Vachellia*) *karroo* suppressed grass growth. In contrast, Ludwig *et al.* (2001) found that grass biomass was no different under the canopy of *Acacia* (= *Vachellia*) *tortilis* to that in the open, but they did, however, find that nutrient concentrations in grasses under the tree canopies were higher than those in the open. They also found that the addition of nitrogen to the experimental plots in the open resulted in higher grass biomass, while the addition of phosphorus increased grass biomass under the tree canopy. They suggested that the trees, being leguminous, were not removing nitrogen from the soil, but phosphorus (Ludwig *et al.* 2001). In their investigation into the effect of

additional nitrogen on the competitive ability of savanna trees when grown in conjunction with grass, Cramer and Bond (2013) found that both non-leguminous and leguminous trees were equally sensitive to grass competition. They concluded that nutrients such as phosphorus, rather than nitrogen, might be limiting with regard to sapling growth (Cramer *et al.* 2010; Cramer and Bond 2013). This was confirmed in a greenhouse experiment performed by N Makhaye and A Magadlela (2017, unpublished data) who found that *Vachellia sieberiana* did not perform well in soils low in phosphorus. Saplings had a lower biomass and fewer nitrogen-fixing nodules than those grown in soils high in phosphorus (Makhaye and Magadlela 2017, unpublished data).

#### *1.2.1.3 Root gaps*

An important component of below-ground competition between plants is the ability of the roots to occupy space (Pärtel and Wilson 2002). This depends on relative growth rate, root-hair density, biomass and total surface area (Aerts *et al.* 1991; Casper and Jackson 1997). In investigating the effect of belowground competition in North America, Jurena and Archer (2003) found that there was no correlation between grass root biomass and grass basal cover. They suggested, therefore, that increases in the belowground grass root biomass inhibited the establishment of the shrub, *Prosopis glandulosa* (Jurena and Archer 2003) i.e. a closure of root gaps, rather than any aboveground competition. Milchunas and Lauenroth (1989), while investigating plant biomass on a shortgrass steppe, made the same observation: that a uniform, horizontal distribution of grass roots would inhibit establishment of other species (see also Coffin and Lauenroth (1990); February and Lewis (2016)). Only with the death of an individual of the more competitive species, will the less competitive species be able to establish itself (Coffin and Lauenroth 1990). Work done by Wakeling *et al.* (2015) in KwaZulu-Natal, South Africa, also suggests that the high density (biomass) of grass roots in moist high-altitude grasslands prevented the establishment of tree seedlings, noting that in these areas the grass roots formed a dense mat. Moreover, interspecific roots compete, not only for space and for resources, but some may also interfere below ground by exuding allelopathic compounds (Alsaadawi *et al.* 1990; Ridenour and Callaway 2001; Bais *et al.* 2003).

#### *1.2.1.4 Light*

Many savanna trees do not tolerate shade (Smith and Shackleton 1988). Trees and grasses also compete for light (Scholes and Archer 1997). Vadigi and Ward (2013) found, in

controlled field trials with eight different savanna tree species, that shading of saplings did not affect survival rate. However, shading did have a negative effect on relative growth rate of six out of the eight species, even in the absence of grass competition and with the addition of nutrients (Vadigi and Ward 2013). Bush and Van Auken (1987) also noted that shading negatively affected growth rates in all parameters of *Prosopis glandulosa* seedlings. A low growth rate would leave the saplings more vulnerable to the effects of frost, fire and herbivory. Hagenah *et al.* (2009) found that grass height, i.e. shading, negatively affected the survival rate of *Dichrostachys cinerea* tree seedlings. This result is contrary to the findings of Tedder *et al.* (2014), who, working with different savanna tree species, found that shading alone had no effect on sapling biomass. They do note that it was possible that the grass productivity in this trial was less than expected and, consequently, the shading of the saplings by the grass sward was not unduly detrimental (Tedder *et al.* 2014).

Shading of the understorey by trees may affect grass production and the species composition of the understorey. Ludwig *et al.* (2001) found that, in the wet season, shading from the tree canopy reduced grass production, whereas in a dry season, shading had a positive effect on grass production. Ludwig *et al.* (2004) recorded an increase in the number of herbaceous dicotyledons under the canopy of shrubs and small trees, which they attributed to these species being more shade tolerant than grasses. Shade-loving grass species, such as *Panicum maximum*, occur beneath large trees (Treydte *et al.* 2007) where shade is not deep enough to exclude them (see also Treydte *et al.* (2008)), while shade intolerant grass species were excluded (Charles-Dominique *et al.* 2018).

#### **1.2.1.5 Facilitation**

Not all interactions between plants are competitive. Treydte *et al.* (2008) expressed concern regarding the impact of decreasing tree populations in certain parts of Africa because of their potentially facilitative effect on the landscape. In a greenhouse experiment, Kambatuku *et al.* (2011) found that the presence of trees increased grass biomass on a sandy substrate.

Belsky *et al.* (1989) found that, in savannas with low tree density, trees positively influenced the microclimate beneath their canopies. Although soil moisture beneath tree canopies initially was lower than in the inter-canopy zone at the start of the rainy season,

as the season progressed soil moisture beneath the canopy was higher than that in the inter-canopy zone. This was the result of reduced soil temperature and evaporation due to shading by the canopy (Belsky *et al.* 1989). However, the benefits of shading by a tree canopy during the dry season may be overridden by depletion of soil moisture by tree water uptake (Ludwig *et al.* 2001).

Under certain conditions, grass productivity may be higher under the canopy of nitrogen-fixing trees than in the open (Belsky *et al.* 1989). Soils under tree canopies were higher in nutrients such as phosphorus, potassium and calcium, as well as organic matter (Belsky *et al.* 1989), although there may be seasonal variations (Ludwig *et al.* 2001) (See also Hudak *et al.* (2003)). Trees utilize nutrients from the subsoils and return the nutrients to the surface as leaf litter and twigs. Animals also congregate in and under the canopy, contributing to soil nutrients through defecation (Belsky *et al.* 1989; Belsky 1994).

The concept of nurse plants is well recognised. Callaway and Walker (1997) reviewed interspecific facilitation, including the role of nurse plants. An adult plant may provide shelter for the seedlings of another species (Callaway 1994), generally providing a buffer against a harsh environment (Inouye 2000; Flores and Jurado 2003) or trampling (Midgley 2009). Ultimately, the balance may shift from facilitation to competition as the beneficiary of the nurse plant gradually out-competes the nurse plant itself (Callaway and Walker 1997; Munzbergova and Ward 2002; Rice *et al.* 2012), although this need not always be the case (Callaway 1994). Grasses (Ludwig *et al.* 2003) and shrubs (Schleicher *et al.* 2011) may benefit in this way from certain African *Acacia* (= *Vachellia*) species that are responsible for hydraulic lift, whereby water from the deep subsoils is brought to the surface.

Grasses, too, may facilitate tree seedling emergence and establishment. Germination of *Vachellia karroo* seeds, for instance, is greatly enhanced when placed within a dense grass clump (O'Connor 1995). Riginos and Young (2007) noted that grasses may protect young saplings from herbivory and trap surface moisture. Grasses also prevent an impervious soil crust from forming, allowing water to penetrate the top soil (Grellier *et al.* 2012a) to become available for young saplings.



### 1.2.2 The role of disturbance (top-down approach)

Niche separation theories are, generally, not adequate to explain the balance between trees and grasses (Higgins *et al.* 2000). In reality, tree-grass interactions are driven by a complex network, invoking resources and disturbances. While a bottom-up impact on tree-grass interactions invokes competition for resources as a driver, disturbances create demographic bottle necks in the recruitment and establishment of trees in savannas and grasslands (Sankaran *et al.* 2004). A typical disturbance may take the form of fire (see e.g. Bond and Keeley (2005)), herbivory (Augustine and McNaughton 2004) or frost (Whitecross *et al.* 2012) or disturbances may interact synergistically, such as fire and herbivory (Archibald *et al.* 2005) or frost and herbivory (Holdo 2007).

#### 1.2.2.1 Fire

It is widely held that fire is a major driver in maintaining grasslands and the open nature of savannas throughout the world (see for example Higgins *et al.* 2000; Bond *et al.* 2003; Kraaij and Ward 2006; Sankaran *et al.* 2008; Accatino *et al.* 2010). Large portions of savanna in the tropics, which are dominated by C<sub>4</sub> grasses, are capable of supporting forest, yet forests are absent except in mosaic patches (Bond 2008; Bond and Zaloumis 2016). Using simulation models, Bond *et al.* (2005) predicted that much of the moist grasslands of Africa and America would transform into forest if fire was withheld. It was assumed that these savannas were the result of deforestation through fires of anthropogenic origin. In his review, Backéus (1992) argues that most of the African savannas and grasslands are secondary formations as a result of fires generated by humans. However, evidence derived from fossil charcoal indicates an increase in fire activity 10 million years ago (reviewed by Bond and Zaloumis (2016)), followed by the spread of the highly flammable C<sub>4</sub> dominated grassy biome (Cerling *et al.* 1997). This was well before hominids started using fire about 1.5 million years ago (Brain and Sillent 1988). It appears then that the savannas and grasslands of Africa are extremely ancient and owe their existence to extensive and frequent fires. Fire, in these biomes, has the potential to create a demographic bottleneck (Higgins *et al.* 2000; Sankaran *et al.* 2004; Bond 2008), whereby saplings are trapped within the fire zone, if not killed (Roques *et al.* 2001).

Savannas of the tropics and subtropics are typified by distinct alternating wet and dry seasons (Scholes and Archer 1997). This pattern results in an accumulation of grass

during the warm, wet summer period. The following dry winter months ensure a continuous fuel load for the grass fires that typically occur in the African savanna. In their work in the Kruger National Park, South Africa, Staver *et al.* (2017) noted that, not only did grass biomass increase with increasing rainfall, but also that fire frequency increased along the rainfall gradient, which, presumably related to the increasing grass biomass. Fire return frequency (Gordijn and Ward 2014), the timing of the fire (Trollope 1982; Zimmermann *et al.* 2008) and the characteristics of the fire (Trollope *et al.* 2002) all have an effect on bush density and the viability of the grass sward.

Optimal fire return frequency for suppressing woody growth depends on the grass fuel load, which, generally, depends on rainfall (Staver *et al.* 2017) and grazing intensity (Van Langevelde *et al.* 2003). Gordijn and Ward (2014) found that, in the old-land grasslands of Ithala Game Reserve, South Africa, a fire return frequency of one year or of 10 years or more resulted in dense woody cover. Too frequent a fire prevents the fuel load from building up sufficiently to create a fire of the requisite intensity to suppress or kill encroaching trees (Higgins *et al.* 2000), although this negative correlation between fire frequency and fire intensity has been questioned by Midgley *et al.* (2006). Uys *et al.* (2004) found that shade-intolerant bunch grasses, such as *Themeda triandra*, increased as fire frequency increased and that shade-tolerant sod-forming grasses decreased with increasing fire frequency. Too long a fire return frequency allows tree saplings to escape the fire zone, making them impervious to subsequent burns (Higgins *et al.* 2000; Bond *et al.* 2003; Bond *et al.* 2005), while frequent fires coupled with low grazing pressure have been shown to prevent sapling establishment (Roques *et al.* 2001).

Fire intensity is also a function of grass biomass (Trollope 1980; Scholes and Archer 1997; Van Langevelde *et al.* 2003) and fuel moisture (Trollope *et al.* 2002): the greater the fuel biomass and the drier the fuel load, the hotter the fire. The timing of the fire will also affect sapling survival. West (1965, cited by Trollope 1982) and Zimmermann *et al.* (2008) suggested that the optimal time would be at the beginning of the rainy season (i.e. at the end of the dry season) when stems are sensitive and trees are about to bud. This is also when fires would be most intense as fuel loads will at their driest (Trapnell 1959; Trollope and Tainton 1986; Wigley *et al.* 2010). Trapnell (1959) noted that, by burning at the end of the dry season, the vegetation “opened up” greatly, resulting in a grass sward

that was denser and taller than before. Consequently, the following burn resulted in even more intense fires and, ultimately, scorching trees up to 1.8 m and more (Trapnell 1959).

Savanna trees are well adapted to survive fire. Saplings, within the flame zone may experience complete top-kill, yet will rapidly resprout from the base of the plant (Schutz *et al.* 2009; Midgley *et al.* 2010; Schutz *et al.* 2011). Once they reach a height between 1.5 and 2 m, depending on species, fire has little effect (Trollope and Tainton 1986). In their review on savanna woody plant dynamics, Midgley *et al.* (2010) state that how fire kills the above-ground biomass of trees is poorly understood. One opinion is that damage to the cambium layer within the stem is the cause of death (Bond and Van Wilgen 1996; Midgley *et al.* 2010). Another opinion is that top-kill may be caused by damage to the phloem (Midgley *et al.* 2010). However, in both these scenarios, death is not fast enough and are unlikely to be the cause of death due to fire damage (Midgley *et al.* 2010). Experimental work done by Balfour and Midgley (2006) and, later, West *et al.* (2016) confirmed a third hypothesis: that top-kill is caused by damage to the xylem, possibly as a result of heat-induced cavitation. The pattern of fire damage matches that of frost damage in that the smaller stems die first and rapidly (within a month) (Holdo 2006; Midgley *et al.* 2010), which, again, excludes the possibility that it is damage to the cambium or phloem that causes top-kill. Holdo (2005) noted that stems with thick and flaking bark are more resistant to fire and frost damage as opposed to thin barked stems (see also Lawes *et al.* 2011).

Generally, fire frequency and fire intensity have no effect on the ability of the grass to recover (Trollope and Tainton 1986; Everson and Everson 2016), although it is possible for fire frequency to change the species composition of a grassland (Fynn *et al.* 2005). Grasses can regrow rapidly after a fire (Linder *et al.* 2017). With the absence of woody tissue, they can mobilise below-ground resources into photosynthetically active tissue, maximising the benefits of pulsed events, such as fire or rainfall (Bond 2008; Linder *et al.* 2017), giving grasses the competitive edge against saplings. Fire may, in fact, improve the standing biomass of grass. In a long-term study in a moist montane grassland, Everson and Everson (2016) demonstrated that whether fires were annual or biennial, grass biomass was higher than the grass biomass in areas protected from fire. While Everson and Everson (2016) do not mention the effect of fire on grass species composition, Fynn *et al.* (2005) noted that timing (winter vs spring) and frequency (annual and biennial vs

triennial) of burning affected species composition with a switch from short to tall grasses in a mesic grassland.

Fire alone is not always able to maintain grasslands or open savannas, but is often advocated to control bush encroachment, particularly in conjunction with herbivory, thus keeping the landscape open (Trollope 1980; Titshall *et al.* 2000; Gordijn and Ward 2014). Frost and fire may also act synergistically to control bush encroachment. Holdo (2005) makes the point that frost, in killing plant tissue, increases the fuel load and, thus, fire intensity.

### **1.2.2.2 Herbivory**

Herbivory plays as important a role in the tree:grass ratio as does fire. It is important, once again, to be cognisant that different species of grass and trees respond differently to herbivory and that different herbivores impact differently on the plants they consume.

#### **1.2.2.2.1 Grazing**

While bulk grazers, such as certain ungulates and bovids, may eat tree seedlings, thereby preventing them from becoming established (Midgley *et al.* 2010), their primary role in the tree-grass dynamic is in the removal of grass biomass (Charles *et al.* 2017). Different grass species respond in various ways to herbivory. For example, Charles *et al.* (2017) demonstrated that productivity of certain grasses may increase under moderate grazing pressure (see also McNaughton (1979); Skarpe (1991); Linder *et al.* (2017)), while some grass forms (stoloniferous vs bunch) are more resistant to heavy grazing pressure (Coughenour 1985), creating short grazing lawns. McNaughton (1979) does point out, however, that grazing limits seed production of grasses. Conversely, in certain circumstances, grazing can weaken the grass sward by reducing belowground biomass (Pucheta *et al.* 2004; Tedder *et al.* 2014), removing competition from grass roots for physical space (Jurena and Archer 2003) and soil moisture (Sankaran *et al.* 2005). However, McNaughton *et al.* (1998) demonstrated that, in the Serengeti National Park, Tanzania, grazing did not reduce grass root biomass. Grazing also removes aboveground grass competition for light (Hagenah *et al.* 2009) as well as adequate fuel load for fire (Trollope 1980; Van Langevelde *et al.* 2003).

#### 1.2.2.2 Browsing

Small browsers limit tree seedling recruitment (Augustine and McNaughton 2004; Sankaran *et al.* 2008; Moe *et al.* 2009), while browsing by mega-herbivores, such as elephant (*Loxodonta africana*) and black rhinoceros (*Diceros bicornis*), impacts on vegetation structure (Prins and van der Jeugd 1993; Augustine and McNaughton 2004; Asner *et al.* 2009). Generally, once established, savanna trees are resilient to browsing as they resprout vigorously (Midgley *et al.* 2010) and the main effects will be a reduction in size (Augustine and McNaughton 2004; Sankaran *et al.* 2008; Staver *et al.* 2009). This, in turn, would affect the rate of transition from one size class to another and thus, potentially, maintaining a tree within the fire zone (Scholes and Archer 1997; Staver *et al.* 2009) and, therefore, susceptible to canopy damage. Riginos and Young (2007), however, noted that browsed saplings of *Acacia* (= *Vachellia*) *drepanolobium* grew twice as tall as protected saplings (see also Vadigi and Ward, 2014) and Stuart-Hill and Tainton (1989) noted that *Acacia* (= *Vachellia*) *karroo* increased browse production with increasing simulated browsing. It is, therefore, not uncommon for plants to reallocate resources in times of stress, such as browsing. For example, a browsed tree may reallocate resources into rapid regrowth to remain competitive at the cost of producing further defences against browsing, such as metabolites or thorns (see for example Herms and Mattson (1992); Pratt *et al.* (2005) Fornara and du Toit (2007)).

Browsing could also impact tree seedling recruitment by influencing flowering, seed production (Louda and Potvin 1995; Goheen *et al.* 2007) and seed dispersal (Tews *et al.* 2004; Midgley *et al.* 2010; Maclean *et al.* 2011). The seeds and pods of many *Vachellia* spp. are eaten by wild and domestic herbivores and the pods may form a substantial part of a herbivore's diet during the dry winter months (Coe and Coe 1987). The large mammalian herbivores tend to act as agents of seed dispersal and, in most cases, also increase the rate of germination, as digestive juices act as a scarifying agent (see review by Or and Ward (2003)).

#### 1.2.2.3 Frost

André *et al.* (2010) suggest that frost is the most significant weather hazard for crops and forests. There are various definitions for frost, most of which state that frost will occur when ambient temperature drops to or below 0°C, measured at a height between 1.25 and 2 m (see e.g. Snyder and de Mel-Abreu (2005)). Possibly, a more appropriate definition

for ecologists has been proposed by Savage (2012): "... [it is] the condition for which the surface and earthbound objects have a temperature at or below 0°C, often resulting in ice on leaves and soil if the temperature of the surface, or air near the surface, is less than or equal to the dewpoint temperature." In South Africa, radiative frosts are most common (Savage 2012). These are a result of radiative cooling (i.e. loss of surface heat) during calm, clear winter nights creating a temperature inversion (Inouye 2000; Schultze 2006; Savage 2012). A white frost (or hoar frost) occurs when water vapour turns to ice on the surface of an object (i.e. when dewpoint temperature is higher than the minimum temperature), whereas a black frost occurs when there is no surface ice (i.e. when dewpoint temperature is less than minimum temperature), but ice forms inside a plant causing the cells to rupture (Snyder and de Mel-Abreu 2005; Schultze 2006; Savage 2012).

Plants react to stress (in this case, chilling or freezing) by tolerance or by avoidance (Levitt 1980; Osmond *et al.* 1987). Plants that are tolerant to stress have mechanisms that permit the plant to maintain metabolic activity when under stress, albeit at a reduced level (Osmond *et al.* 1987). Tolerance increases as a result of exposure to chilling before stress sets in (Taiz and Zieger 2006), a process known as hardening. It is accomplished by changes in the plant's cell biology (Beck *et al.* 2004), for example, by increasing the percentage of unsaturated fatty acid chains within the cell membrane (Taiz and Zieger 2006) which are less likely to solidify at low temperatures (see also Beck *et al.* (2004)). Avoidance of freezing may take the form of dormancy (Osmond *et al.* 1987); dehydration; the development of dense, bulky organs (e.g. thick bark which retards heat exchange) (Schultze 2006); or an accumulation of solutes in the cell sap, such as sugars or proteins, which lower the critical temperature at which freezing will take place (Levitt 1980; Taiz and Zieger 2006).

Many plants of the tropics and subtropics are vulnerable to chilling (Beck *et al.* 2004). Such plants may experience chilling damage at temperatures below 12.5 °C, but generally, most damage occurs when ice forms within the plant tissue (Snyder and de Mel-Abreu 2005) at temperature of 0°C and below. Damage may be tissue specific or specific to particular life stages such as death of apical meristems, reproductive organs, or new leaves, which would impact sapling structure (Silberbauer-Gottsberger *et al.* 1977; Inouye 2000).

The role of frost as an agent of disturbance is seldom considered in African savannas (Holdo 2006; Muller *et al.* 2016). When frost is taken into account, it is, generally, in conjunction with fire. Holdo (2005) found that trees that had been damaged by frost prior to fire, were more likely to experience topkill (see also Holdo 2007). If the frost is severe enough, complete top-kill could result (Brando and Durigan 2005; Muller *et al.* 2016), requiring the sapling to resprout from the base, or death of the whole plant. Whitecross *et al.* (2012) suggest that, in an African savanna, sufficient freeze events will create a freeze trap, maintaining trees and shrubs below their potential size, not unlike a fire or browse trap, although at a different height. In the case of grasses, frost could kill off the entire above-ground portion of the plant resulting in drier fuel loads and, therefore, more intense fires and higher rates of topkill of trees than might be expected (Childes and Walker 1987), but if the tillering node of the grass is not affected, the grass may recover (Snyder and de Mel-Abreu 2005). Muller *et al.* (2016) speculated that frost might be a significant agent of disturbance in arid or semi-arid environments where fires are infrequent and of low intensity (see also Brando and Durigan (2005)).

Elevated atmospheric carbon dioxide (CO<sub>2</sub>) may also affect a plants response to chilling and freezing. Lutze *et al.* (1998) found that frost damage occurred in *Eucalyptus pauciflora* seedlings at higher temperatures when grown under elevated CO<sub>2</sub> concentrations compared to those seedlings grown at current atmospheric CO<sub>2</sub> concentrations.

#### **1.2.2.4 Seed predation**

The larvae of a variety of insects, including Coleoptera, Hemiptera, Lepidoptera and phytophagous Hymenoptera will attack all species of *Vachellia* seeds (Coe and Coe 1987), with contrasting effects on seed viability (Mucunguzi 1995; Or and Ward 2003). The most frequently observed insect predator is the bruchid beetle (Bruchidae) (Coe and Coe 1987; Mucunguzi 1995; Okello and Young 2000; Or and Ward 2003). Experimental work done by Mucunguzi (1995) demonstrated that the germination rate of *V. sieberiana*, in particular, was actually enhanced by bruchid infestation.

### **1.3 Tree-line effect**

The foregoing discussion has covered the drivers of tree-grass dynamics and what, potentially, maintains open savanna systems and grasslands. However, there are instances

of high-altitude grasslands which could support trees, yet trees are absent or, at least, are only present in patchy mosaic patterns. The transition zone from forest or woodland to grassland is often referred to as the treeline. While the term “treeline” suggests an abrupt boundary, the transition, generally, is a gradual one (Körner 1998). These instances have been documented by researchers such as Fensham and Kirkpatrick (1992); Bond *et al.* (2005); Overbeck *et al.* (2007); Bond *et al.* (2008). Various hypotheses have been proposed as to why these grasslands exist. Some of these hypotheses are: anthropogenic activity; shallow, poorly drained soils; frost; and fire (see for example O'Connor and Bredenkamp (1997)). These drivers are not necessarily mutually exclusive and may act synergistically (Wakeling *et al.* 2012). That these grasslands were largely anthropogenically derived has been largely discounted (Meadows and Linder 1993; Coetzee *et al.* 1994; Bond *et al.* 2003). Although fire is commonly accepted as a principal agent for maintaining grasslands (see e.g. Bond *et al.* (2005)), it is surprising that fire-adapted trees, such as certain *Vachellia* spp. are absent in high-altitude grasslands. In his review on high altitude treelines, Körner (1998) reflects that trees are limited by a thermal boundary which, at times, may be coupled with “regional modulatory” agents, such as fire or herbivory, particularly in the Mediterranean and the southern hemisphere (see also Körner and Paulsen (2004)). Körner (1998) suggests that there is a minimum temperature below which cell production and tissue differentiation is inhibited in higher plants. The apical meristems within the tree canopy cannot benefit from radiant energy warming during the day because of convective cooling. Nor can they benefit from the release of stored warmth in the topsoil during the night as the low growing grass species and forbs would (Körner 1998). Consequently, structural growth is blocked. Grasslands of southern Africa are, mostly, not cold enough to invoke the hypothesis that trees are excluded because of thermal boundaries (Bond *et al.* 2003) as many savanna tree species such as *Vachellia karroo* and some of the *Searsia* spp. are frost tolerant. Wakeling *et al.* (2012) suggest a combination of low temperatures and fire in the high-altitude grasslands and that trees, consequently, do not escape the fire trap, whereas in the warmer lower altitudes, the trees grow faster, thereby escaping the fire trap.

There is general consensus that global climate is changing and that it impacts on species distribution (see for example Parmesan and Yohe (2003); Tkemaladze and Makhashvili (2016)). If treelines are, indeed, limited by a thermal boundary which may or may not be



modified by disturbance, then long term climate change will affect the position of the treeline. In their investigation into the upward shift of the treeline in the Swiss Alps, Gehrig-Fasel *et al.* (2007) noted that, while change in land-use was the most significant cause for the shift in the treeline, a small portion of the upward migration of the treeline was due to global warming (see also Harsch *et al.* (2009)). Feeley *et al.* (2011) also recorded an upward migration of the treeline in the Andean mountains of Peru in response to increases in ambient temperature. An indirect consequence of the effect of global warming on the treeline, is the effect on interspecific interactions. Experimental work done by Hillyer and Silman (2010) predicted that seed predation rates would be affected with an upward shift in the treeline. Their model showed that, assuming there is no shift in the range of the seed predators, e.g. rodents, seed survival rate increased significantly at the higher elevations. This in turn would result in an increase in plant population growth rate (Hillyer and Silman 2010). Another experiment done by Alexander *et al.* (2015) investigated novel interspecific competition between plants as a consequence of climate change. They found that, where species did not migrate upwardly, their performance was hampered by those species that did successfully migrate from a lower altitude (Alexander *et al.* 2015). Although Alexander *et al.* (2015) experimented with forbs, the same principle would apply to woody species, resulting in changes in species composition and plant biodiversity.

#### **1.4 Seed dispersal and germination**

The seed pods are either dehiscent (where the pod splits and the seeds are dispersed by wind or merely fall to the ground) or indehiscent (where the pods remain on the tree until eaten by browsers or fall to the ground where they are either eaten by grazers or merely remain there until broken through trampling or fungal degrading and, over time, form a seed bank in the soil) (Sabiiti and Wein 1988; Miller 1994). The seed pods of *Vachellia* are readily consumed by browsers and cattle alike, being high in nutrients (Coe and Coe 1987). The seeds are, thus, dispersed in the dung of the herbivores and germination is aided by the softening of the hard testes of the seed while in the gut and by the destruction of the bruchid beetle (which predate the seeds) within the seed (Coe and Coe 1987; Sabiiti and Wein 1988; Miller 1994). Some *Vachellia* species, such as *V. karroo*, develop a seedling bank, being able to survive in a dense grass sward for up to a year (O'Connor 1995), while other *Vachellia* seeds are capable of remaining dormant in the soil for

“years” (Sabiiti and Wein 1988) and will readily germinate after a fire. Sabiiti and Wein (1988) found that a hot fire significantly increased germination success rate of *V. sieberiana* seeds, probably as a result of two factors: fire will crack open the testa and fire will kill the bruchid larva within the seed, preventing further damage to the content of the seed.

### **1.5 The C<sub>4</sub> photosynthetic pathway and atmospheric carbon dioxide (CO<sub>2</sub>)**

Changing levels in atmospheric CO<sub>2</sub> have elicited much interest, particularly regarding the rise of C<sub>4</sub> photosynthetic pathways and responses of plant growth to the changes. There are three photosynthetic pathways used by plants to assimilate carbon: C<sub>3</sub>, C<sub>4</sub> and CAM. The C<sub>3</sub> pathway is, by far, the most common, while the C<sub>4</sub> pathway is the least commonly used pathway (Ehleringer *et al.* 1991). The great interest in C<sub>4</sub> plants is that they are mostly grasses, dominating warm-climate grasslands and savannas at low altitudes and latitudes (Edwards *et al.* 2010). These biomes are extremely important as primary producers (see for example the reviews by Wand *et al.* (1999); Sage (2004); Grace *et al.* (2006)).

Early plants evolved at a time when atmospheric CO<sub>2</sub> concentrations were high and utilized the C<sub>3</sub> pathway (Ehleringer *et al.* 1991). However, under conditions of low CO<sub>2</sub> concentrations, the rate of photosynthesis is reduced (Ehleringer *et al.* 1991; Cerling *et al.* 1997). Evidence suggests that the C<sub>4</sub> pathway evolved as early as Oligocene epoch (24 – 35 million years ago) (Sage 2004) at a time when CO<sub>2</sub> concentrations were lower than current levels. The C<sub>4</sub> pathway concentrates CO<sub>2</sub>, increasing its photosynthetic efficiency (Sage 2004). An added benefit of the C<sub>4</sub> pathway is that it increases water-use efficiency (Pagani *et al.* 1999). However, the C<sub>4</sub> pathway is energetically expensive and, therefore, operates well in conditions of high light intensity and high temperature (30°C and above) (Ehleringer and Björkman 1977; Ehleringer and Pearcy 1983) whereas the C<sub>3</sub> pathway performs best at temperatures lower than 30°C (Kirschbaum 1994) and can tolerate a lower light intensity. By the early to middle Miocene, CO<sub>2</sub> levels were as low as ~180 p.p.m. and then steady rose to stabilize at around preindustrial levels of ~350 to ~280 p.p.m (Van Der Burgh *et al.* 1993; Pagani *et al.* 1999). However, C<sub>4</sub> grasses rose to dominance only 7 – 8 million years ago, during the late Miocene and Pliocene (Cerling *et al.* 1997; Retallack 1997; Sage 2004) when fire, concurrent with a change in climate,

became a major disturbance (Keeley and Rundel 2005; Beerling and Osborne 2006; Scheiter *et al.* 2012).

Since the industrial revolution, from about 1790 to between 1820 and 1840, atmospheric CO<sub>2</sub> concentrations have been steadily increasing to the current level of 406.82 p.p.m., as measured at Mauna Loa, Hawaii (Tans 2018). A continued increase in CO<sub>2</sub> levels will, in all likelihood, be beneficial for the C<sub>3</sub> plants. Experimental work done by Morgan *et al.* (2007), demonstrated that C<sub>3</sub> shrubs grown at a CO<sub>2</sub> concentration of 720 p.p.m. had a 20 – 40-fold increase in biomass, while the biomass of the C<sub>4</sub> grasses did not respond to the increase in CO<sub>2</sub> concentration. Cerling *et al.* (1997) predict that once CO<sub>2</sub> concentrations exceed 500 p.p.m. the C<sub>3</sub> pathway will be favoured under all conditions, except under extremely high temperatures. Bond and Midgley (2000) proposed that trees would be favoured by increased CO<sub>2</sub> because of an increase in stored carbon, enabling them to resprout after injury even more rapidly than before, rather than CO<sub>2</sub> impacting photosynthetic responses differentially. Glasshouse experiments confirm that *Acacia* (= *Vachellia*) *karroo*, a typical African savanna tree, responds significantly to increased CO<sub>2</sub> concentrations, with increases in root and shoot biomass (Kgope *et al.* 2010). Regardless of the mechanism, elevated CO<sub>2</sub> concentrations will have implications for the future tree (C<sub>3</sub>):grass (C<sub>4</sub>) ratios of savanna and grasslands (see *inter alia* Polley *et al.* (1994); Kgope *et al.* (2010); Bond and Midgley (2012); Bellasio *et al.* (2018)), if the shift to CO<sub>2</sub> driven woody encroachment has not already begun (Buitenwerf *et al.* 2012).

## **1.6 Mechanisms of stress tolerance and avoidance**

Osmond *et al.* (1987) states that every form of disturbance within a plant community will result in stress and, in a stressful environment, plants will exhibit one of two types of behaviour: tolerance or avoidance. The dominating sources of stress are temperature and water availability (Levitt 1980; Osmond *et al.* 1987). The effects of frost on plants is of great economic importance because of its impact on commercial crops and forestry. Consequently, much research has been dedicated to understanding the mechanisms of frost damage and frost acclimation in plants.

### **1.6.1 Freezing**

Snyder and de Mel-Abreu (2005) refer to direct damage within the plant cell protoplasm (intracellular freezing) and to indirect damage within the plant, but not within the cells

(extracellular freezing). The extent of intracellular damage depends on the speed at which the temperature drops (rapid) and may be lethal, while extracellular freezing occurs when temperatures drop slowly and the plant may make full recovery (Levitt 1980; Snyder and de Mel-Abreu 2005). The duration of the freeze event does not influence the extent of the damage. Once ice has formed within the plant tissue, cells are subjected to dehydration and mechanical damage through the collapse of cell walls and the presence of large ice crystals (Wisniewski and Fuller 1999; Snyder and de Mel-Abreu 2005).

### 1.6.2 Freeze tolerance and avoidance

Plant may acquire a certain degree of tolerance to freezing by being exposed to non-lethal temperatures prior to the freeze event (see e.g. Pollock *et al.* (1986); Greer *et al.* (1991); Sung *et al.* (2003)). Tolerance may be lost when temperatures increase, making the plants vulnerable to an unexpected late spring frost. Avoidance appears to be a generalised plant trait, although there is some overlap between the two strategies. Plants may be tender (those which have no mechanism to tolerate or avoid intracellular freezing), slightly hardy, moderately hardy or very hardy (those which have mechanisms that tolerate or avoid intracellular freezing as well as damage caused by desiccation. The stage of phenology is also important as to how well a plant may withstand freezing (Jacobsen *et al.* 2005; Snyder and de Mel-Abreu 2005). The acclimation and avoidance mechanisms can take various forms by, for example:

- Increased solute concentrations (such as sugars, salts of organic acids, proteins, anthocyanins or tannins) within the protoplasm (Levitt 1980).
- Increased fluidity of the plasma membranes by increasing the proportion of unsaturated fatty-acids of the membrane lipids (Levitt 1980; Taiz and Zieger 2006).
- Increased tolerance to desiccation due to plasmolysis (a shrinking) of the protoplasm (Snyder and de Mel-Abreu 2005).
- Development of bulky organs, such as thick bark, with high heat capacity to act as an insulation (Snyder and de Mel-Abreu 2005).
- Supercooling of the plant tissue (Levitt 1980; Wisniewski and Fuller 1999).
- Withdrawal of water from the xylem vessels (to prevent splitting with the onset of freezing) and dormancy (Taiz and Zieger 2006).

- Desiccation of plant material, such as seeds, to survive freezing (Wisniewski and Fuller 1999).

### **1.6.3 Water stress**

The most rapid changes in water availability take place within the top soil and, while water stress is most frequently related to high temperatures, it can also occur when soils are cold or frozen (Osmond *et al.* 1987). Mechanisms employed by plants to avoid water stress include stomatal closure; lower stomatal density; leaf movement; leaf shedding; and extensive and deep rooting systems. Certain drought tolerant plants (e.g. *Acacia* (= *Vachellia*) *tortilis*) have been shown to increase soluble sugars, as well as starch, within the plant tissue (Kebbas *et al.* 2015), thereby reducing water and osmotic potential.

## **1.7 Monitoring vegetation over time**

### **1.7.1 Remote sensing**

Geographic information systems (GIS), coupled with remote sensing, are used extensively to survey current landscapes or to follow the dynamics of landscape change over time. Earlier exercises in remote sensing involved the use of aerial photographs with minimal processing (see for example Trollope *et al.* (1998) O'Connor and Crow (1999); Kakembo (2001); Puttick *et al.* (2011)), although, with the use of a GIS programme, the photographs could be mosaiced into a composite photograph and/or georectified to match other photographs of the same area but of a different time sequences. With minimal processing, the aerial photographs were, generally, inspected manually. Manual classification of aerial photographs is time consuming and not particularly accurate (Carmel and Kadmon 1998; Hudak and Wessman 1998). Consequently, Carmel and Kadmon (1998) advocated using computerised classification of vegetation types on scanned aerial photographs, having first rectified them for radial tilt and relief distortion. The best degree of accuracy was obtained by employing a form of supervised classification, considering, not only the spectral characteristics of individual pixels, but also the characteristics of neighbouring pixels (Carmel and Kadmon 1998). Still using scanned aerial photographs, Hudak and Wessman (1998) standardised brightness and contrast across the set of photographs, as well as grain size, having first rectified them. Thereafter they applied textual analysis, which is a measure of local pixel variance or

textural index, using GIS software (Hudak and Wessman 1998). They found this technique also offered a high degree of accuracy in classifying vegetation types.

Satellite imagery, which has now replaced aerial photography, gives repeated coverage at short intervals and consistent image quality. There are a number of satellites orbiting the earth, but the two most frequently used sensors in the field of vegetation mapping are SPOT 5 and Landsat ETM+. Both are passive sensors, utilising sunlight and thermal radiation reflected off the earth's surface. Combined with normalised difference vegetation index (NDVI), it has been possible to map different vegetation types with satellite images (Justice *et al.* 1985; Batista *et al.* 1997; Archer 2004; Adjorlolo *et al.* 2012), to as fine a detail as to C<sub>3</sub> versus C<sub>4</sub> grasses. As with aerial photographs, satellite images may need correction to overcome scattering and absorption by water vapour and aerosols (Saltz *et al.* 1999; Hayes and Sader 2001). Cloud cover may also prove to be problematic (Tottrup and Rasmussen 2004). However, potential problems are small and thematic mapping, using satellite imagery to reveal spatial distribution of a single topic, has become a popular tool for observing change (see for example Wachendorf *et al.* (2018).

### **1.8 The study area**

Van Reenen's Pass (Figure 1.1) rises from from 1 105 m above sea level to 1 768 m, taking road traffic on the N3 motorway from the province of KwaZulu-Natal up the Drakensberg escarpment into the province of the Free State, South Africa. It also transitions the traveller from Northern KZN Moist Grassland, a sub-escarpment grassland, into Eastern Free State Sandy Grassland, a mesic Highveld grassland (Mucina and Rutherford 2006). The two dominant tree species in the area are *Vachellia sieberiana* var. *woodii* and, from mid-escarpment to the top, *Acacia dealbata*, an invasive alien species. *V. sieberiana* is absent from the top of the pass and at mid-escarpment its range overlaps with that of *A. dealbata*. Historic photographs and farmers' accounts of the area on either side of Van Reenen's Pass suggest that the presence of *V. sieberiana* is a relatively recent phenomenon. Dominant grass species observed over the study period were *Aristida junciformis*, *Cymbopogon excavatus*, *Elionurus muticus*, *Melinis repens*, *Setaria sphacelata* and *Sporobolus pyramidalis* in the mid-escarpment, and *Cymbopogon validus* and *Sporobolus pyramidalis* at the bottom of the escarpment. Both the areas fall within the Norther KZN Moist Grassland. At the high-altitude site, within the Eastern

Free State Sandy Grassland, the dominant grass species were *Aristida junciformis*, *Cymbopogon plurinoides*, *Diheteropogon filifolius*, *Eragrostis racemosa*, *Harpochloa falx*, *Panicum natalense*, *Sporobolus pyramidalis* and *Themeda triandra*.

Mean annual precipitation ranges from 836 mm in the sub-escarpment grassland to 701mm in the Highveld grassland (Mucina and Rutherford 2006). Both biomes receive most of their rainfall in the summer months – October to March – and both experience subzero temperatures in winter with frost and occasional snow in the Highveld. Mean annual temperature (MAT) is 16.2°C and mean annual frost days (MAF) is 20, while MAT is 13.6°C and MAF is 51 days for the sub-escarpment and the Highveld respectively (Mucina and Rutherford, 2006). Three farms were selected to set up field trials. Zandspruit at 1 165m asl (above sea level) (S28° 26' 30.8"; E29° 32' 49.4") was situated at the foot of Van Reenen's Pass and Wyford at 1 350m asl (S28° 24' 20"; E29° 25' 30.7") was about midway up the pass. Both farms fell within the Northern KZN Moist Grassland biome (Mucina and Rutherford 2006), described as a savannoid grassland.

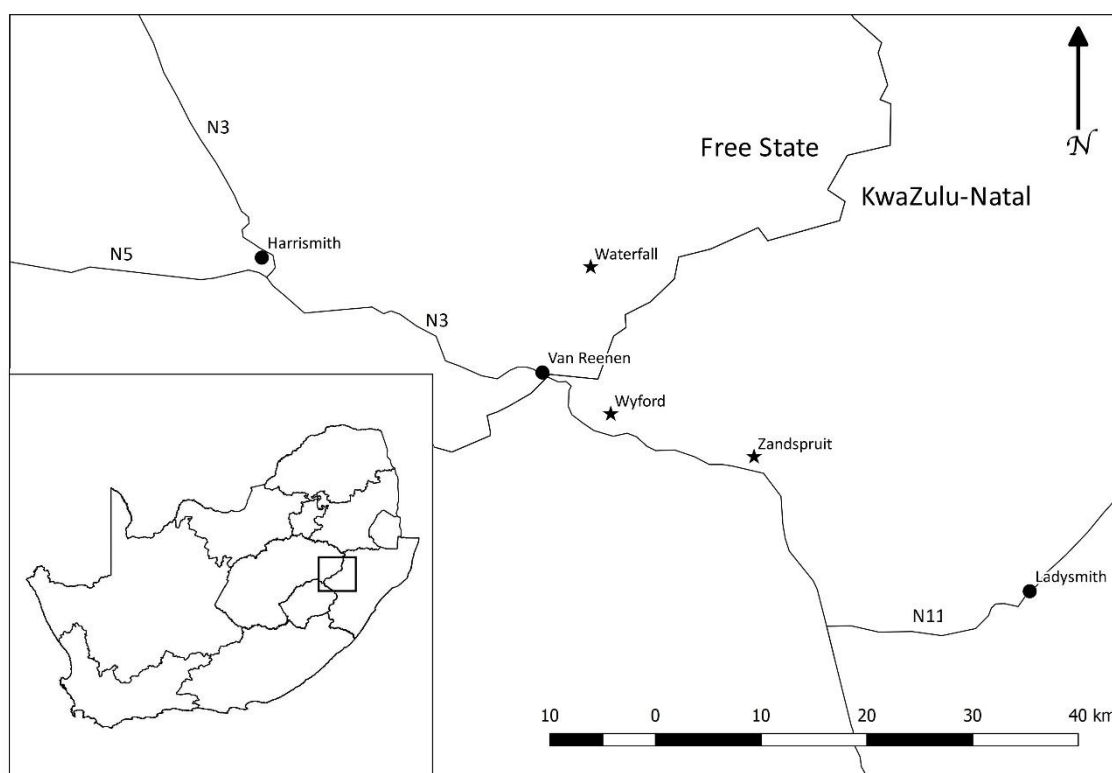


Figure 1.1: Map of KwaZulu-Natal and Free State, showing key towns and the study sites marked with a ★.

Underlying the Northern KZN Moist Grasslands are mudstones, shales, siltstones and sandstone of the Karoo supergroup with dolerite intrusions. The soils are largely litholic, derived from basic rock (Van der Eyk et al. 1969). At Zandspruit the catenal effect of being at the base of the gradient is evident as the soils there are high in nutrients and organic carbon. The third site was on the farm Waterfall at 1697m asl (S28° 16' 50.2"; E29° 24' 29.2), at the top of the escarpment and 11 km from the pass itself. This area fell within the Eastern Free State Sandy Grassland biome (Mucina and Rutherford 2006). *A.dealbata* was absent from the Waterfall site and surrounds. Underlying this biome are mudstones, sandstones and shales of the Beaufort group with pseudo-podzolic soils (Soils Research Institute 1965).

The area has been held in private landownership and extensively farmed since the mid-1800s. Cattle ranching is the primary commercial enterprise combined with limited commercial crop farming. Stocking rates vary according to the season and to annual rainfall, with many commercial farmers owning or renting multiple farms, moving livestock out of, or into, the area as grazing conditions permit (Oates, 2014<sup>1</sup>, pers. comm.; Russell, 2014<sup>2</sup>, pers. comm.; pers. obs.). Non-commercial farmers in the area keep livestock with no regard to carrying capacity and with no opportunity to practice the more traditional form of transhumance which is the seasonal migration of livestock, and the people who tend them, between lowlands and adjacent mountains (for examples of transhumance see for example Rohde *et al.*, 2006; Samuels *et al.*, 2013). There is no coordinated burning regime between land owners in the area. Some land owners burn every year, some do not burn at all (Russell, 2018, pers. comm.<sup>3</sup>).

### **1.9 Thesis structure**

This thesis has been prepared as five chapters. In the second chapter, the extent to which *V. sieberiana*, not only has increased in density, but also has extended its range up an altitudinal gradient in the study area, is explored. Aerial photography has been successfully used to observe changes in landscapes over time (see, for example Britz and Ward, 2007; Russell and Ward, 2014). Accordingly, aerial photographs and satellite

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<sup>1</sup> Mr E Oates, Zandspruit Farm, Sand River, Van Reenen District, KwaZulu-Natal

<sup>2</sup> Mr S Russell, Wyford Farm, Van Reenen District, KwaZulu-Natal

<sup>3</sup> Mr S Russell, Wyford Farm, Van Reenen District, KwaZulu-Natal



images, taken between 1955 and 2013 of the area in the vicinity of Van Reenen's Pass and covering the three study sites, were obtained from the Chief Directorate of National Geo-Spatial Information, Mowbray, South Africa. In this chapter, the photographs are compared and changes in the woody cover quantified, giving an indication of the changes in the vegetation over time.

The aerial photographs show there has been an increase in *V. sieberiana* density and an extension of its range into higher altitudes, although there was no evidence of *V. sieberiana* at the top of the escarpment. The composition and structure of grasslands and savannas are continually being influenced by biotic drivers such as interspecific competition and herbivory and by abiotic drivers such as climate and fire. Field trials, therefore, were undertaken along an altitudinal gradient, investigating the effect of fire, frost and simulated grazing on growth rates and survival of *V. sieberiana* saplings to ascertain whether their exclusion was a result of environmental conditions or management strategies. Chapter three describes the trials done at three sites of different altitudes and the outcome of these trials.

The effect of frost on vegetation structure is frequently overlooked as an agent of disturbance (Whitecross *et al.*, 2012). In chapter four the role of frost on recovery rates and survival of *V. sieberiana* saplings is described. Experiments were performed in a controlled environment on the effects of white frost and black frost on the saplings. Preliminary investigations into avoidance strategies of *V. sieberiana* saplings and seed viability after freezing were also conducted.

Chapter five is the discussion which draws together the preceding three chapters and draws conclusions. Suggestions for further investigation are also made.

## Chapter 2

Aerial photographs track woody cover changes on Van Reenen's Pass, KwaZulu-Natal, South Africa, over a 60-year period, with particular focus on *Vachellia sieberiana* var. *woodii*.

This chapter has been submitted to the *African Journal of Range & Forage Science* and has been formatted accordingly. It is currently under review.

### Chapter 3

*Vachellia sieberiana* var. *woodii*, a high-altitude encroacher: the effect of fire, frost, simulated grazing and altitude in north-western Kwazulu-Natal, South Africa.

This chapter has been submitted to *Global Change Biology* and has been formatted accordingly. It is currently under review.

### Chapter 4

Frost tolerance in *Vachellia sieberiana* var. *woodii* (formerly *Acacia sieberiana* var. *woodii*).

This chapter has been submitted to *Applied Vegetation Science* and has been formatted accordingly. It is currently under review.

### Chapter 5

#### Discussion

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**CHAPTER 2: AERIAL PHOTOGRAPHS TRACK WOODY COVER CHANGES ON VAN REENEN'S PASS, KWAZULU-NATAL, SOUTH AFRICA, OVER A 60-YEAR PERIOD, WITH PARTICULAR FOCUS ON *VACHELLIA SIEBERIANA* VAR. *WOODII***

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**Abstract**

Bush encroachment into grasslands and savannas is a cause for concern as it impacts on the primary productivity of these areas. The grasslands of South Africa are not exempt from this phenomenon. *Vachellia sieberiana* var. *woodii*, a typical savanna tree, has been encroaching into the grasslands along the escarpment of the Drakensberg, KwaZulu-Natal, South Africa, over several decades. We acquired aerial and satellite images covering the Van Reenen's Pass area, north-western KwaZulu-Natal, dating from 1955 to 2015. Three specific areas were examined: a low-altitude site, a mid-altitude site and the top of the escarpment. These images showed that *V. sieberiana* was increasing in density along the escarpment of the Drakensberg. At the low-altitude site, 1.6% of the area could be considered encroached by *V. sieberiana* by 2015. At the mid-altitude site, the area that was encroached had reduced. This was due to clearing of alien species, but the number of *V. sieberiana* trees had increased. On the top of the escarpment, < 1% of the site could be considered encroached. This was due to the presence of alien trees. There were no *V. sieberiana* present on top of the escarpment, despite the successful establishment of the other tree species. It also appeared that *V. sieberiana* was extending its range into higher altitudes. We recommend further experimental and field studies to ascertain the cause of these changes in land-cover.

**Key words:** Aerial imagery, bush encroachment, grasslands, tree-line, *Vachellia sieberiana*

## 2.1 Introduction

Bush or woody plant encroachment into open savanna and invasion into grasslands has become a worldwide phenomenon (see *inter alia* Eldridge *et al.* 2011). The grasslands of Africa are not exempt and, as early as the 1920s, Bayer (1933) noted woody invasion into the grasslands of KwaZulu-Natal (KZN), South Africa. In their study on the dynamics of bush encroachment in an African savanna, Roques *et al.* (2001) considered that, at 40% woody cover, an equilibrium between woody plants and grasses should have been attained within a mesic savanna with an annual rainfall of 675mm. In a semi-arid savanna, equilibrium would be reached at a lower percentage cover (Meyer *et al.* 2007). In a mesic savanna, therefore, woody cover above 40% (or 31% in a semi-arid savanna) would be considered encroachment.

Impacts of woody encroachment may include soil erosion caused by the loss of herbaceous cover (Bayer 1933; Martin and Morton 1993) or underground piping as the result of stemflow (Grellier *et al.* 2012); a loss of faunal diversity (Sirami *et al.* 2009; Stanton *et al.* 2017; Leitner *et al.* 2018), but see Blaum *et al.* (2009); a change in composition of the herbaceous layer (Gibbens *et al.* 2005; Mugunga and Mugumo 2013); a decrease in below-ground carbon storage (Coetsee *et al.* 2013); reduction in primary production (Smit 2004); and a change in soil hydrology (Jackson *et al.* 2005; Honda and Durigan 2016). Conversely, a moderate increase in woody cover may be beneficial to the environment (Maestre *et al.* 2016). Soil fertility may improve as a result of leaf litter and defecation from animals sheltering within and below woody plants (Mugunga and Mugumo 2013). An increase in above-ground carbon storage (Jackson *et al.* 2005; Coetsee *et al.* 2013) and increased water infiltration (Dunkerley 2002) have also been described. However, the consensus is that, in an African savanna, woody encroachment results in overall degradation of the environment.

Anecdotal (Russell, 2014, pers. comm.<sup>4</sup>; personal observation) and photographic evidence (Figure 2.1) show that *Vachellia sieberiana* var. *woodii* (formerly *Acacia sieberiana* var. *woodii*) is not only increasing in density within southern African savannas, but is also invading high-altitude grasslands i.e. the treeline is moving up an

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altitudinal gradient. This observation is supported by Grellier *et al.* (2012) who reported both an increase in *V. sieberiana* along the Drakensberg escarpment of KZN, as well as a tree-line shift into higher altitudes. Tree-line migration in other parts of the world has been documented by, *inter alia*, Gehrig-Fasel *et al.* (2007) and Lutz *et al.* (2013) and the phenomenon has been reviewed by authors such as Grace *et al.* (2002) and Harsch *et al.* (2009). The general opinion is that the tree-line is controlled by a thermal boundary (Körner 1998) with moderating factors such as herbivory (Cairns and Moen 2004), soil nutrients (Wakeling *et al.* 2010) and increasing atmospheric CO<sub>2</sub> (Buitenwerf *et al.* 2012).

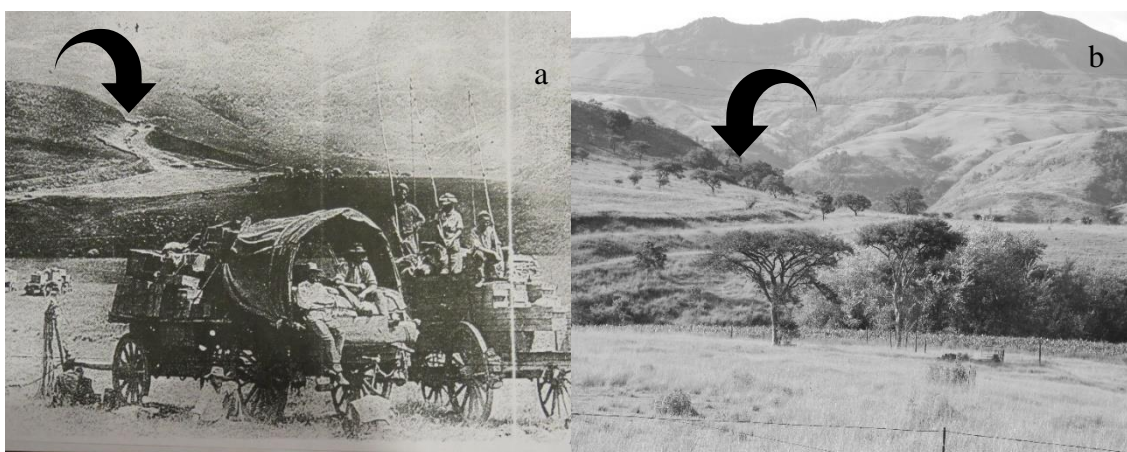


Figure 2.1: Repeat photographs taken of the original Van Reenen's Pass at Wyford Farm, KwaZulu-Natal in a) c. 1890 (photograph supplied courtesy of S Russell) and b) 2014. The camera was brought forward to replicate the photograph in 2014 as trees obscured the view of the pass. The arrows indicate the cutting on the hillside. In 1890 photograph no *Vachellia sieberiana* trees are evident. A number of *V. sieberiana* are present in the photograph taken in 2014. In the valley and drainage lines behind the pass in b) dense stands of *Acacia dealbata* are visible.

*Vachellia sieberiana* is described by Boon (2010) as a deciduous, flat-topped savanna tree. It is widespread in KZN, sometimes forming pure stands in wooded grasslands. They are a food source for game and cattle. High in hydrocyanic acid, the pods may be consumed sparingly. An iconic tree of the South African savannas, it is increasingly regarded by stock farmers as a detrimental encroacher (Oates<sup>5</sup>, 2014, pers. comm.;

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<sup>5</sup> Mr E Oates, Zandspruit Farm, Sand River, Van Reenen District, KwaZulu-Natal

Russell<sup>6</sup>, 2014, pers. comm.), impacting on grass species composition and, consequently, grass palatability for grazing livestock and game (Mugunga and Mugumo 2013).

To assess the encroachment of *V. sieberiana* in the Van Reenen's Pass area of north-western KZN, we examined a series of aerial photographs taken of the area between 1955 and 2013. Analysis of aerial photographs and, particularly, of satellite images is commonly undertaken to monitor environmental change over time. Various techniques have been employed, some more sophisticated than others: from manual comparisons, using aerial photographs (Eckhardt *et al.* 2000; Russell and Ward 2014b), sometimes in combination with textual analysis (Hudak and Wessman 1998), to Advanced Very High Resolution Radiometry (Batista *et al.* 1997) and Normalised Difference Vegetation Indices (NDVI) (Adjorlolo *et al.* 2012) using satellite imagery. We used primarily aerial photographs which predate satellite imagery and, therefore, allowed us to observe landscape changes, if any, over a longer period. We anticipated that we would observe: 1) an increase in tree density in areas that were already open woodland and 2) that the tree-line of *V. sieberiana* would advance up the altitudinal gradient over time.

## 2.2 Methods

### 2.2.1 Study site

Van Reenen's Pass crosses the Drakensberg escarpment from the province of KZN into the province of the Free State, at the village of Van Reenen. The pass itself rises from 1 105m above sea level (asl) to 1 768m asl. The vegetation below the escarpment has been classified as Northern KZN Moist Grassland, a sub-escarpment grassland, with a mean average precipitation (MAP) of 836 mm (Mucina and Rutherford 2006). On top of the escarpment the vegetation is classified as Eastern Free State Sandy Grassland and is a little drier than below the escarpment with a MAP of 701mm (Mucina and Rutherford 2006). Three localities on the pass were examined: at the foot of the pass incorporating the farm Zandspuit at 1 165m asl (S28° 26' 30.8"; E29° 32' 49.4"); approximately half way up the pass, on the farm Wyford at 1 350m asl (S28° 24' 20"; E29° 25' 30.7"), and on top of the escarpment including the farm Waterfall at 1697m asl (S28° 16' 50.2"; E29°

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24' 29.2). All three localities receive the bulk of their rain in the summer months (October to March).

### 2.2.2 Data acquisition and preparation

Aerial photographs and satellite images of the Van Reenen's Pass area, taken between 1955 and 2015, were obtained from the Chief Directorate of National Geo-Spatial Information, Mowbray, South Africa (Table 2.1). Using GIS software, ArcGIS 10.2®, the photographs were georeferenced against the 2015 satellite image and the portion to be analysed clipped. Aerial photographs have inherent errors which need to be recognised. Variations in scale may occur when the topography is highly variable (Anders and Byrnes 1991). As a result, features closer to the camera lens, such as crest lines, appear at a larger scale than those features further from the lens. Another source of error occurs when the camera is not parallel to the earth's surface, causing tilt distortion (Anders and Byrnes 1991) as the aircraft pitches and yaws in air turbulence. Despite these drawbacks, the use of aerial photographs offers certain advantages over satellite imagery. Predating the use of satellites, aerial photographs are particularly useful in the field of long-term landscape change detection (Kakembo 2001; Fensham and Fairfax 2003). We used the spline transformation method with a minimum of 10 ground control points (GCPs) per photograph to smooth out distortion caused by radial tilt of the camera and/or the topography of the terrain. The optimum number of GCPs proved to be between 20 and 40. Beyond 40 GCPs further distortion of the photograph occurred. Areas of high reflectance could not be smoothed out without losing detail and resolution between the different time series was not consistent. In some years, certain areas had been burnt prior to the photographs being taken, adding to the inconsistencies between the time series already mentioned. Also, because of the heterogeneity of the landscape, geometric accuracy was not possible, despite using spline transformation in the georectification of the photographs. Consequently, the use of computerised classification or change detection was not possible. We, therefore, chose to analyse our aerial photographs manually, placing 50 x 50 m grids over the areas of interest. The areas of interest at the three localities each amounted to 800 ha. Our classification was based on vegetation structure i.e. woody versus herbaceous cover, and estimations of percentage woody canopy cover within each cell was based on pixel darkness and the shape of the objects. Canopy cover is defined by Trollope *et al.* (1990) as "the proportion of the ground area [expressed as a



percentage] covered by the vertical projection of the plant canopy”. Woody cover estimations were grouped into five categories: 1) 0 cover; 2) woody cover between 1% and 24%; 3) woody cover between 25% and 49%; 4) woody cover between 50% and 74%; and 5) woody cover above 75%.

Table 2.1: A list of aerial photographs and the satellite image supplied by the Chief Directorate of National Geo-Spatial Information

Date of photographs		Scale of photographs
Year	Month	
1955	July	1:36 000
1964	May	1:40 000
1975	April/June	1:30 000
1987	May	1:50 000
1992	June/July	1:50 000
2000-2001	August/February	1:30 000
2013	Not supplied	Satellite image

## 2.3 Results

Aerial photographs from 1987 were not included in the analysis as the resolution was too poor to discern features with any confidence. Photographs used were, therefore, taken in 1955, 1964, 1976, 1992, 2000 and the satellite image of 2015. The entire clipped photograph was analysed.

### 2.3.1 Zandspruit (1 165m asl)

The groves of introduced trees such as of *Carya illinoensis* (pecan trees) and *Populus* sp. (poplar trees) were conspicuous and thus ignored. Estimations of woody cover included, therefore, only *V. sieberiana*. In 1955 *V. sieberiana* was present at the Zandspruit site has a light scattering of trees with canopy cover well below 25% (Figure 2.2a). Thereafter, there was a steady increase in spread and in density, except for the period between 1976 and 1992 where the loss of canopy cover exceeded the gains (Figure 2.2a). The small decrease in woody cover may be a consequence of the severe drought experienced throughout the whole of KZN during the 1980s. By 1976, canopy cover, in small, select areas, began to approach 50% cover, i.e. woody encroachment was becoming evident and by 2000 certain portions of the area of interest had reached 50 – 75% woody cover. This trend continued through to 2015 with continued increase in the number of individual trees, as well as increased density of stands of *V. sieberiana* (Figure 2.2b). In 1955, the Zandspruit area of interest could be considered open woodland as canopy cover

of *V. sieberiana* was less than 25% on 6.2% of the 800 ha and between 25% and 49% on 0.6% of the area. By 2015, *V. sieberiana* covered 38.59% of the area, of which 1.6% was severely encroached by *V. sieberiana* with canopy cover exceeding 50%. At this point the Zandspruit area could be still be considered as open woodland, but with heavily encroached patches.

Physical inspection of the area highlighted that there were many more *V. sieberiana* trees than could be discerned from the aerial photographs. The shorter trees were trapped within the grass layer and were mown or burnt every year, never reaching full height and not discernible from the air. Consequently, the percentage land encroached is much greater than indicated in the aerial photographs and the satellite image.

### **2.3.2 Wyford (1 350m asl)**

It was not possible to distinguish with confidence exotic trees from *V. sieberiana* in the aerial photographs of the area of interest incorporating Wyford (Figure 2.3a and b). Therefore, all woody cover was included in the analysis.

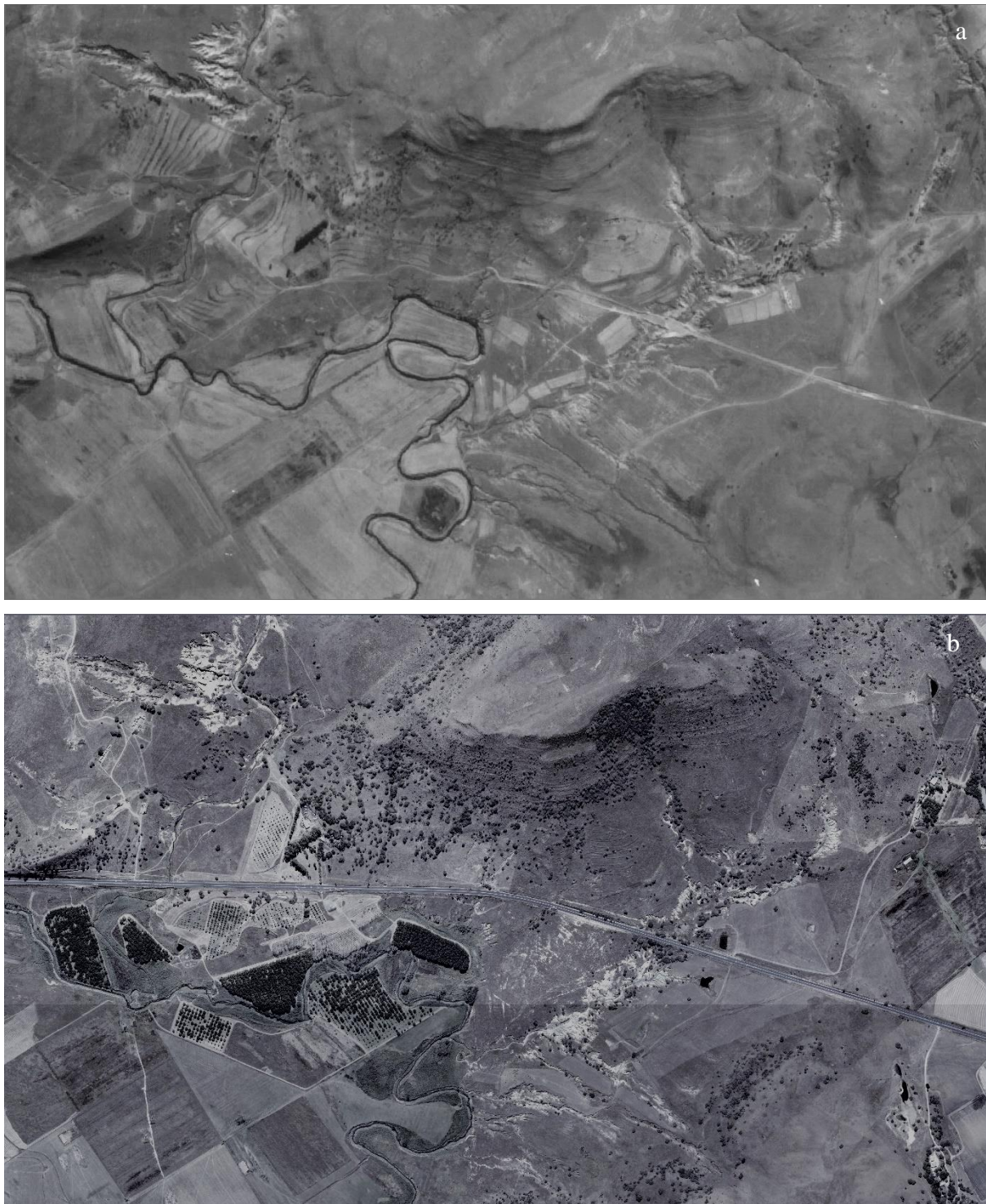


Figure 2.2: a) Aerial photograph of the Zandspruit area, KwaZulu-Natal taken in 1955 and b) a satellite image of the same area taken in 2015. There is a marked increase in *Vachellia sieberiana*. The groves of *Carya illinoensis* can be distinguished in the bottom left quadrant of b).

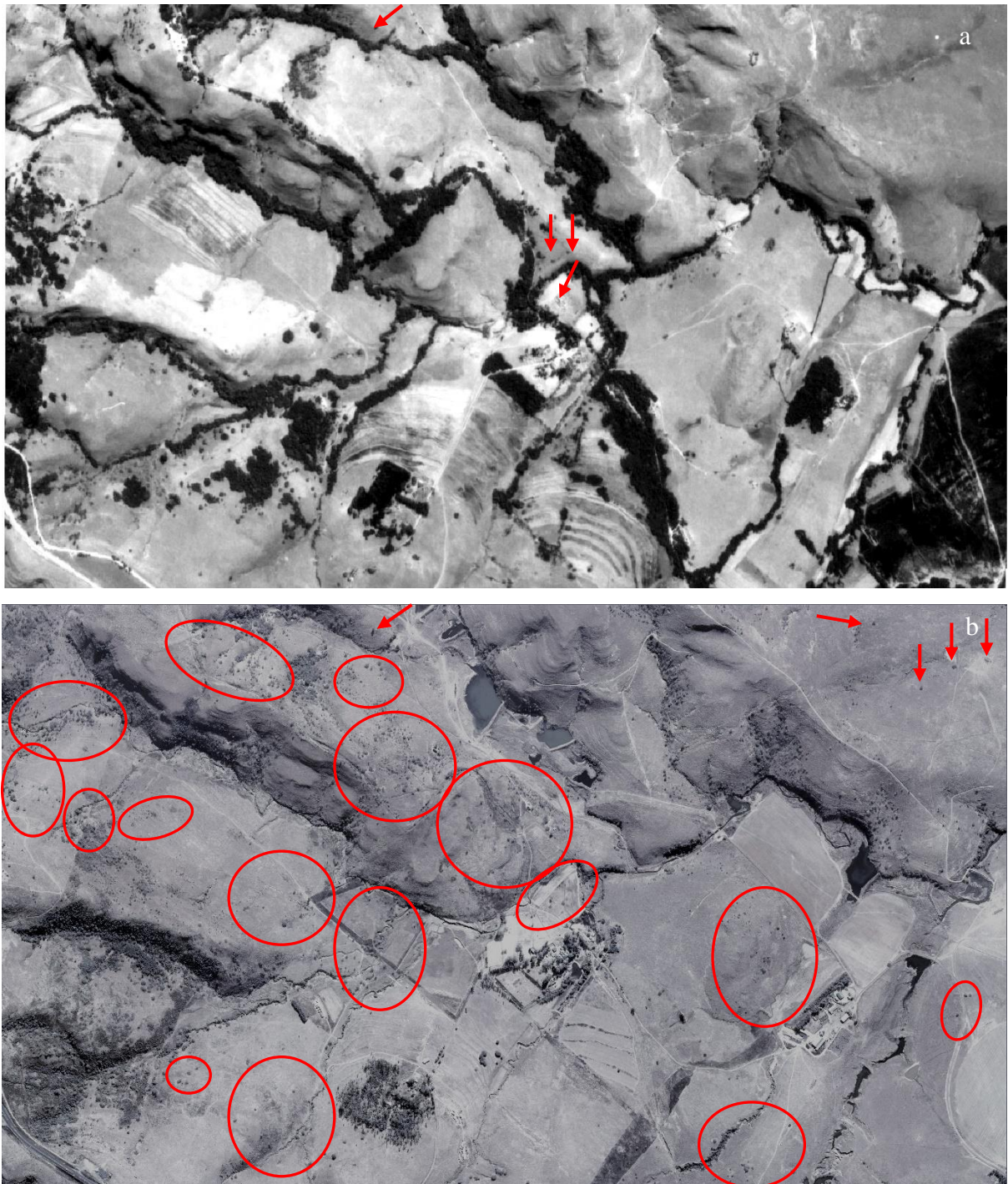


Figure 2.3: a) Aerial photograph of the Wyford area, KwaZulu-Natal taken in 1955 and b) a satellite image of the same area taken in 2015. The reduction in general woody cover between 1955 and 2015 is evident as is the increase in *Vachellia sieberiana* in b). The position of *V. sieberiana* has been marked with a circle or an arrow.



Figure 2.4: a) Aerial photograph of the Waterfall area, Free State taken in 1955 and b) a satellite image of the same area taken in 2015. Woody cover in both a) and b) is largely *Eucalyptus* sp. around homesteads. In the 2015 image firebreaks are evident along the district roads.

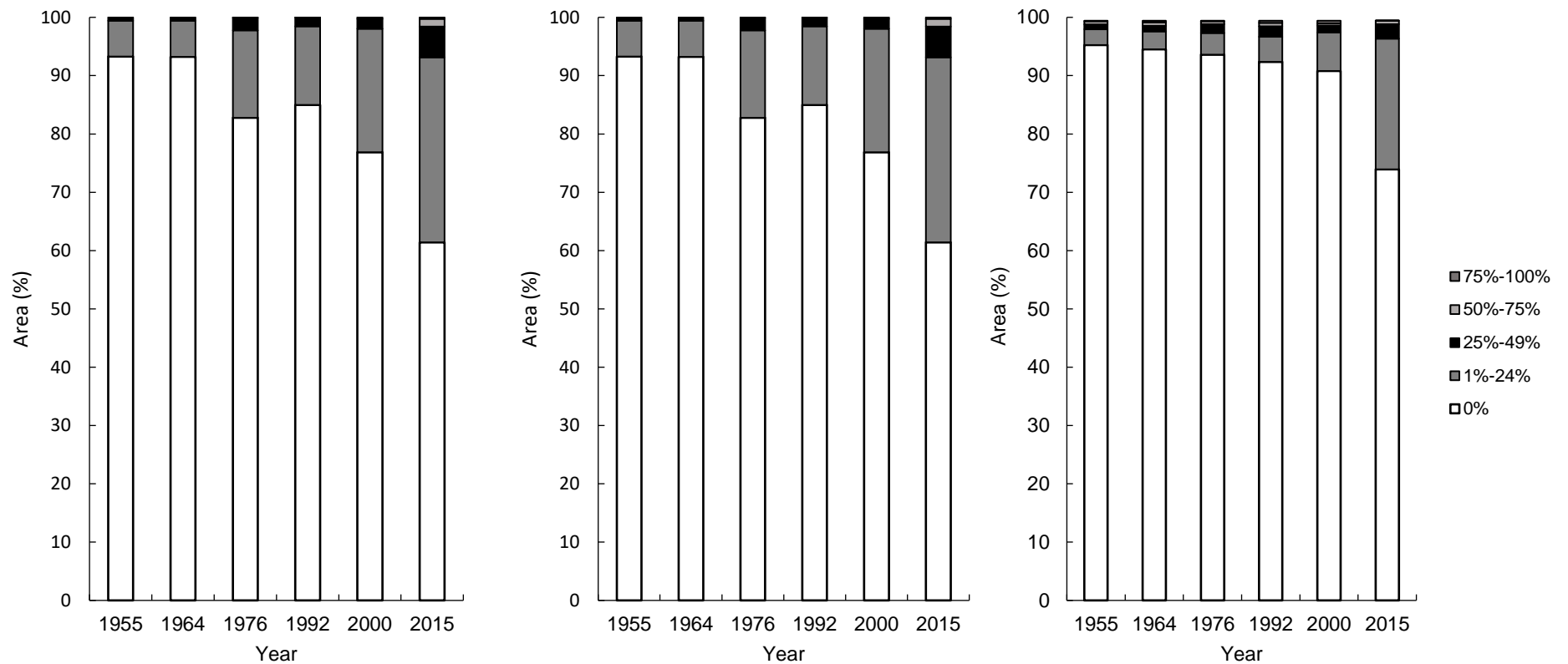


Figure 2.5: Changes in total woody cover between 1955 and 2015 in the district of Van Reenen. a) The Zandspruit area, KwaZulu-Natal (KZN) (1165m asl), showing the gradual increase in cover in all cover density categories with the exception of 1992, when there was a decrease in cover. b) The Wyford area, KZN (1350 m asl). Woody cover remained fairly static until after 1992, when there was a drive to eradicate alien tree invasion. Thereafter, there was an increase in the lowest cover density category (1-24% woody cover). c) The Waterfall area, Free State (1697m asl). Overall change was a thinning in encroached areas (> 49%).

Overall, woody cover fluctuated during the study period and showed no consistent trend (Figure 2.5**Error! Reference source not found.**b). In 1955, 23.9% of the 800 ha had only a light woody cover (between 1% and 24%), and 9.3% of the area could be considered encroached (> 50% woody cover). Initially, the woody cover was found almost exclusively in the drainage lines, in disturbed areas such as the original wagon track up the escarpment, and around homesteads, with woody cover being almost absent on the exposed hillsides and crest lines. *Acacia dealbata* (wattle), in particular, was very invasive, radiating out from the drainage lines (J Russell, pers. obs.). During the 1990s a concerted effort was made by government agencies and landowners to eradicate *A. dealbata* which would explain the decrease in woody cover in all cover classes by the year 2000 (see Figure 2.5b). However, the satellite image of 2015 showed that much of the *A. dealbata* is regenerating from cut stumps. The effect of the 1980s drought was not evident here, possibly as the trees were mostly in sheltered areas. The numbers of the few isolated trees present in the open grassy slopes appear to be stable until the year 2000 onwards. By 2015 the numbers of isolated trees had increased and were beginning to coalesce into small dense clumps. After the year 2000 there was evidence of regrowth in many of the cleared sites resulting in an increase in overall cover but still with further reduction of woody cover in the encroached area (50 – 100% cover reduced from 11.1% in 1976 to 2.6% in 2015).

Inspection of the area showed that the individual trees on the open grassy slopes, of which many eventually formed dense clumps, were *V. sieberiana*. The *V. sieberiana* did not form mixed stands with *A. dealbata* or other trees. The trees around the homesteads were *Eucalyptus* sp., providing windbreaks, and ornamental trees. As with the Zandspruit site, encroachment by *V. sieberiana* is potentially a greater problem than is evident from the air.

### 2.3.3 Waterfall (1 697m asl)

There was very little woody cover in this area (Figure 2.4a and b). Most of the woody cover was in the vicinity of homesteads. In 1955, 3.5% of the 800 ha had light woody cover (< 50%) and 0.7% could be considered encroached (> 50% woody cover). Between 1955 and 2015, the area with woody cover steadily increased with a very marked increase in light cover by 2015 and a decrease in the area that was encroached (22.4% and 0.03% respectively) (Figure 2.5c). The rapid increase in the area with light woody cover (< 25%)

between 2000 and 2015 was largely due to the thinning of existing dense stands of trees, possibly as the result of devastating fires that swept through the area in 2014, but the overall increase in tree density was a result of planting and thickening of wind breaks and ornamental trees around homesteads. Physical inspection of the area showed that most of the trees were *Eucalyptus* sp. and *Populus* sp., which provided wind breaks. Along the water course *Leucosidea sericea* was present. There were no *V. sieberiana* trees in the area and the open grassy slopes and ridges formed pure grasslands.

## 2.4 Discussion

The invasion of native trees into grasslands is not unique. In North America, Knapp *et al.* (2008) identified several grasslands over a wide rainfall gradient that had been invaded by native woody plant species. Skowno *et al.* (2016) tracked woody encroachment into South African savannoid grasslands between the years 1990 and 2013, using satellite imagery. A pattern of encroachment by *V. sieberiana*, similar to our observations, was also described by Grellier *et al.* (2012), working along the Drakensberg foothills, 45 km to the south-west of Van Reenen's Pass, although encroachment commenced later than observed in the Van Reenen's Pass area.

There is a degree of inaccuracy using aerial photographs to estimate tree density and it can be assumed that individual trees below a certain canopy diameter will not be detected, depending on the resolution of the photograph. Russell and Ward (2014b) found that the mean minimum detection threshold was 2.88 m<sup>2</sup> (SD = 1.09). Other researchers recorded detection thresholds of 4 m<sup>2</sup> (Le Roux, 1999, cited by Hottman and O'Connor, 1999), 2.9 – 3.3 m<sup>2</sup> (Robinson *et al.* 2008) and 3.8 m<sup>2</sup> (Browning *et al.* 2009). Aerial photographs also cannot reveal changes in the composition nor structure of the vegetation (Hottman and O'Connor 1999). Despite these limitations, as well as those described previously, the use of aerial photographs in change detection on a landscape scale is justified. As we sought to describe changes in general trends and patterns over time, one could compensate for these limitations using manual classification.

Our aerial and satellite imagery showed that, while both areas below the escarpment (Zandspruit and Wyford, respectively) are classified as grassland (Mucina and Rutherford 2006), by 1955 these areas could be described as savanna. In 1955, the dominant tree at Zandspruit was *V. sieberiana* at low density (< 25% cover). For the next 60 years *V.*



*sieberiana* density increased steadily, eventually forming dense stands of mature trees within the area. At Wyford, woody cover provided by *A. dealbata* increased steadily until the 1990s when various eradication programmes were implemented. Consequently, the presence (and eradication) of *A. dealbata* in the Wyford area of interest obscured any general trends. However, the number of *V. sieberiana* trees in the Wyford area appeared to remain unchanged from 1955 until 2015, when there was a noticeable increase in *V. sieberiana*. This could be interpreted as a shift of the tree-line towards higher altitudes. At Waterfall (at the top of the escarpment), there was no evidence of *V. sieberiana* in the photographs. This was confirmed by a ground inspection.

Invasion of woody species into grasslands is ascribed to drivers such as changes in climate, fire intensity and frequency, herbivory and atmospheric carbon dioxide concentrations. Determinants of land-cover change seldom act in isolation. While there may be an overarching driver, there will generally be other modifying drivers.

Rainfall is often considered to be a dominant determinant of woody cover. Sankaran *et al.* (2005) suggested that woody cover was constrained when mean annual rainfall (MAP) was less than 650 mm. However, in all our three study areas, MAP is above 650 mm and, therefore, any further increase in MAP would not explain the increase in the density of *V. sieberiana* at both the areas around Zandspruit and Wyford. Neither is rainfall a constraint in the Waterfall area as there, too, it is more than 650 mm per annum and, yet, *V. sieberiana* is absent. Ambient temperature may also limit the presence of woody plants, particularly those of subtropical origin which are not tolerant of frost. Mean annual temperature (MAT) below the escarpment is 16.2°C with 20 mean annual frost (MAF) days, while in the area around Waterfall, at the top of the escarpment, MAT is 13.6°C and MAF is 51 days (Mucina and Rutherford 2006). Wakeling *et al.* (2012) recorded that *V. sieberiana* experienced frost damage at high altitudes and, where temperature dropped to -8°C, frost mortality. Temperature data recorded at the Van Reenen police station and obtained from South African Weather Services indicated that, as a rule, minimum temperatures in the Van Reenen area did not drop below -5°C. Very little is known about the limits of frost tolerance in *V. sieberiana*. It is, therefore, not possible to state whether -5 °C is cold enough to exclude them and climatic records for Van Reenen are not sufficiently complete to ascertain whether there have been changes in climatic conditions since 1955. Records from Dundee, KwaZulu-Natal, 80 km to the east-north-east of Van

Reenen's Pass suggest a slight overall increase in MAT and a decrease in MAF between 1873 and 2012 (Russell and Ward 2014a). It is possible that the area in the vicinity of Van Reenen and surrounds has followed the same climatic trend. Shifts in montane tree-lines have also been attributed to changes in ambient temperature (see for example Körner, 1998 and Harsch *et al.*, 2009). However, trees in the mesic grasslands of South Africa are not excluded by cold as these grasslands are well below the global tree-line (Bond *et al.* 2003). Temperatures in the Waterfall area are lower than those at Wyford and Zandspruit, with more frost days (JR, unpublished data). It is possible that this could have a bearing on the absence of *V. sieberiana* in the area, although certain introduced trees and *L. sericea* are present as they are better adapted to withstand frost. While minimum temperatures at Wyford and Zandspruit (which were not as low as at Waterfall) may not have influenced tree density in those two areas, an increase in the length of the growing season could enable saplings to escape the fire zone and, thereby, reach reproductive maturity.

In their review, Bond and Keeley (2005) demonstrated that fire has the potential to exclude woody cover in grasslands that have the climatic potential to support forest. Consequently, many of the grasslands in South Africa, particularly those east of the Drakensberg, cannot be described as true grasslands, but are maintained by fire (see for example Acocks (1953), cited by Bond *et al.* 2003). In simulation studies, Bond *et al.* (2003) confirmed that woody plants are excluded from these mesic "false" grasslands by fire (see also Bond *et al.* 2005). Most savanna trees are fire tolerant and rapidly coppice after a fire. However, a hot fire will cause top-kill in saplings, which could trap the trees within the fire zone if fire return frequencies are adequate (Higgins *et al.* 2000). Land-use in the vicinity of Van Reenen's Pass is primarily commercial stock farming. It is possible that the fire return period is not optimal for control of woody plant encroachment. However, the Waterfall area is also commercial rangeland with infrequent fires, yet *V. sieberiana* does not occur in this area.

Herbivory frequently acts together with fire in maintaining grasslands and open savannas. Browsing, in conjunction with fire can reduce woody plant biomass (Trollope 1974; Van Langevelde *et al.* 2003; Staver *et al.* 2009) and, ultimately, an encroached grassland could revert to its former state. Augustine and McNaughton (2004) found that the presence of mixed native browsers in commercial rangelands had a marked impact on woody plant

biomass. Farming in the district around Van Reenen, above and below the escarpment is extensive cattle ranching. Except for the occasional flock of goats, there are no domestic browsers of significance in the area which could have had an inhibitory effect on woody plant encroachment. There are, however, small herds of native browsers, such as kudu (*Tragelaphus strepsiceros*), which do browse on *V. sieberiana* saplings (personal observation). However, their numbers are too few to impact on tree density. Cattle are bulk grazers, effective in reducing fuel loads (i.e. the grass biomass) and, consequently, reducing fire intensity. The cooler fires have less impact on woody plants (Van Langevelde *et al.* 2003), particularly on *V. sieberiana* which is fire adapted. Consequently, with fire return period less than optimal, coupled with less intense fires, *V. sieberiana* has become a significant encroacher.

In the recent years, the increase in atmospheric carbon dioxide and its potential impact on vegetation has created great interest (see for example Rogers *et al.* 1994; Bond and Midgely 2000; Donohue *et al.* 2013). While some researchers favour the hypothesis that the rise in atmospheric carbon dioxide is behind, at least in part, the global increase in woody plant encroachment (Bond and Midgley 2012), other researchers advise caution (Archer *et al.* 1995; Körner 2006). In the Van Reenen's Pass area, one could speculate that the increase in *V. sieberiana* in the two lower areas, below the escarpment, is due to increasing carbon dioxide and that temperature is overriding the effect in the area around Waterfall.

## **2.5 Conclusion**

Aerial photographs confirm that *V. sieberiana* is encroaching into the high-altitude grasslands in the vicinity of Van Reenen's Pass. The phenomenon commenced before 1955, but although there are stands of exotic trees at the top of the escarpment, *V. sieberiana* has not established itself in the Waterfall area. It is, therefore, likely that while there may be an overarching global driver, such as changes in climate or atmospheric carbon dioxide concentrations causing the increase in *V. sieberiana*, there are local, modifying factors at play. To understand the reasons behind the increase and to establish management practices that will limit further encroachment, further work in the field needs to be undertaken.

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**Appendix A: AERIAL PHOTOGRAPHS**

**2A.1 Waterfall (1 697 m asl)**





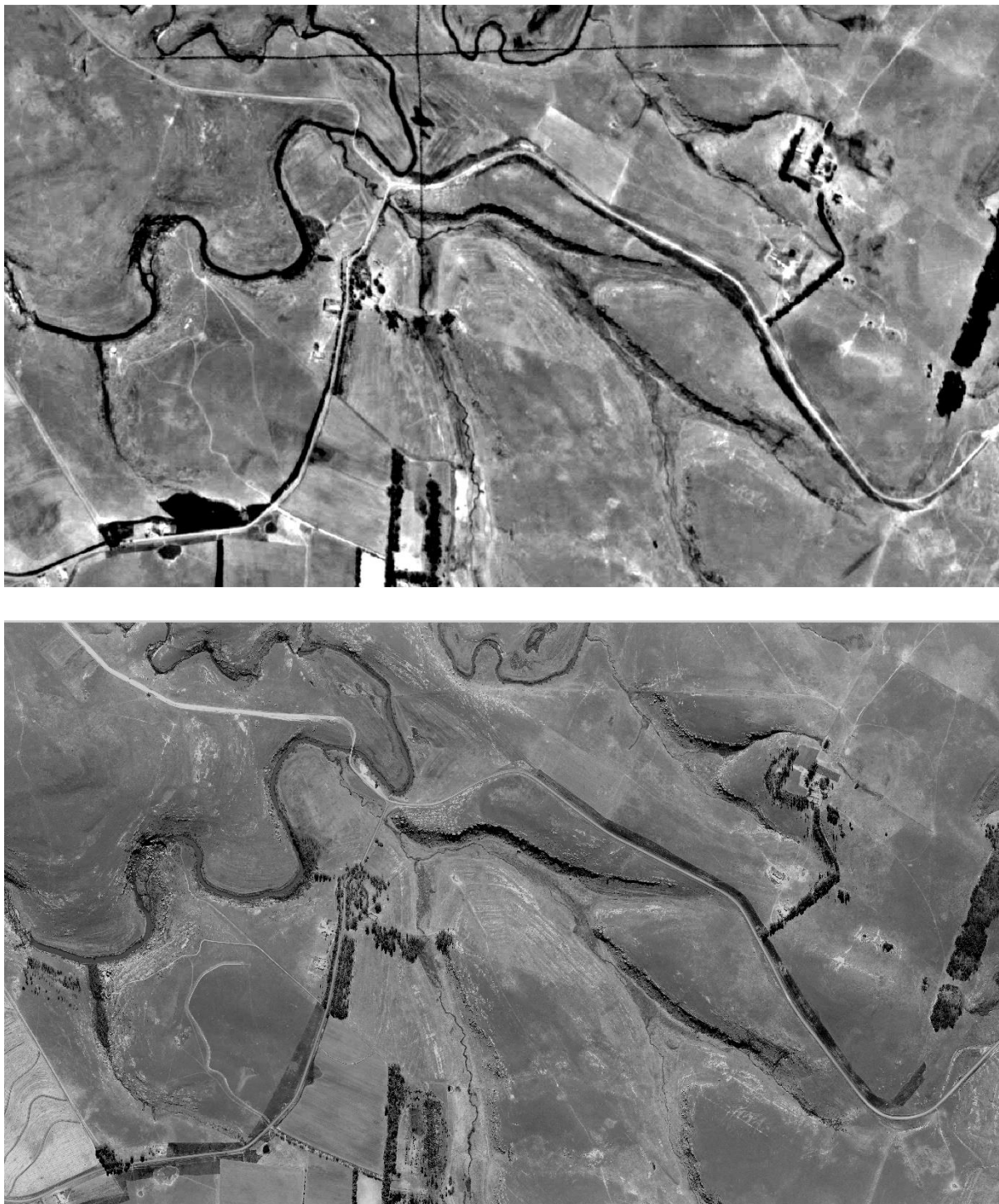
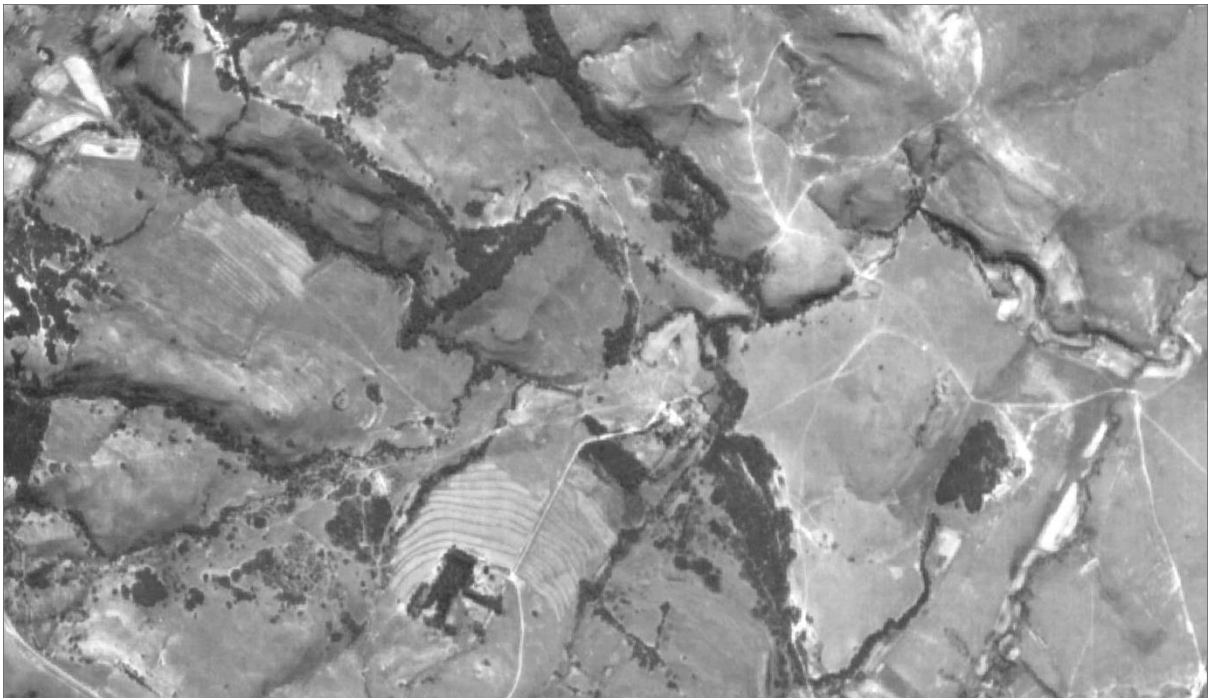
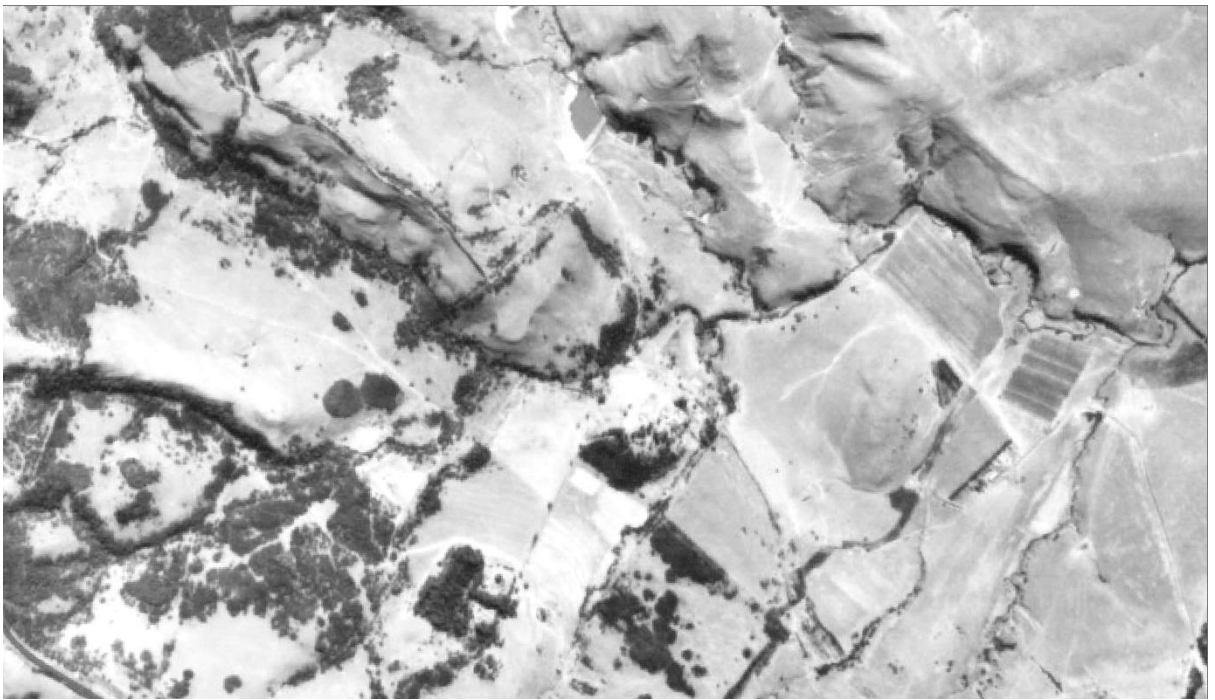


Figure 2A.1: Aerial views of the study area at the top of the Drakensberg escarpment in the vicinity of Van Reenen's Pass at 1 697 m, taken in 1955, 1964, 1976, 1992, 2000 and 2015 with the Wilge River in top left-hand quadrant. The treeless nature of the landscape is evident with conspicuous wind breaks of Eucalyptus trees. Fire breaks are also evident in the photographs.

2A.2 Wyford (1 350 m asl)





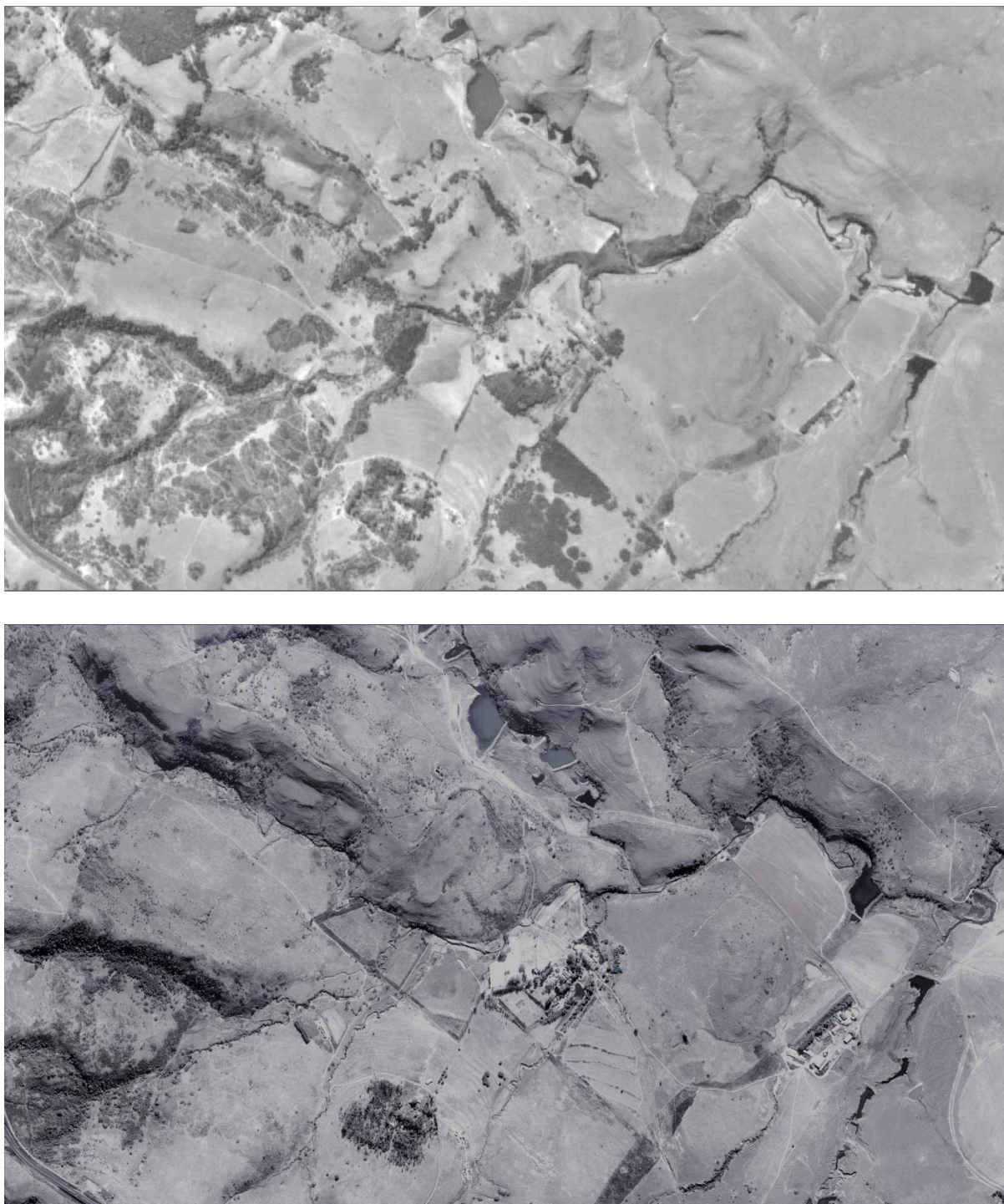
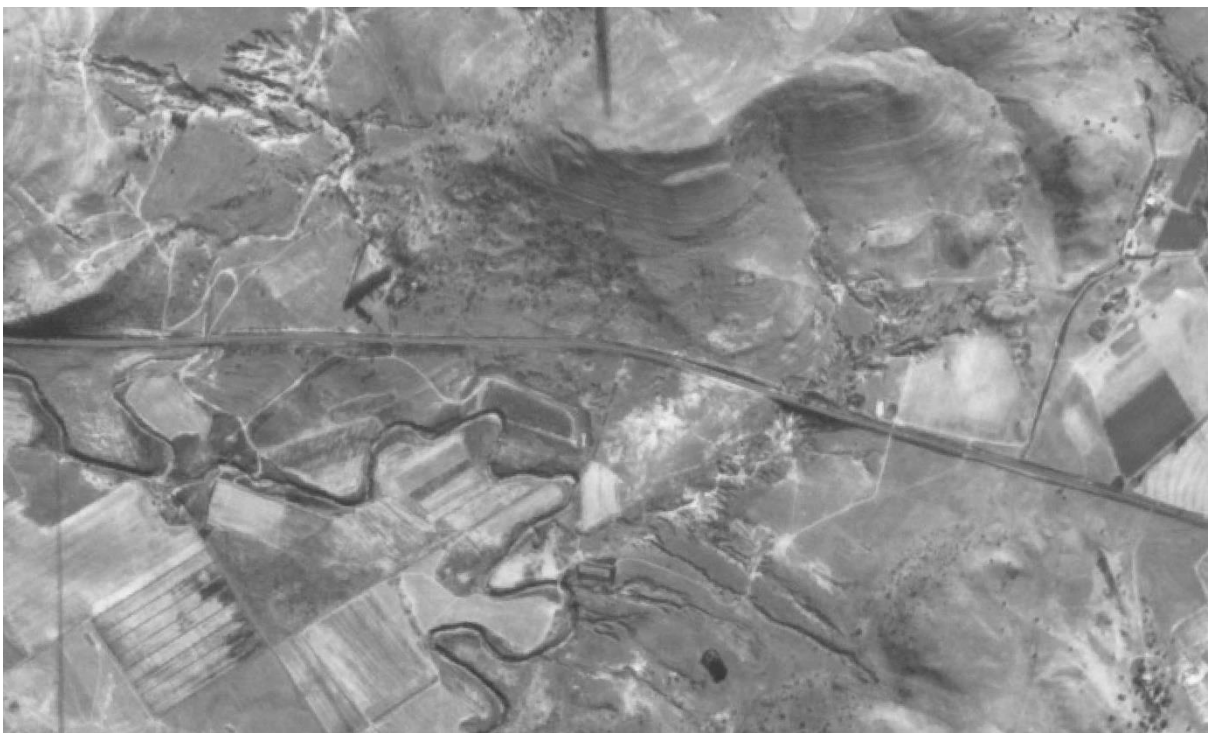


Figure 2A.2: Aerial views of the study area midway along Van Reenen's Pass at 1 350 m asl as the pass climbs up the Drakensberg escarpment, taken in 1955, 1964, 1976, 1992, 2000 and 2015 with the pass itself just visible in the bottom left-hand corner. Dense stands of *Acacia dealbata* are conspicuous along water courses and drainage areas in the earlier photographs. During the 1990s an attempt was made to eradicate these trees, but 2015 there was evidence of regrowth. Individual *Vachellia sieberiana* trees can be discerned in the open areas.



**2A.3 Zandspruit (1 165 asl)**



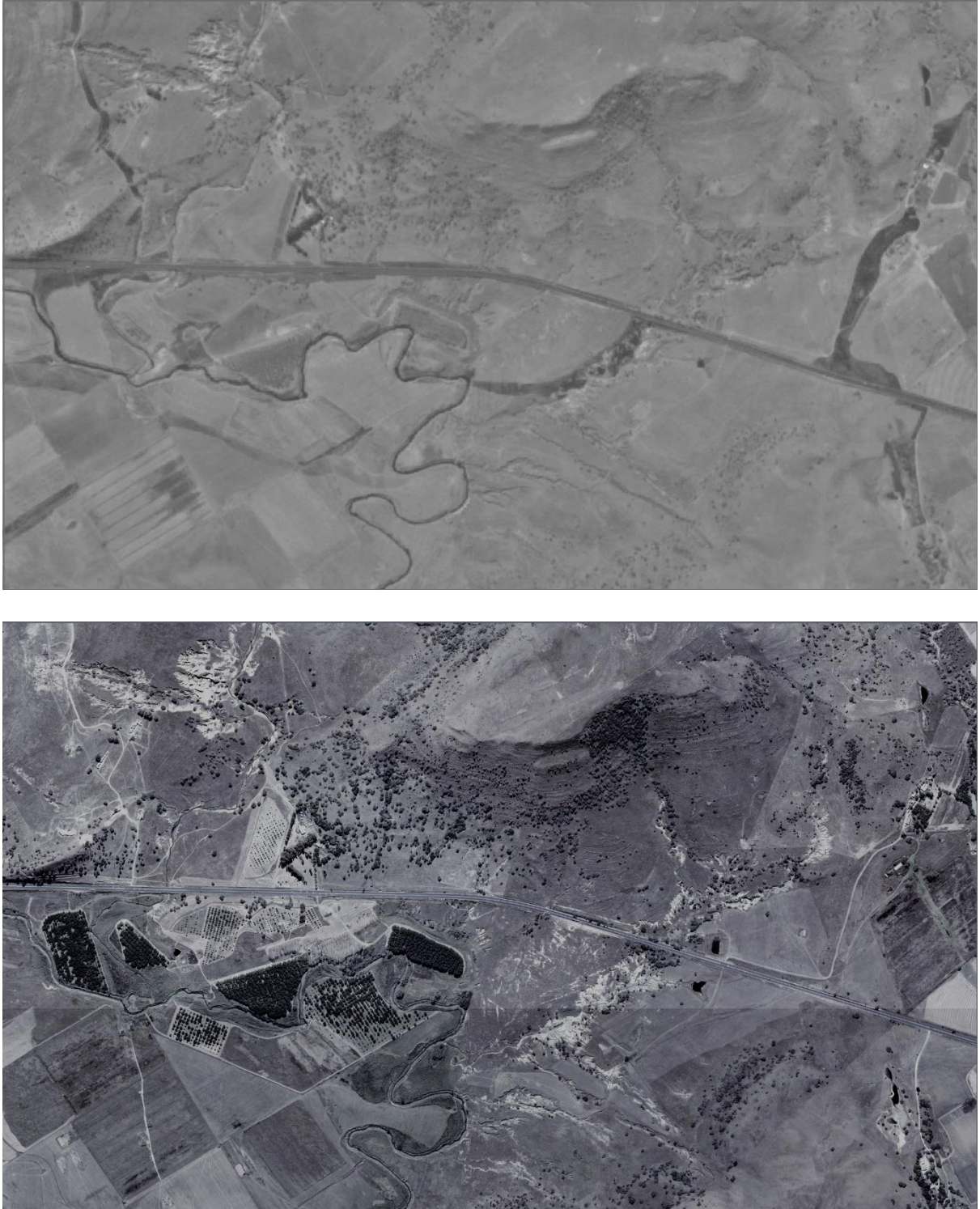


Figure 2A.3: Aerial views of the study area at the foot of Van Reenen's Pass at 1 165 m asl as the pass commences its climb up the Drakensberg escarpment, taken in 1955, 1964, 1976, 1992, 2000 and 2015 with the Sand River in the bottom left-hand quadrant. The pass itself bisects the photographs horizontally. The groves of pecan nuts trees are easily discernible in the 2015 image. The *Vachellia sieberiana* trees can be seen gradually increasing in number over time.

**CHAPTER 3: *VACHELLIA SIEBERIANA* VAR. *WOODII*, A HIGH-ALTITUDE ENCROACHER: THE EFFECT OF FIRE, FROST, SIMULATED GRAZING AND ALTITUDE IN NORTH-WESTERN KWAZULU-NATAL, SOUTH AFRICA**

*Vachellia sieberiana*: a high-altitude encroacher

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**Abstract**

There is increasing evidence that savannas and grasslands throughout the world are experiencing bush encroachment which has serious consequences for net primary productivity. In South Africa, *Vachellia sieberiana* (formerly *Acacia sieberiana*), is encroaching into the savannas and invading high-altitude grasslands of KwaZulu-Natal. We investigated the role of fire, frost and simulated grazing on the establishment of transplanted *V. sieberiana* saplings along an altitudinal gradient in north-western KwaZulu-Natal at three different altitudes. The effect of nutrients and tree:grass competition for root-gaps were briefly taken into consideration, although they were not the main thrust of the study. Saplings were smallest at the high-altitude site and largest at the low-altitude site after two growing seasons in the field. There was no response to treatments at the high-altitude site; at the mid-altitude site sapling stem decreased in response to fire treatment and increased in response to, frost and simulated grazing; and at the low-altitude site there was a negative response to frost and a positive response to simulated grazing. There was no correlation between root-gap size and tree density, but there was a positive correlation between soil nutrients and sapling biomass, with sapling size increasing as nutrients increased down the gradient. There was no interactive effect between treatments. While there were significant responses to the various treatments, none of the treatments or combinations of treatments were able to prevent the establishment of *V. sieberiana*. With the dual spectres of global warming and increasing levels of atmospheric CO<sub>2</sub> looming, *V. sieberiana* may become an even more aggressive encroacher. Managers of grassy biomes, therefore, will need to be vigilant in their monitoring of the presence of potential woody invaders and devise novel methods in mitigating the risks.

**Key words:** altitudinal gradient, bush encroachment, fire, frost, herbivory, *Vachellia sieberiana*

### 3.1 Introduction

Savannas are ecosystems that comprise mixed herbaceous-woody systems, forming part of a continuum of biomes, from open grassland to closed woodland or forest. About one fifth of the human population and most of the domestic and wild herbivores are dependent on these ecosystems (Lehmann *et al.* 2009) and yet the nature and dynamics of savannas are poorly understood (Scholes and Archer 1997; Jeltsch *et al.* 2000). In Africa, over half of the continent is covered by savanna (Scholes and Archer 1997). The drivers behind the tree-grass interactions within a savanna system are, generally, seen as competitive or disturbance-based (Sankaran *et al.* 2004) and often act in concert with one another.

Trees and grasses compete for resources such as soil moisture, nutrients, light and physical space. Spatial partitioning or niche separation reduces below-ground competition for soil moisture. Partitioning may be vertical (Kulmatiski and Beard 2013; Ward *et al.* 2013) or horizontal (February *et al.* 2013). Resource partitioning may also be temporal, with the deeper rooting trees being able to access deep-level water long after the rainfall event (Casper and Jackson 1997; Kulmatiski and Beard 2013). Whether trees and grasses compete directly for nutrients and for which nutrients they compete appears to be a moot point. Cramer and Bond (2013) found that savanna trees, both leguminous and non-leguminous, do not compete with grasses for nitrogen, but, possibly, for other nutrients (see also Vadigi and Ward 2013). It is possible, however, that increased nitrogen causes an increase in grass biomass, which in turn exerts a negative effect on tree sapling growth through shading. Many savanna trees are shade intolerant (Smith and Shackleton 1988) and competition with grass for light may impact the survival rate of the seedlings of many leguminous species (Cramer *et al.* 2007; Hagenah *et al.* 2009) or, at least, affect growth rate negatively (see also Bush and Van Auken 1987). Trees and grasses may also compete for physical space within the topsoil (Pärtel and Wilson 2002). A dominant species may prevent the establishment of a less competitive species by merely occupying below-ground space (Coffin and Lauenroth 1990). Wakeling *et al.* (2015) suggested that the dense below-ground mats of grass roots in certain grasslands, prevent tree seedlings from becoming established.

The four main determinants of savanna structure are soil moisture, nutrients, fire and herbivory (Sankaran *et al.* 2008). Water availability limits the amount of woody cover (see for example Lehmann *et al.* 2011), with edaphic features, fire and herbivory acting

as modifying agents. However, once mean annual precipitation (MAP) exceeds 650 mm, soil moisture is no longer limiting and disturbances such as fire and herbivory, along with soil nutrients and texture (Tomiolo and Ward 2018), determine the proportion of woody cover to grass (Van Langevelde *et al.* 2003; Sankaran *et al.* 2008). The grasslands and savannas of the tropics are generally able to support higher tree densities than observed (Bond 2008) and are thought to be derived largely from fire. Fire traps saplings within the fire zone, creating a demographic bottleneck (Higgins *et al.* 2000). Savanna trees, on the other hand, have developed strategies to survive fire and to escape the fire zone. Many savanna tree species can rapidly resprout after top-kill by fires (Balfour and Midgley 2008; Lawes and Clarke 2011), thereby growing above the fire zone before the next fire. Other species avoid stem damage by developing particularly thick bark (Lawes *et al.* 2011). Herbivory may act in concert with fire or as a separate agent in tree-grass dynamics. Browsers have the potential to maintain savanna trees and shrubs within the fire zone (Staver *et al.* 2009), again creating a demographic bottleneck. Browsing, alone, by megaherbivores such as elephant (*Loxodonta africana*) (Augustine and McNaughton 2004; Goheen *et al.* 2007) or by smaller browsers, such as domestic goats, after fire (Trollope 1980), has been known to successfully reduce tree density. However, in certain circumstances, browsing can stimulate vigorous shoot production (Fornara and Du Toit 2007; Riginos and Young 2007), causing the browsed plant to reallocate resources to remain competitively strong. Grazing acts indirectly on tree density in one of two ways. Intense grazing of the grass sward removes the competitive effect of grasses upon trees (Sankaran *et al.* 2008), removing the need for tree saplings to compete for soil moisture or for light. Alternately, grazing removes the potential fuel load required for intense fires (Case and Staver 2017), thereby reducing the damage that fire could inflict upon the trees. Frost generally acts in conjunction with fire or herbivory (Holdo 2007). Frost also has the potential to influence fire intensity by accelerating the curing of the grass layer (Childes and Walker 1987). Consequently, the proportion of trees experiencing top-kill is higher than expected. Muller *et al.* (2016) suggested that in semi-arid and arid environments, frost alone might be a significant agent of disturbance (also Whitecross *et al.*, 2012).

It has become increasingly evident that savannas and grasslands throughout the world are experiencing a phenomenon generally referred to as woody or bush encroachment (see for example Archer *et al.*, 1995; Stevens *et al.*, 2016). As early as 1917, Bews (1917)

recorded, what he termed, bush “invasion” into the savannas and grasslands of KwaZulu-Natal, South Africa. The drivers of this phenomenon are hotly debated. The debates invoke drivers on both a global and a local scale (Devine *et al.* 2017).

In recent years, much attention has been paid to increasing atmospheric CO<sub>2</sub> and its impact on vegetation. In their review, Bond and Midgley (2000) proposed that increasing CO<sub>2</sub> concentrations favoured the increase in woody cover in grasslands and savannas (see also, for example, Stevens *et al.* 2016). The effect of an increase in global temperatures on vegetation structure is best seen in the shift of Alpine tree-lines into higher altitudes. In his review of what may possibly limit the establishment of trees in high altitude grasslands, Körner (1998) concluded the overarching limitation was a thermal boundary, but with regional modifying factors (see also, for example Harsch *et al.* 2009). As global temperatures rise so the tree-line will move up into higher altitudes (see also Lutz *et al.* 2013).

Local drivers of vegetation change include fire and herbivory. Changes in fire regime may lead to changes in tree density. Historically, in southern Africa, fires were either actively discouraged or there was a shift from late season burning to early season burning when the grass layer was still slightly moist, resulting in cooler fires (reviewed by O'Connor *et al.*, 2014). This had a positive effect on woody encroachment (see also Gordijn *et al.* 2012). Similarly, a fire regime that is too frequent also encourages woody encroachment (Higgins *et al.* 2000). The uncontrolled introduction of bulk grazers, such as cattle, into savannas and grasslands has led to effectively removing grass competition and allowing recruitment and establishment of trees (Archer *et al.* 1995) as well as cooler fires (Van Langevelde *et al.* 2003). The loss of browsing megaherbivores exacerbates the problem of bush encroachment (Van Langevelde *et al.* 2003; Staver *et al.* 2009).

*Vachellia sieberiana* var. *woodii* (formerly *Acacia sieberiana* var. *woodii*) is a typical savanna tree, widespread throughout Kwazulu-Natal, South Africa. It's leaves and seed pods are eaten by both wild and domestic herbivores. Seeds are ingested by herbivores and dispersed in their dung. Farmers' perceptions and historic photographs suggest that *V. sieberiana* is invading the grasslands of the Drakensberg mountains of KwaZulu-Natal, in the vicinity of Van Reenen's Pass. Analysis of aerial photographs (J Russell and M Tedder (2018) unpublished data) confirmed that *V. sieberiana* was not only increasing in



density within its original range, but was, possibly, also increasing its range into higher altitudes. We, therefore, designed a controlled field study to investigate the effects of fire, frost and simulated grazing, as well as the interactions between these variables, on the establishment of *V. sieberiana* saplings along an altitudinal gradient. Our hypotheses were that:

1. Fire would:
  - a. Result in significant reduction in relative growth rates of *V. sieberiana* saplings.
  - b. In conjunction with exposure of the saplings to frost, result in higher sapling mortality than on its own.
  - c. In conjunction with simulated grazing, would be less intense and, therefore, there would be a high rate of sapling survival.
2. Exposure of *V. sieberiana* saplings to frost would
  - a. Result in a reduction of sapling biomass.
  - b. In conjunction with simulated grazing, the reduction in sapling biomass would be greater than the reduction caused by frost alone.
3. Simulated grazing would result in higher relative growth rates of *V. sieberiana* saplings in the absence of fire and frost.
4. Relative growth rates of saplings would be lower as altitude increased.
5. Saplings grown from seeds from trees growing in an area which experiences more frost would survive chilling better than those saplings grown from seed from an area with less frost, i.e. they would be preadapted to tolerate colder temperatures.

We also considered the effects of soil nutrients and below-ground grass competition for physical space, but did not test for these possible effects.

## **3.2 Methods**

### **3.2.1 Site description**

Van Reenen's Pass in north-western KwaZulu-Natal (South Africa) provided a steep altitudinal gradient over a relatively short distance. The pass rises from 1 105m above sea level to 1 768m, crossing the Drakensberg escarpment into the Free State. It is an area that was historically open savanna and grassland, but is now becoming encroached by *V. sieberiana* (pers. obs.). We selected three farms as being suitable to set up the field trials.

The high-altitude site was located on top of the escarpment on the farm Waterfall (S28° 16' 50.2"; E29° 24' 29.2") at 1 697 m. These grasslands fall within the Eastern Free State Sandy Grassland, a mesic Highveld grassland (Mucina and Rutherford 2006) with MAP of 701 mm. The soils are pseudo-podzolic soils (Soils Research Institute 1965) with a shallow topsoil. There were no *V. sieberiana* at this site. *Leucosidea sericea* was present, mostly along the water courses. Other conspicuous trees were ornamental trees and trees, such as *Eucalyptus* sp., that have been introduced as wind breaks. The middle site was at Wyford (S28° 24' 20"; E29° 25' 30.7") at 1 350 m. It is just below the *V. sieberiana* tree-line. Along with *V. sieberiana*, small macrophyllous woody species were present along sheltered water courses, as well as exotic trees such as *Acacia dealbata* and *Eucalyptus* species. Mucina and Rutherford (2006) describe the vegetation in the middle to lower reaches of the pass as Northern KZN Moist Grassland, a sub-escarpment grassland with mean annual precipitation (MAP) of 836 mm. The lowest site was at Zandspruit (S28° 26' 30.8"; E29° 32' 49.4") at 1 165 m. Besides a predominance of *V. sieberiana*, there are introduced trees such as *Carya illinoensis* and *Populus* sp. The soils of the mid- and low- altitude sites are largely litholic (Soils Research Institute 1965; Van der Eyk et al. 1969). The topsoil at the mid-altitude site is shallow, but at the low altitude site the topsoil is deep and nutrient levels are high. All three farms are used for extensive cattle ranching. Stocking rates vary according to season and current rainfall. (See Appendix A.) All three sites fall in the summer rainfall area, with cold dry winters.

All three sites were burnt prior to the commencement of the trials in the preceding winter. After the burn, 0.5 ha at each site was fenced with Bonnox® fencing, a square mesh fence, 1.2 m high, to exclude large herbivores. All woody plants within each site were spot poisoned with a mix of diesel and the herbicide Triclon®. Poisoning of self-seeded woody plants continued throughout the trial. Eight plots were laid out within each fenced area, each with eight stations spaced 3 m apart to avoid the risk of treatments unintentionally interfering with each other (Figure 3.1).

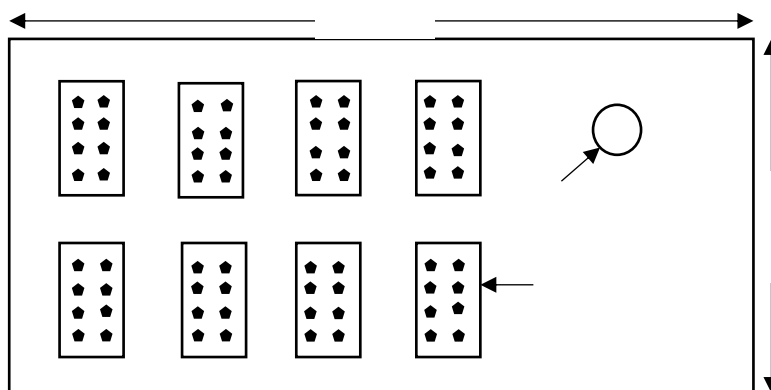


Figure 3.1: Schematic diagramme of the layout of the field study site. Within the 100 m x 50 m fenced site were eight plots measuring 15 m x 9 m. The plots were 2 m away from fence-line and 5 m apart, with a wide corridor down the centre of the sight for easy vehicle access. Within each plot were eight stations (◆) at which a sapling was planted. The rain gauge was placed within the fenced site and is demarcated as a ○. Four of the plots were burn, four remained unburnt.

### 3.2.2 Measurement of ambient temperature and rainfall

At each site, we set up a HOBO Pro v2® Temperature/Relative Humidity data logger with an external temperature/relative humidity sensor, manufactured by Onset, USA. As we were interested in ambient temperature and frost at sapling canopy level, the sensor was placed at sapling height, i.e. approximately 10 cm above the ground (see Savage 2012). Ambient temperature and relative humidity were measured at five-minute intervals and the data stored in the logger. Data was downloaded and the logger was cleared once a month.

We set up a bulk rain gauge at each site. Each comprised an aluminium frame which supported a funnel 1.5m above ground that fed into a 25-liter tank placed below the funnel. The rain gauges were cleared once a month and rainfall determined using the equation:

$$Vr = Vt/Ao$$

where  $Vr$  = amount of rain;  $Vt$  = volume rain water in tank; and  $Ao$  = area of funnel orifice.

### 3.2.3 Propagation

Ripe seed pods were collected from *V. sieberiana* trees growing at Zandspruit and Wyford. The pods were harvested from all trees bearing pods, by knocking the pods out of the trees and collecting the fallen pods. Where pods had already dropped to the ground,

these were also collected. The pods from the two sites were kept separate and the seeds removed. In September, at the start of spring, the seeds were scarified by clipping the end opposite the hilum and soaked overnight before planting out into 16 seed trays in a 50:50 compost:sand mix. Each seed tray held 128 seeds. Once the seedlings had reached approximately 7.5 cm in height, they were transplanted into 1-liter plastic bags of river sand and kept under shade cloth in the arboretum at the University of KwaZulu-Natal, Pietermaritzburg. They were watered for two hours twice a week between 9h00 and 11h00, using an automatic sprinkler system. In February of the following year, the seedlings were transplanted into the field at the study sites. This was undertaken late in the summer season as the anticipated summer rains were late. Three seedlings were planted at each station i.e. in each of the eight plots at each site, 24 seedlings were planted, a total of 192 seedlings per site. Once the seedlings were established, after a full year in the field, they were thinned to one seedling per station, i.e. 64 seedlings per site (Figure 3.1).

### 3.2.4 Treatments

During the first year, while the saplings established, any that died were replaced with saplings that had been kept in reserve (Table 3.1). These saplings were of the same age as those already in the field. By the time the treatments started, there was no observable difference between the replacement saplings and the others.

Table 3.1: Survivorship of transplanted saplings after the first growing season in the field. Those that died were replaced the following spring of 2015 in October.

Site name	No. sapling survived	No. saplings died
Waterfall (high-altitude)	56	8
Wyford (mid-altitude)	54	10
Zandspruit (low-altitude)	0	64

Treatment of the saplings comprised two levels: saplings grown from seeds collected from Wyford and saplings grown from seeds collected from Zandspruit. Within those two levels, three further treatments, and combinations thereof, were applied:

- 1) Fire: All sites were protected from fire during the first winter while the saplings established themselves. Treatment commenced during the second winter. At each site, four of the plots were burnt. The remaining four plots were left unburnt. Plots were burnt using a head fire in conjunction with the landowners' fire-break

burning regime and weather conditions. Consequently, the high-altitude site (Waterfall) was burnt 2 August 2016, the mid-altitude site (Wyford) was burnt 17 May 2016 and the low-altitude site was burnt 21 June 2016. All the burns occurred after the first winter frost.

- 2) Frost: All the saplings were protected from frost while they established during the first winter. Sheets of frost-fleece, 1.5m x 1.5m, reinforced with wire mesh, were suspended horizontally from stakes between 45cm and 50cm above the tree saplings. Treatment started at the start of the second winter of the field trials. In each plot, four of the stations were protected from frost, four were not.
- 3) Simulated grazing: In each plot, the grass layer was mown in a circle of 1m radius around four of the eight tree stations in each plot. Mowing around each sapling commenced at the start of the second growing season, after good rains had fallen in January 2016. The sward was cut to a height of 2 cm  $\pm$  1 cm and maintained at that height until the end of the following growing season, when then trial ended in May 2017. The primary concern was to remove competition for light.

Treatments of fire, frost and simulated grazing were randomly assigned to stations, using computer generated random numbers. The burning treatment was applied to a whole plot. The plots assigned for burning were randomly selected. The eight plots ensured four replicates of all treatments. There were, therefore, eight treatments per plot with a full factorial design with all possible combinations: seed source, fire, frost and simulated grazing (Table 3.2).

Table 3.2: Table of the different treatment combinations a burnt plot and at an unburnt plot. B=Burnt, F=Frost, C=Clipped, UB=Unburnt, NF= No Frost, NC= Not clipped

Burn treatment		No burning	
Wyford	BxFxC	Wyford	BxFxC
	BxFxNC		BxFxNC
	BxNFxC		BxNFxC
	BxNFxNC		BxNFxNC
Zandspruit	BxFxC	Zandspruit	BxFxC
	BxFxNC		BxFxNC
	BxNFxC		BxNFxC
	BxNFxNC		BxNFxNC

The treatments were laid out in a completely randomized design including four replications per treatment. Shortly before each burn, grass biomass was estimated for each site, using a disc pasture meter. Eighty disc pasture meter readings (half within each study site where the sward was undisturbed and half adjacent to the study site) were taken, ten of which, distributed across a range of sward heights, were clipped and dried in order to develop site specific regression equations. The results were then averaged to estimate total grass biomass per hectare for each site.

During each burn, wind speed and relative humidity were measured using a Kestrel® 4000 Pocket Weather Tracker. At the same time, six samples of grass clippings were collected and used to calculate fuel moisture content. The clippings were weighed, oven dried for 48 hours at 60°C and then reweighed. Moisture content was expressed as a percentage of the wet weight of the clippings. Fire intensity was calculated, using the regression equation based on research published by Trollope *et al.* (2004):

$$FI = 2729 + 0.8684x_1 - 530 \sqrt{x_2} - 0.1907x_3^2 - 596 1/x_4$$

where  $FI$  = fire intensity (kJ/s/m);  $x_1$  = fuel load (kg/ha);  $x_2$  = fuel moisture (%);  $x_3$  = relative humidity (%); and  $x_4$  = wind speed (m/s).

### 3.2.5 Root gap, soil nutrients and tree density

Ten soil cores, each 7 cm diameter and 20 cm deep, were taken from each site. Plant root material was removed from the individual cores by hand in the manner described by Wakeling *et al.* (2015). The roots were washed, oven dried for 96 hours at 60°C and weighed. Root biomass from the three sites were compared to ascertain the degree of below ground competition the saplings may experience.

Further soil samples from the top 20 cm of soil were taken from within each site where there were no trees except for the small transplanted saplings. Four samples were taken from each site from each corner. The soils from each site were then pooled and well mixed. Subsamples from each site were sent to the Fertilizer Advisory Service of Research & Technology Development: Analytical Services, Cedara, KwaZulu-Natal, for nutrient analysis. We conducted a survey of the number of *V. sieberiana* trees per site. Four 2 x 50 m belt transects, outside the study site, at both Wyford and Zandspruit were laid out and the number of trees counted. The results per site were averaged to give an




estimate of tree density per site. As there were no *V. sieberiana* at the top of the pass, this was not done at the Waterfall site.

### 3.2.6 Analysis of data from the field trials

The trial period ended in April 2017, the year following the application of treatments, which was the end of the growing season. Saplings stems were harvested, the remaining root-stock spot poisoned to prevent regrowth. Despite our best efforts to exclude large herbivores from the trial sites, saplings at Zandspruit were heavily browsed by kudu (*Tragelaphus strepsiceros*) which jump fences with ease. Consequently, we used sapling stem diameter as a measure of the response of the saplings to treatments, rather than plant biomass or shoot length. However, we measured pre- and post-treatment sapling heights to ascertain whether or not saplings were able to escape the fire trap within one growing season.

In analysing the data, we first used the outlier labelling rule to identify outliers, using the value of 2.2 as the most appropriate multiplier (Hoaglin and Iglewicz 1987). The outlier labelling rule is defined as the difference between the 25<sup>th</sup> and 75<sup>th</sup> percentile values of the data distribution curve multiplied by 2.2. That value is then subtracted from the 25<sup>th</sup> percentile and added to the 75<sup>th</sup> percentile. Any data points falling beyond these subsequent values are considered outliers. Outliers were then winsorized, giving them the same value as the highest most reliable value in the data set (Tukey 1962). Outliers were found only in the data set from the Waterfall site. Graphical representation of the outliers can be found in Appendix D. Using IBM SPSS Statistics® software, version 25, we analysed the data using Generalized Linear Models (GLMs), the best fit being the gamma distribution with the log function. A *post hoc* test was performed using the least significant differences.

Table 3.3: A comparison of the characteristics during the duration of the trials and fire intensity of the three study sites: Waterfall (1 697 m ASL), Wyford (1 350 m ASL) and Zandspruit (1 165 m ASL). Soil nutrient data is derived from the soil analysis as provided by the Fertilizer Advisory Service of Research & Technology Development: Analytical Services

	Waterfall	Wyford	Zandspruit
Altitude (m)	1 697	1 350	1 165
MAP (mm) May to April	1 437.3	1 853.3	1 630.4
Min. temperature (°C)	-5.95	-3.25	-3.58
Max. temperature (°C)	35.07	38.46	39.61
Average Temperature (°C)	15.4	18.33	18.77
No. potential frost days	64	15	29
Length of growing season (months)	7	9	9
No. <i>V. sieberiana</i> per ha	0	850	4 125
Below-ground dry grass root biomass (g/m <sup>3</sup> )	1 837.66	1 022.078	1 414.29
Dominant grass spp.	<i>Harpochloa falx</i> <i>Panicum natalense</i>	<i>Aristida junciformis</i> <i>Elionurus muticus</i>	<i>Cymbopogon validus</i> <i>Sporobolus pyramidalis</i>
			
Soil nutrients			
pH	4.11	3.78	4.73
P (ml/L)	5	7	31
N (%)	0.16	0.18	0.33
Organic carbon (%)	1.3	2.2	3.5
Clay (%)	17	39	21
Fire intensity data:			
Fuel load (kg/ha)	6 591	10 965	15 884
Fuel moisture (%)	14	39	41
Relative humidity (%)	36	42	68
Windspeed (m/s)	2.5	1.7	0.9
Fire intensity (kJ/m/s)	6 020.148	8 260.04	11 584.74

### 3.3 Results

#### 3.3.1 Temperature and rainfall

During the twelve months (May to April the following year) over which the treatments were run, Waterfall was the coldest site. Between May and October, there were 64 potential frost days, the lowest temperature being -5.95°C. At Zandspruit, at the foot of



the pass, there were 29 potential frost days between May and September. The lowest temperature was  $-3.58^{\circ}\text{C}$ .

Wyford experienced 15 potential frost days between May and September, with the lowest temperature being  $-3.25^{\circ}\text{C}$ . At Wyford, between 5 and 10 of May 2015, the logger malfunctioned and data for those five days were missing. During those days Zandspruit temperatures did not drop below  $4^{\circ}\text{C}$  and at Waterfall there was one day during which the temperature fell to  $-0.44^{\circ}\text{C}$ . It is, therefore, unlikely that Wyford experienced frost during that time and those days were regarded as frost free. Annual rainfall was much higher than the predicted mean at all three sites. Waterfall received a total of 1 437.3 mm. Wyford received 1 853.3 mm and Zandspruit received 1 630.4 mm (Table 3.3). Soil moisture, therefore, was not a limiting factor at any of the sites.

### 3.3.2 Fire intensity

Although fuel loads decreased as altitude decreased, the grass biomass at each site was sufficient to create fires of high fire intensity (Table 3.3). Trollope and Potgieter (1985) classified a fire as “extremely hot” if fire intensity was above 3 000 kJ/m/s. (See appendix B.)

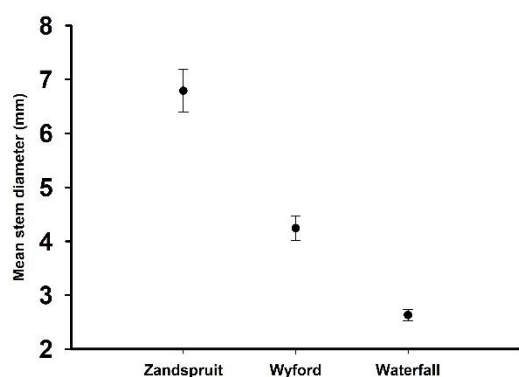


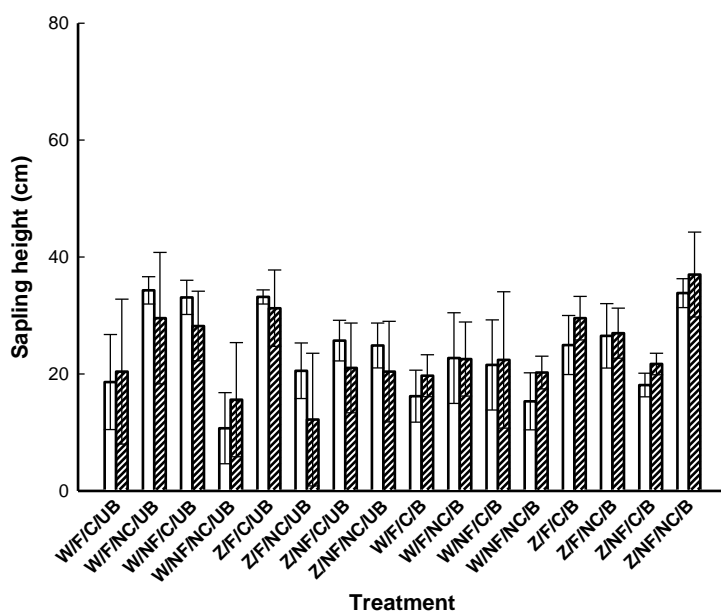
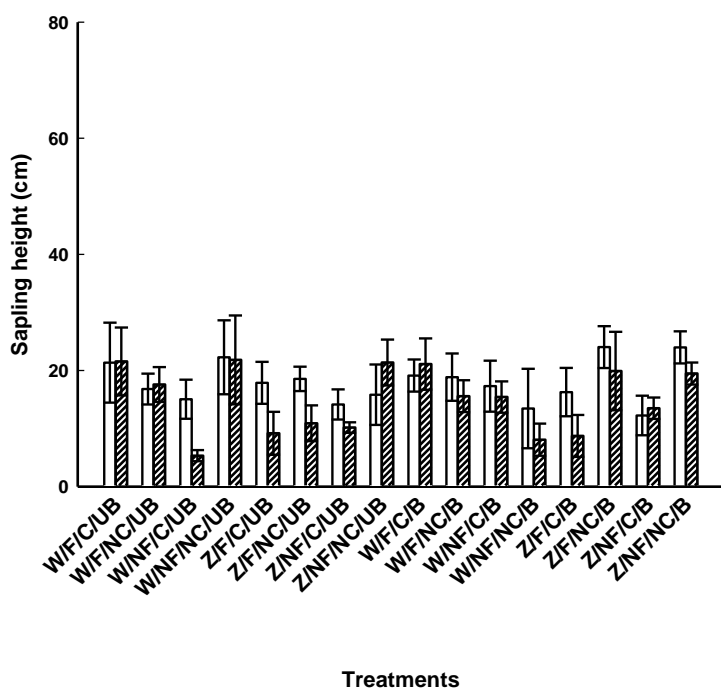
Figure 3.2: Mean ( $\pm$  SE) stem diameter of *Vachellia sieberiana* saplings along an altitudinal gradient from Zandspruit (1165m asl) to Wyford (1350m asl) to Waterfall (1697 m asl), Van Reenen’s Pass, South Africa.

### 3.3.3 Effects of altitude and treatments

In general, *V. sieberiana* saplings were less robust at the highest the altitude, regardless of treatment effect (Figure 3.2). At Waterfall, the high-altitude site, one of the saplings ( $n = 64$ ) died during the trial period, at Wyford, the mid-altitude site, four saplings died during the trial period ( $n = 64$ ) and at the low-altitude site, Zandspruit, six saplings die during the same period ( $n = 64$ ). Mortality rate at all three sites was low and not related to treatment.

### 3.3.4 Effect of treatment on stem height

At Waterfall, the high-altitude site, there was little change in sapling height pre- and post-treatment (Figure 3.3a). Sapling heights at Wyford, the mid-altitude site, varied in height pre- and post-treatment (Figure 3.3b). At Zandspruit, the low-altitude site, 72% of the saplings were heavily browsed. Despite this increase in height was marked (Figure 3.3c).



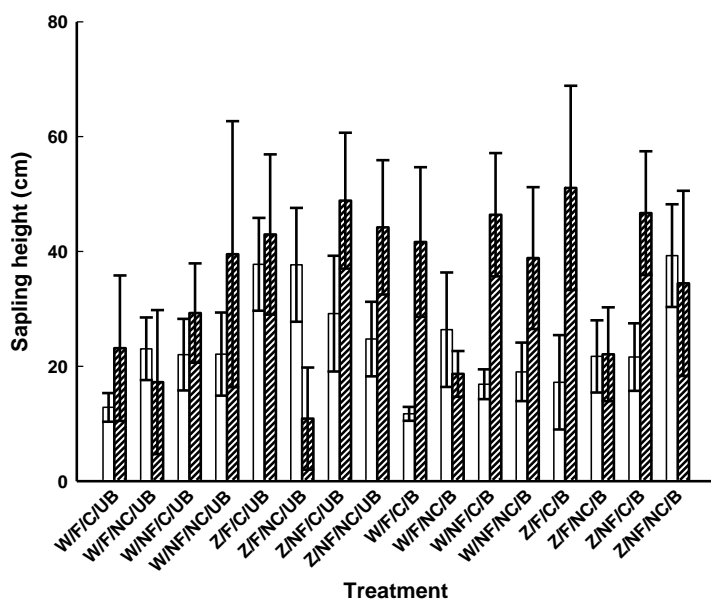


Figure 3.3 A comparison of sapling heights before and after treatment at the three sites a) Waterfall (high-altitude); b) Wyford; and c) Zandspruit. The different treatments are: W = Wyford (seed source); Z = Zandspruit (seed source); F = Frost; NF = no frost; C = Clipped; NC = Not Clipped; B = Burnt; NB = Not burnt. The empty columns denote sapling height before treatment, the hatched columns denote sapling height after one growing season post-treatment.

### 3.3.5 Effect of treatment on stem diameter

#### Waterfall

There was no significant effect of treatment or any combination thereof on sapling stem diameter (Table 3.4).

Table 3.4: The effects of seed source, fire, frost and mowing, and their combinations on stem diameter of *Vachellia sieberiana* saplings at three sites along an altitudinal gradient at van Reenen's Pass, South Africa, using a generalized linear model, with the gamma probability distribution with the log function. *p*-values in bold and with an \* are significant

Treatment	Waterfall (1 700 m)			Wyford (1 330 m)			Zandspruit (1 165 m)		
	Likelihood Ratio Chi-Square	df	Sig.	Likelihood Ratio Chi-Square	df	Sig.	Likelihood Ratio Chi-Square	df	Sig.
Seed source	0.145	1	0.703	3.936	1	<b>0.047*</b>	2.038	1	0.153
Fire	1.401	1	0.237	10.848	1	<b>0.001*</b>	2.966	1	0.085
Frost	1.322	1	0.25	5.362	1	<b>0.021*</b>	5.719	1	<b>0.017*</b>
Mown	1.1	1	0.294	10.303	1	<b>0.001*</b>	6.754	1	<b>0.009*</b>
Seed source x Fire	0.01	1	0.921	0.471	1	0.492	1.484	1	0.223
Seed source x Frost	2.498	1	0.114	0.091	1	0.763	0.792	1	0.374
Seed source x Mown	2.99	1	0.084	0.506	1	0.477	0.042	1	0.838
Fire x Frost	1.229	1	0.268	3.246	1	0.072	2.888	1	0.089
Fire x Mown	0.398	1	0.528	0.404	1	0.525	0.369	1	0.544
Frost x Mown	2.49	1	0.115	1.369	1	0.242	0.974	1	0.324
Seed source x Fire x Frost	0.058	1	0.81	1.57	1	0.21	0.653	1	0.419
Seed source x Frost x Mown	0.39	1	0.532	2.359	1	0.125	0.497	1	0.481
Seed source x Fire x Mown	0.109	1	0.742	0.569	1	0.451	0.966	1	0.326
Fire x Frost x Mown	0.792	1	0.374	0.32	1	0.571	0.054	1	0.816
Seed source x Fire x Frost x Mown	0.783	1	0.376	0.092	1	0.761	0.01	1	0.919

#### Wyford and Zandspruit

All the main treatments had a significant effect on stem diameter (Figure 3.4a, b, c and d). Those saplings that had been protected from fire were significantly more robust than those that had been burnt. Sapling stems that had been exposed to frost had larger stem diameters than those that had been protected from frost. Sapling stems had larger diameters where the grass sward had been mown. Saplings grown from seeds collected from Zandspruit had a greater stem diameter than those grown from seeds collected from Wyford. There was no interaction between treatments (Table 3.4).

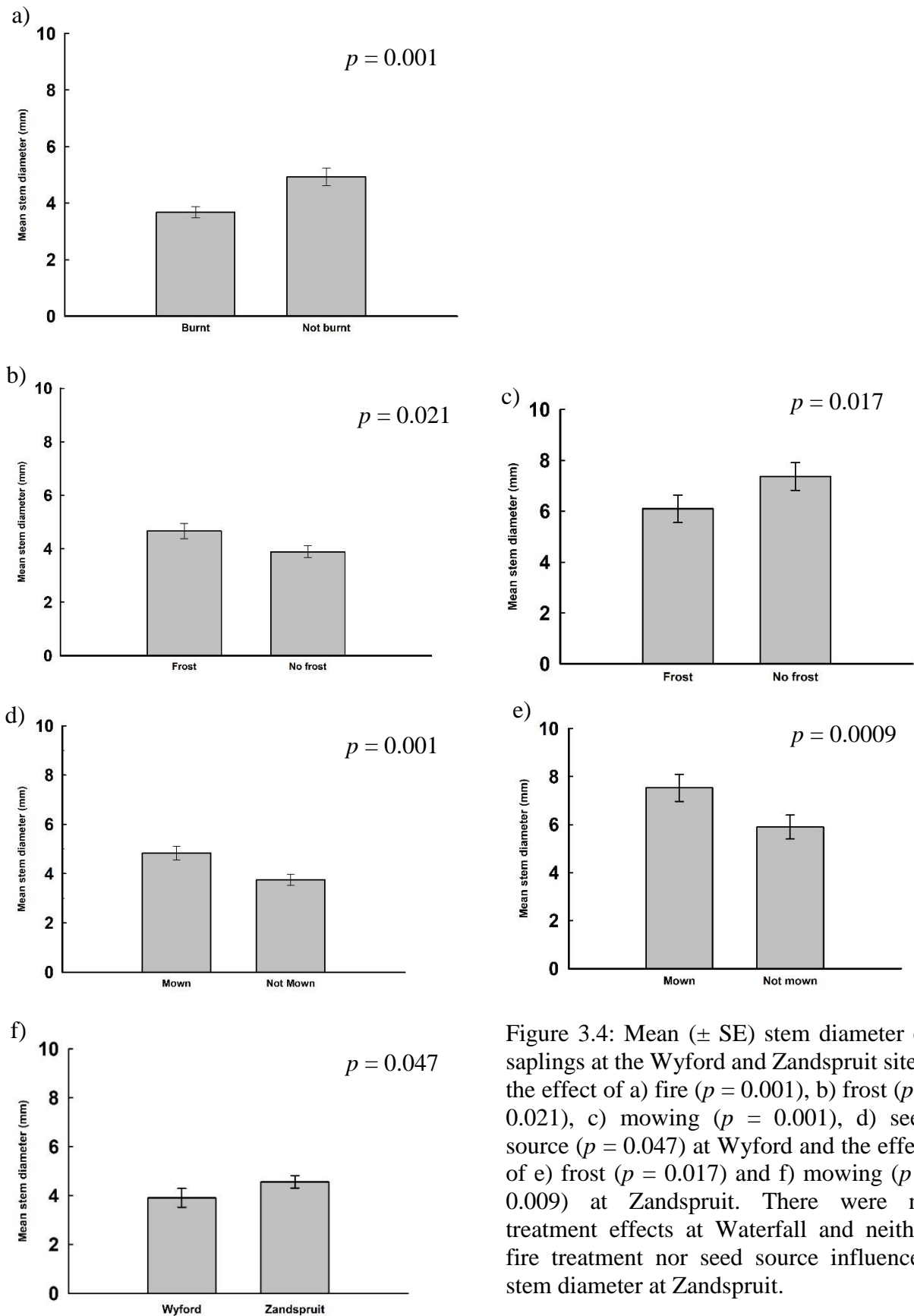


Figure 3.4: Mean ( $\pm$  SE) stem diameter of saplings at the Wyford and Zandspruit sites: the effect of a) fire ( $p = 0.001$ ), b) frost ( $p = 0.021$ ), c) mowing ( $p = 0.001$ ), d) seed source ( $p = 0.047$ ) at Wyford and the effect of e) frost ( $p = 0.017$ ) and f) mowing ( $p = 0.009$ ) at Zandspruit. There were no treatment effects at Waterfall and neither fire treatment nor seed source influenced stem diameter at Zandspruit.

### 3.3.6 Root gap, soil nutrients and tree density

Dry grass root biomass was 1 837.66 g/m<sup>3</sup>; 1 022.08 g/m<sup>3</sup> and 1 414.29 g/m<sup>3</sup> for Waterfall, Wyford and Zandspruit respectively (Table 3.3). In, general, soil fertility increased as altitude decreased (Table 3.3; See also Appendix B). Of interest was phosphorus and nitrogen content as these are the nutrients that are potentially most limiting to tree growth. Both these elements were particularly abundant at the bottom of the pass, at Zandspruit. *V. sieberiana* density at each site was 0 trees at Waterfall; 350 and 4 125 trees at Wyford and Zandspruit respectively.

## 3.4 Discussion

### 3.4.1 Treatment effects

Each site appeared to have its own set of characteristics, directly or indirectly because of altitude, which influenced sapling responses to fire, frost and simulated grazing. Although not included in the statistical analysis, it appeared that fire, frost and mowing had little effect on the height of the saplings at the Waterfall (high-altitude) and the Wyford (mid-altitude) sites, although Wyford there were measurable differences in stem diameters. It suggests, therefore, that something other than these potential agents of disturbance is limiting sapling growth. At Zandspruit (the low-altitude site) there were marked differences between pre- and post-treatment stem heights, in many cases post-treatment heights were greater, despite the saplings having been browsed. Soils at this site are high in nutrients which could have contributed to rapid recover after the treatments. However, the grass sward at Zandspruit was still well over the height of the saplings at the culmination of the trials, due to the preponderance of *Cymbopogon validus*. Therefore, the saplings, potentially, had not been able to escape from the fire trap after one growing season after the treatments were applied.

At Waterfall, contrary to expectation, none of the treatments, nor any combination of treatments, had a significant effect on stem diameter of the *V. sieberiana* saplings. Fire, generally regarded as an effective method of controlling the proliferation of woody plants (see for example Higgins *et al.*, 2000), had no significant effect on sapling stem diameter. Although we achieved a high intensity fire which resulted in 100% top-kill, the saplings recovered by coppicing from the remaining rootstock, confirming that, even at a very tender age, *V. sieberiana* is highly fire resistant. Trees grown at high altitudes with lower

temperatures and shorter growing seasons have been shown to have a slower growth rate than the same species grown at lower altitudes (Wakeling *et al.*, 2012). The overall, slower growth rate of the *V. sieberiana* saplings at Waterfall may have masked the effect of fire.

As with the fire treatment, there was no significant difference in stem diameter between those saplings that were protected from frost and those that were exposed to frost. Frost protection has the potential of raising ambient temperature beneath the covering up to 2°C and, with some types of frost guard, even higher (Teitel *et al.* 1996), although any air disturbance will reduce the effectiveness of the protective screen. Smit (1990), working in the Sourish Mixed Bushveld, noted that when temperatures dropped to -3.5°C several species of *Vachellia* (amongst others) suffered extensive top-kill and recovered by coppicing from the base of the trunk. However, at Waterfall, where temperatures dropped to -5.95°C, there appeared to be only a small amount of damage to side shoots of the transplanted *V. sieberiana* saplings. This suggests some degree of frost tolerance or avoidance in *V. sieberiana*. The control saplings appeared to have the same amount of damage as the unprotected saplings and all resprouted from the side shoots at the start of the growing season. Preliminary trials indicate that there is no significant difference in *V. sieberiana* saplings that had been exposed to frost at temperatures between -3.0°C and -6.0°C (J Russell and M Tedder (2018), unpublished data). As the control saplings would have been subjected to temperatures close to -4.0°C, despite the frost protection, the preliminary trials explain the lack of difference between the two groups.

There was no significant response to simulated grazing. We had expected the grass sward to compete with the saplings for light, but at no stage did the saplings appear affected by the sward, even when well below grass height (see Tedder *et al.* 2014). Although the grass sward was sufficient to create a fire of high intensity, it was not sufficiently dense to limit sapling growth through shading.

We surmise, therefore, that growth rates of *V. sieberiana* at the Waterfall site were suppressed by low temperatures and a short growing season, which may have overridden any treatment effect. However, even though *V. sieberiana* did not flourish at high altitudes, it could survive. Despite this, none were naturally present in the area. Careful inspection of the grass surrounding the trial site revealed no ‘gullivers’ (small, but

suppressed, trees), a term coined by Bond and Van Wilgen (1996), which may have been trapped within the grass sward through fire or frost. Archival temperature data obtained from Meteoblue (2018), suggests that minimum temperatures at the nearby village Van Reenen are generally  $-8^{\circ}\text{C}$ . Considering that temperatures at ground level may be as much as  $5^{\circ}\text{C}$  lower than temperatures measured by weather stations (Savage, 2012), *V. sieberiana* seedlings may well experience killing frosts during a normal winter. As the minimum temperature measured at Waterfall during the field trials was  $-5.9^{\circ}\text{C}$ , we conclude that the winter of 2015 was unusually mild for that area and this may have contributed to the survival of the transplanted saplings.

The negative effect of fire on stem diameter at Wyford was as expected and confirmed that fire has the potential to slow down the growth rate of *V. sieberiana* saplings. Fire, therefore, will trap the sapling within the fire zone at each successive fire, remaining as a 'gulliver' (Bond and Van Wilgen 1996) until it can escape the fire trap. At Zandspruit, however, there was no effect of fire on stem diameter, despite the fire being potentially extremely intense. However, while the basal leaves of the most dominant grass species, *Cymbopogon validus*, burned, the stems and inflorescences which were the bulk of the grass, although scorched, were left standing. Consequently, the shorter grass species present, which were less abundant than *C. validus*, provided most of the fuel for the fire and the fire, therefore, would have been much cooler than computed. This would account for the lack of effect of fire on stem diameter of the saplings. Fire frequency, however, could shift grass species composition. For example, in long-term burning trials, Kirkman *et al.* (2014) found that frequent burning resulted in the grass sward being dominated by short grass species, while in unburnt plots, *C. validus* was one of the dominant species. At a site such as Zandspruit, frequent burning could reduce the density of *C. validus* and, with a sward composition of predominantly short grass, the fire intensity would be higher and, therefore more damaging to saplings. However, it is debatable whether this treatment could reverse the observed encroachment without the introduction of high numbers of browsers.

Frost had a positive effect on stem diameter of the transplanted *V. sieberiana* saplings at Wyford. Just as fire has been referred to as a herbivore (Bond and Keely, 2005), so frost could be regarded in the same way. Certain savanna tree species are known to reallocate resources to the shoots to compensate for browse damage to remain competitive (Stuart-



Hill and Tainton, 1988; Fornara and du Toit, 2007). Light frost damage could have a similar effect on stem elongation as light browsing. Riginos and Young (2007) recorded an over-compensation in stem elongation in certain trees in response to light browsing. This may be the reason for the greater accumulation of biomass as seen by the larger stem diameters of the frosted *V. sieberiana* saplings. A similar response of overcompensation because of frost damage was observed in *V. xanthophloea* (P Blacklaw, pers. comm<sup>7</sup>., 2018). At Zandspruit, however, frost had a negative effect on stem diameter. Generally, frost induced freezing within plant tissue is extracellular (Levitt 1980) and the damage is the result of desiccation of the cells. The saplings at Zandspruit experienced twice the number of possible frost events than those at Wyford. Consequently, the damage to the plant cells may have been greater at Zandspruit than that at Wyford, bringing about a disruption of cell function and slowing down sapling growth. In extreme cases, a number of frost events of a particular magnitude has the potential to determine the biome boundary (Muller *et al.* 2016).

Stem diameter of *V. sieberiana* had a positive response to simulated grazing (mowing) at both Wyford and Zandspruit. While it is unlikely that simulated grazing affected below-ground competition between grasses and saplings (McNaughton *et al.* 1998), removal of above-ground competition for light would have had a positive effect on stem diameter (Smith and Shackleton 1988; Hagenah *et al.* 2009). *V. sieberiana* is sensitive to shading, as noted by Vadigi and Ward (2013), who found that shading had a negative effect, *inter alia*, on stem diameter.

Although Wakeling *et al.* (2012) reported preadaptation in *Acacia* (= *Vachellia*) *karroo* saplings to cold and frost, in our trials with *V. sieberiana* saplings there were no strong preadaptation traits. Although there was a significant response to seed source in the saplings grown at Wyford, the response was only marginal. As the saplings at both Waterfall and at Zandspruit did not show a significant response to seed source, we suspect that the response at Wyford was due to a few saplings that were particularly robust, but not sufficiently so to be eliminated by the outlier labelling rule.

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### 3.4.2 The effect of altitude and below-ground competition regarding nutrients and root gaps

As anticipated, the trend was for the *V. sieberiana* seedlings to decrease in stem diameter as altitude increased. Temperature data, collected during the trials, suggest that, at Waterfall, the longer winter period and lower ambient temperatures contributed to this trend. Wakeling *et al.* (2012) also observed that several typical savanna tree species, including *V. sieberiana*, growing in a colder climate had a lower biomass than their counterparts growing in warmer areas.

Soil nutrients, on the other hand, increased with decreasing altitude, i.e. there was a catenal gradient (Scheidegger 1986), with soils poor in nutrients at the top of Van Reenen's Pass and nutrient rich soils at the bottom of the pass. Although the limited nutrients in the soils at Waterfall should not prevent *V. sieberiana* from establishing (Mason, Fertilizer Advisory Services: pers. comm.<sup>8</sup>, 2017), N Makhaye and A Magadlela (2017, unpublished data) demonstrated, in a greenhouse experiment, that *V. sieberiana* seedlings, grown in soils collected from Waterfall, had a significantly lower biomass than those grown in soils collected from Wyford and Zandspruit. The inference is that the different levels of soil nutrients caused the differences in plant biomass (see Wakeling *et al.* 2010). This work replicated the trend we observed in the field. The most likely limiting nutrient is phosphorus (Cramer and Bond 2013; Magadlela *et al.* 2014), rather than nitrogen (Vadigi and Ward 2013). However, soil fertility is complex, with various factors affecting the ability of plants to absorb nutrients and trace elements, factors such as soil cation exchange capacity and soil pH.

In their investigation into the impact of root gaps on woody plant density, Wakeling *et al.* (2015) found a negative correlation between grass root biomass and tree density. Grass root biomass ranged from 1 326 g/m<sup>3</sup> in the high-altitude grasslands to 600 g/m<sup>3</sup> in the lowland savannas (Wakeling *et al.* 2015). In the Van Reenen's Pass area, we found no such correlation. Although, in general, the root biomass was much higher than those described by Wakeling *et al.* (2015), we did not observe a mat of grass roots, even at the highest biomass. In our study area, the dominant grass species were *Harporchloa falx* and

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*Panicum natalense* at the Waterfall site, *Aristida junciformis* and *Elionurus muticus* at the Wyford site and *Cymbopogon validus* and *Sporobolus pyramidalis* which were different to the grasses observed in the study Wakeling *et al.* (2015). This may have a bearing on the differences in root biomass.

### 3.4.3 Global warming

Our results show that neither fire, frost nor management strategy exclude *V. sieberiana* trees from the grasslands at the top of the Drakensberg escarpment during the study period. However, the unusually mild winter may have been an anomaly. Considering climate of the past, low temperatures and frost may well have precluded *V. sieberiana* from the high altitudes. Our results give a clear indication of the consequences of global warming. Fewer frost days and higher minimum temperatures will accelerate the rate of encroachment. *V. sieberiana* may possibly even overcompensate in response to mild frost events exacerbating the problem.

### 3.5 Conclusion

*Vachellia sieberiana*, has the potential to be an aggressive encroacher into the savannoid grasslands of the Drakensberg escarpment. Fire, a popular method used by field managers to control bush encroachment, had only limited success in maintaining *V. sieberiana* within the fire trap. Temperature, on the other hand, which decreases with increasing altitude, may well limit the success of sapling establishment, but with global warming resulting in warmer temperatures in the high-altitude grasslands of the Drakensberg, this limiting factor may be removed and *V. sieberiana* may well start to invade these grasslands in the future. This will pose a severe management problem in maintaining the grasslands free of *V. sieberiana*. Traditional management methods will need to be revised and new and creative strategies considered.

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## Appendix A: Study sites



Figure 3A.1: Photograph of the fenced high-altitude study site at Waterfall ( $S28^{\circ} 16' 50.2''$ ;  $E29^{\circ} 24' 29.2''$  at 1 697 m). The track is outside the study site.



Figure 3A.2: Photograph of the fenced mid-altitude site at Wyford ( $S28^{\circ} 24' 20''$ ;  $E29^{\circ} 25' 30.7''$  at 1 350 m).



Figure 3A.3: Photograph of the low-altitude study site at Zandspruit (S28° 26' 30.8"; E29° 32' 49.4" at 1 165 m) just prior to fencing.

## Appendix B: Summary of soil analysis

A summary of the soil analysis as supplied by the Fertilizer Advisory Service. Order of soil samples: Waterfall at 1 697 m asl (WF 10/15); Wyford at 1 350 m asl (WY 10/15); and Zandspruit at 1 165 m asl (ZS 10/15)

Page 1



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### SUMMARY OF ANALYTICAL RESULTS

(These results may not be used in litigation)

Batch : 732 Year : 2015 Printed : 02/11/2015

Your sample ID	Lab number	Sample density g/mL	P mg/L	K mg/L	Ca mg/L	Mg mg/L	Exch. acidity cmol/L	Total cations cmol/L	Acid sat. %	pH (KCl)	Zn mg/L	Mn mg/L	Cu mg/L
WF 10/15	F16682	1.10	5	141	293	93	0.48	3.07	16	4.11	2.6	24	0.6
WY 10/15	F16683	0.91	7	317	334	131	2.03	5.59	36	3.78	5.2	22	1.3
ZS 10/15	F16684	0.88	31	238	1181	378	0.04	9.65	0	4.73	19.7	20	2.7

Your sample ID	Lab number	Mid-Infrared Estimates		
		Org. C %	N %	Clay %
WF 10/15	F16682	1.3	0.16	17
WY 10/15	F16683	2.2	0.18	39
ZS 10/15	F16684	3.5	0.33	21

These samples have no associated fertiliser recommendation. This may be because:

1. We cannot supply the recommendation for the intended crop indicated on the submission form.
2. No intended crop was indicated on the submission form.
3. The samples submitted were described as subsoil samples, for which no recommendations are given.

### Appendix C: Burning trials



Figure 3C.1: Fire treatment at the high-altitude site at Waterfall



Figure 3C.2: Fire treatment at the mid-altitude site at Wyford



Figure 3C.3: Fire treatment at the low-altitude site at Zandspruit. In this photograph the remaining standing biomass of *Cymbopogon validus* is evident.



### Appendix D: Identifying outliers

Outliers were identified using IBM SPSS Statistics® software, version 25. Where an outlier has a value of more than 3 times the interquartile range (IQR), it labels the value as extreme and denotes it with an asterisk (\*). Where the outlier has a value between 1.5 and 3 times the IQR it labels the value with a (o). However, Hoaglin and Iglewicz (1987) found that a multiplier of 3 was too extreme and that the most appropriate multiplier was 2.2. Consequently, the identification of outliers was done manually, using 2.2 as the multiplier and the output from IBM SPSS Statistics® used as a guide. The manual calculation for outliers, using 2.2 as the multiplier identified the value at position 23 in the data set as an outlier in the Waterfall data set, which is the same value identified by IBM SPSS Statistics®.

Reference:

Hoaglin DC, Iglewicz B. 1987. Fine-Tuning Some Resistant Rules for Outlier Labeling. *Journal of the American Statistical Association* 82: 1147-1149. DOI:10.1080/01621459.1987.10478551

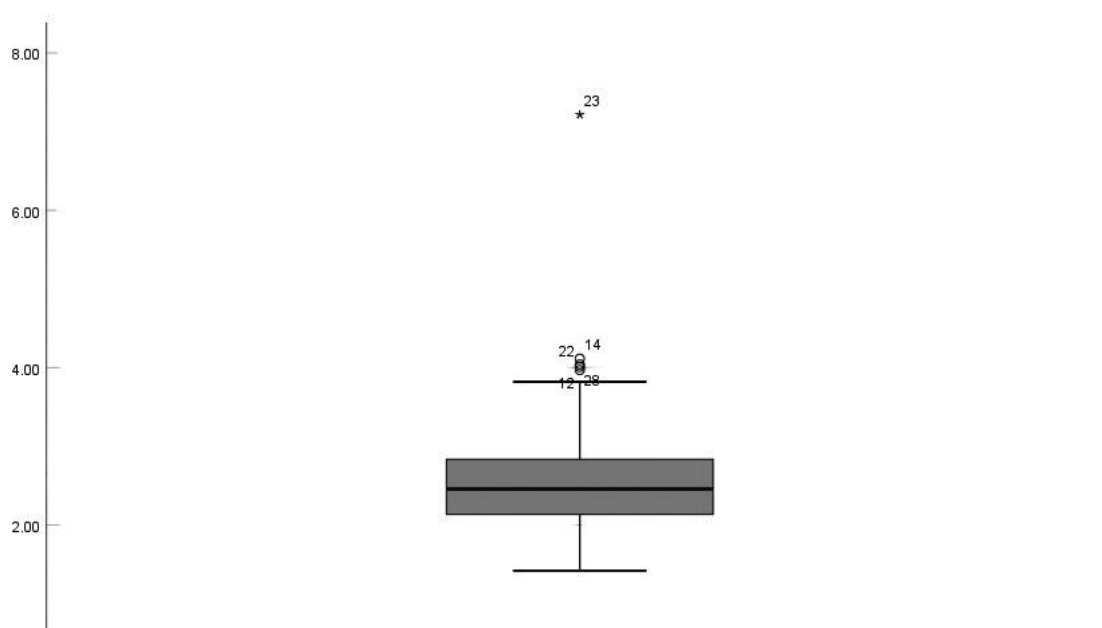


Figure 3D.1 Box and whisker plots generated by IBM SPSS Statistics® denoting outliers in the data from the study site at Waterfall (high-altitude site). The (o) denotes a value between 1.5 and 3 times the IQR, the (\*) denotes a value more than 3 times the IQR. The numbers denote the position of the data in the data set. Stem diameter is measured in mm on the y axis.

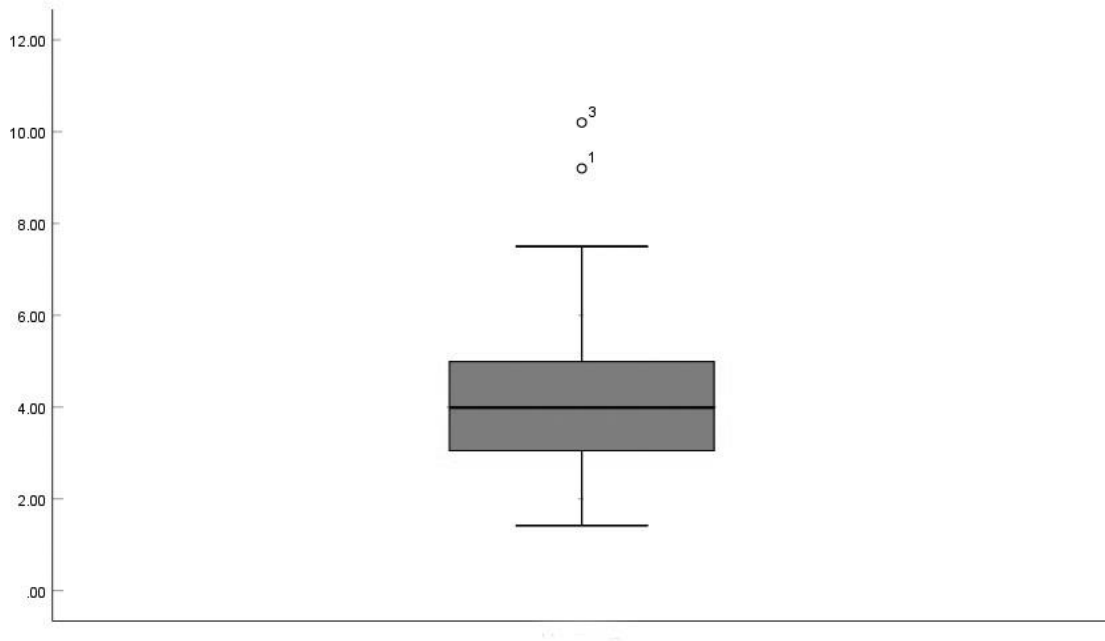


Figure 3D.2: Box and whisker plots generated by IBM SPSS Statistics® denoting outliers in the data from the study site at Wyford (mid-altitude). The (o) denotes a value between 1.5 and 3 times the IQR. The numbers denote the position of the data in the data set. Stem diameter is measured in mm on the y axis.

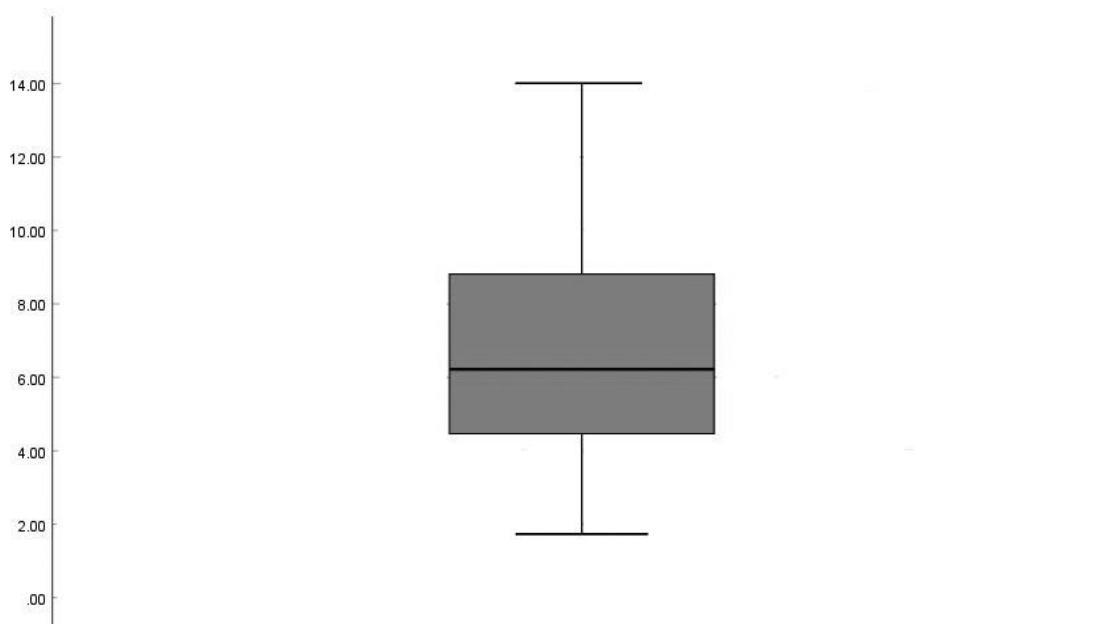


Figure 3 D.3: Box and whisker plots generated by IBM SPSS Statistics® denoting outliers in the data from the study site at Zandspruit (low-altitude). No outliers were identified. Stem diameter is measured in mm on the y axis.

## Appendix E: Graphs of all treatments

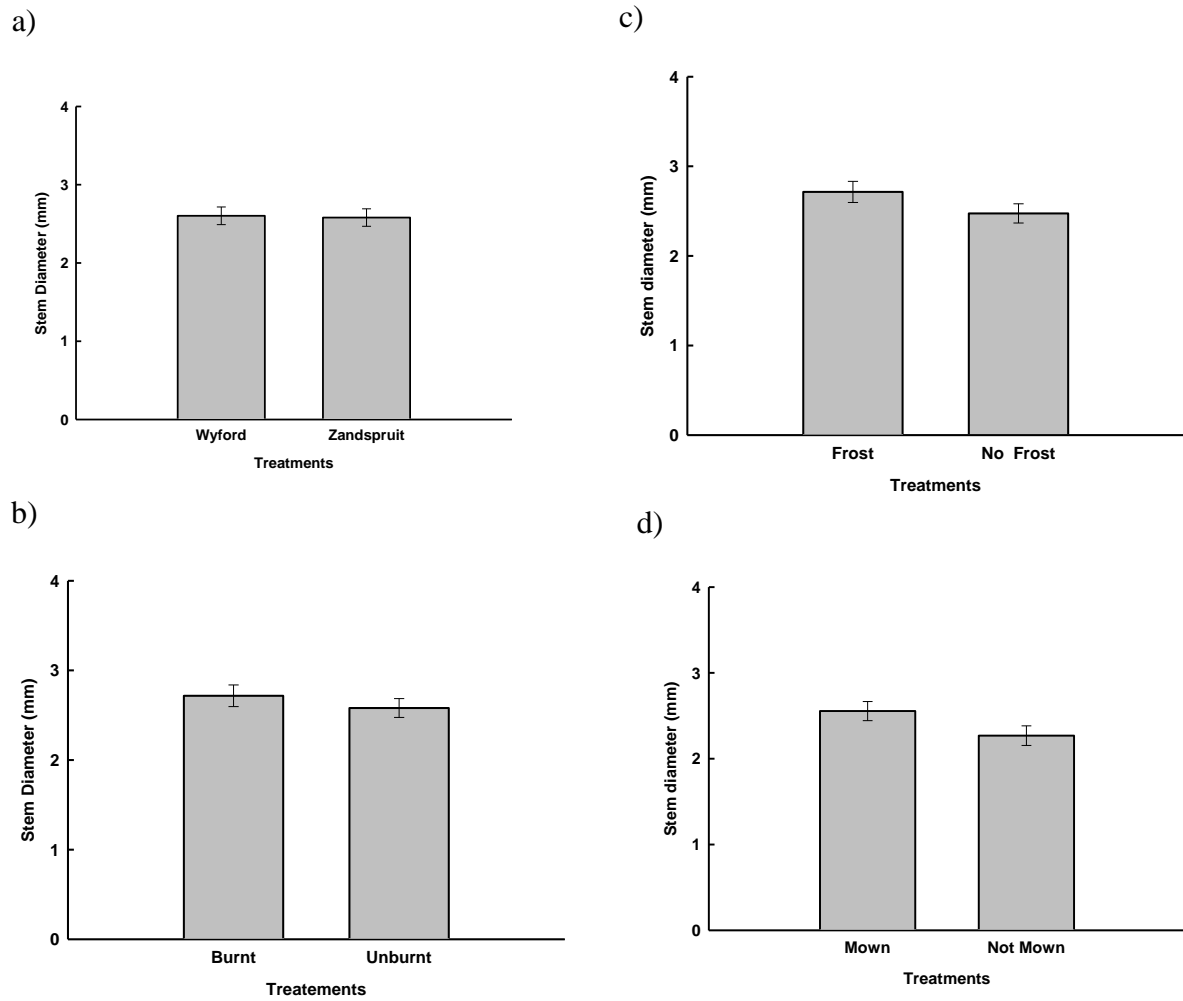


Figure 3E.1: Waterfall site (high altitude): The effect of all treatments and combinations thereof on stem diameter (mean  $\pm$  SE). None of the treatments were significant: a) seed source; b) fire; c); frost; and d) mowing of the grass sward.

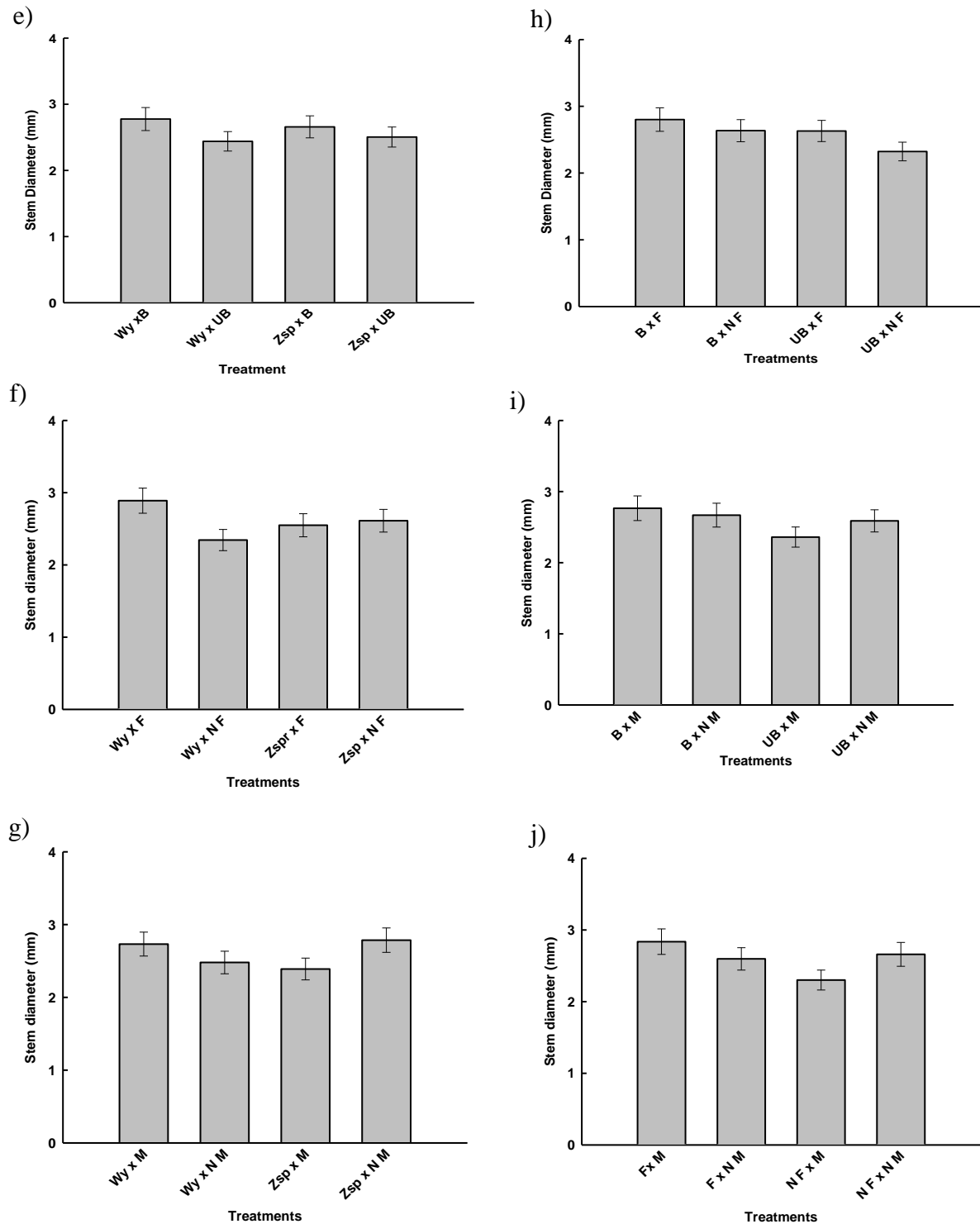


Figure 3 E.2: Waterfall site (high-altitude): The effect of all treatments and combinations thereof on stem diameter (mean  $\pm$  SE). None of the treatments were significant: e) seed source x fire; f) seed source x frost; g) seed source x mowing of the grass sward; h) fire x frost; i) fire x mowing of the grass sward; and j) frost x mowing of the grass sward. Wy = seed sourced from Wyford; Zsp = seeds sourced from Zandspruit; B = burnt; UB = unburnt; F = frost; N F = no frost; M = mowing of the grass sward; N M = no mowing.

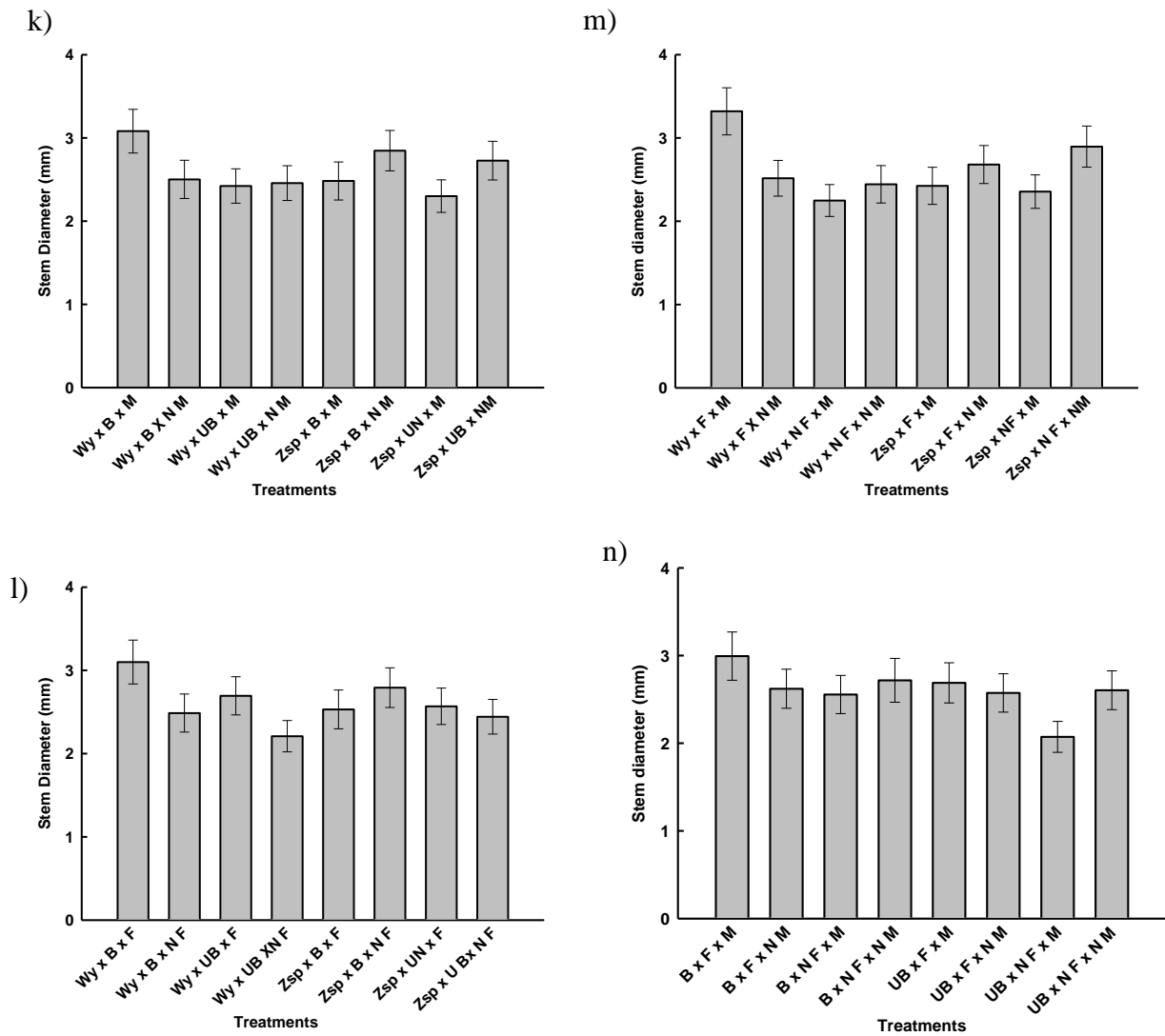


Figure 3E.3: Waterfall site (high-altitude): The effect of all treatments and combinations thereof on stem diameter (mean  $\pm$  SE). None of the treatments were significant: k) seed source x fire x mowing of the grass sward; l) seed source x fire x frost; m) seed source x frost x mowing of the grass sward; and n) fire x frost x mowing of the grass sward. Wy = seed sourced from Wyford; Zsp = seeds sourced from Zandspruit; B = burnt; UB = unburnt; F = frost; N F = no frost; M = mowing of the grass sward; N M = no mowing.

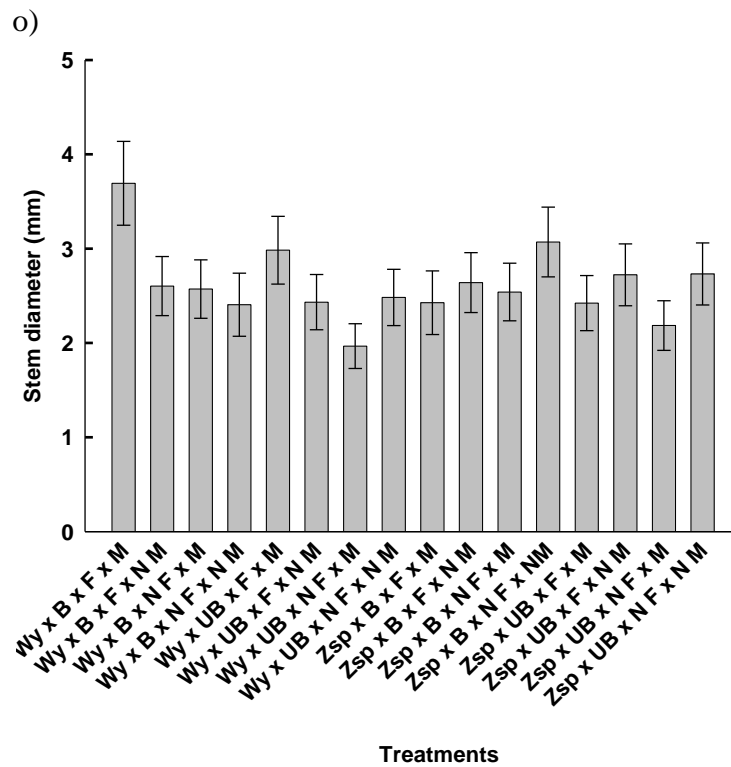


Figure 3E.4: Waterfall site (high-altitude): The effect of all treatments and combinations thereof on stem diameter (mean  $\pm$  SE). None of the treatments were significant. o) seed source x fire x frost x mowing of the grass sward. Wy = seed sourced from Wyford; Zsp = seeds sourced from Zandspruit; B = burnt; UB = unburnt; F = frost; N F = no frost; M = mowing of the grass sward; N M = no mowing.

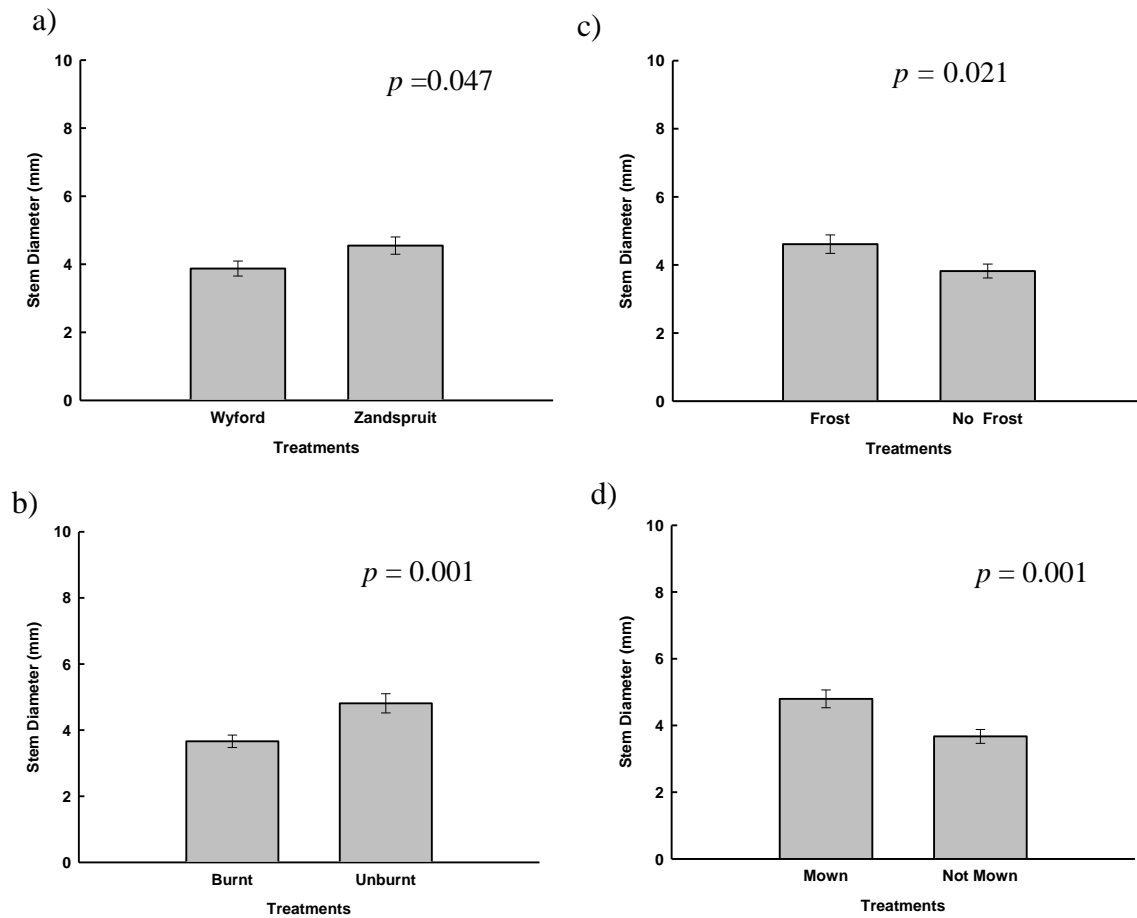


Figure 3E.5: Wyford site (mid-altitude): The effect of all treatments and combinations thereof on stem diameter (mean  $\pm$  SE). The four main treatments (seed sourced from Wyford and Zandspruit, fire, frost and mowing of the grass sward) were significant: a) seed source; b) fire; c) frost; and d) mowing of the grass sward.

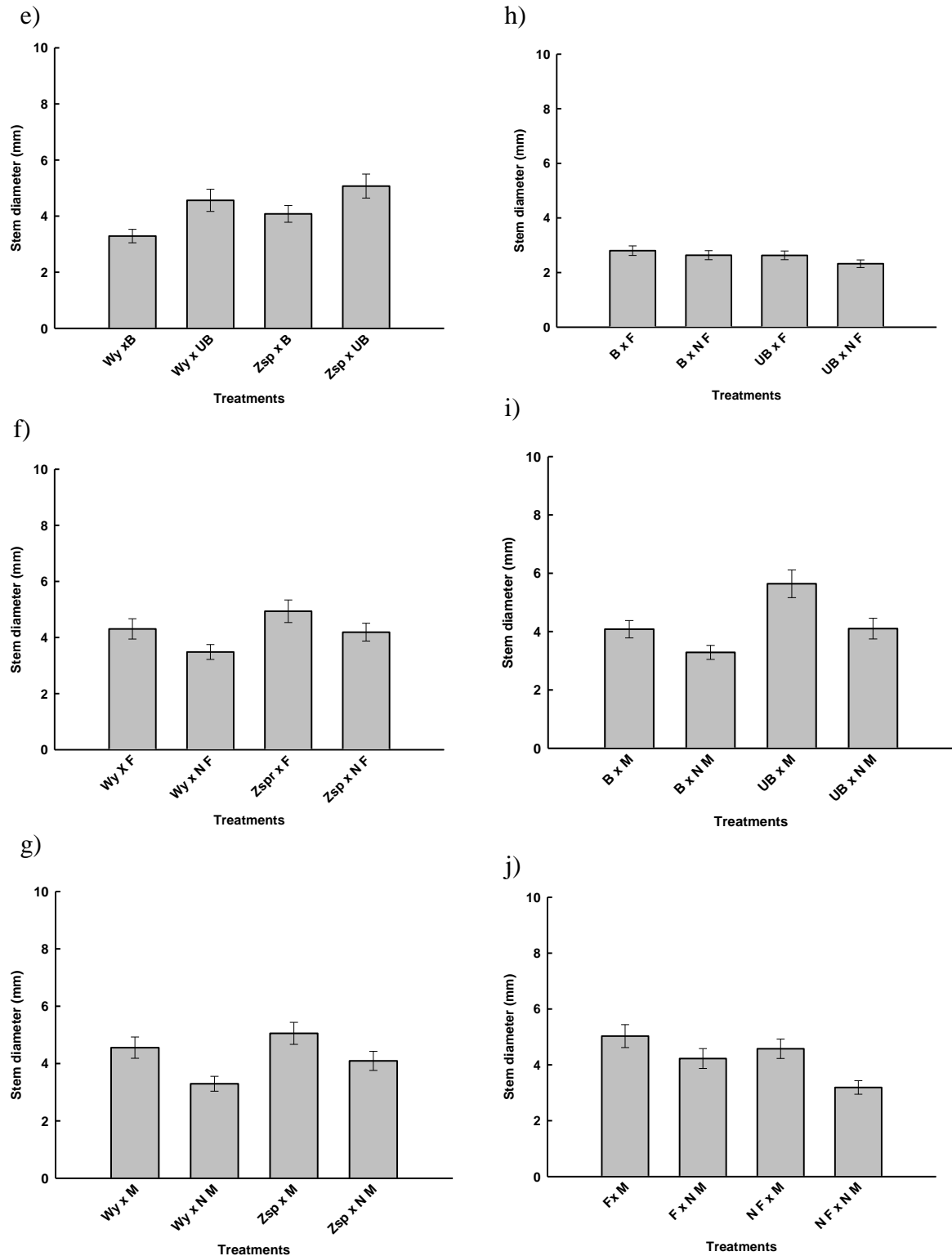


Figure 3E.6: Wyford site (mid-altitude): The effect of all treatments and combinations thereof on stem diameter (mean  $\pm$  SE). None of the following treatments were significant: e) seed source x fire; f) seed source x frost; g) seed source x mowing of the grass sward; h) fire x frost; i) fire x mowing of the grass sward; and j) frost x mowing of the grass sward. Wy = seed sourced from Wyford; Zsp = seeds sourced from Zandspruit; B = burnt; UB = unburnt; F = frost; NF = no frost; M = mowing of the grass sward; NM = no mowing.



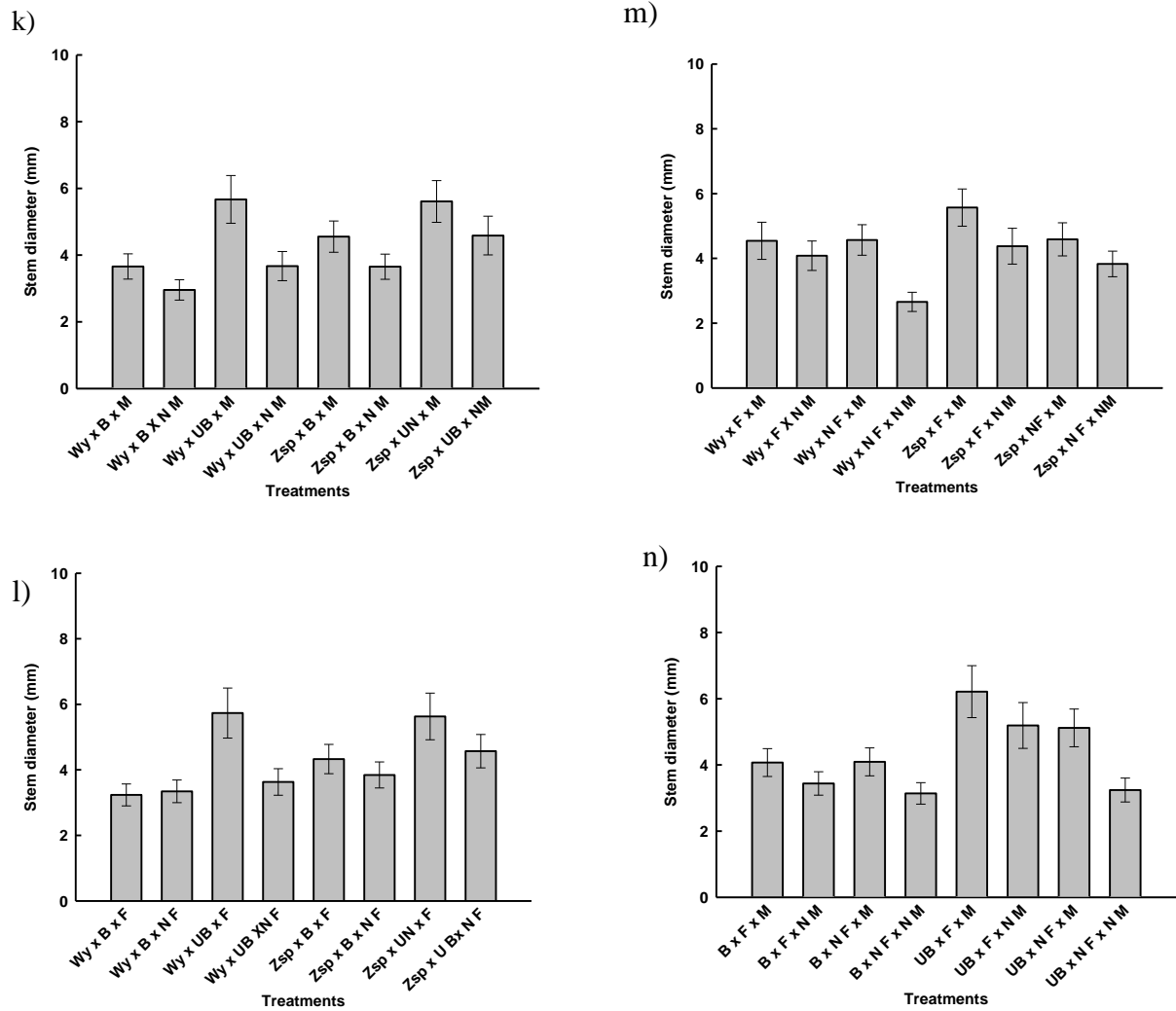


Figure 3E.7: Wyford site (mid-altitude): The effect of all treatments and combinations thereof on stem diameter (mean  $\pm$  SE). None of the treatments were significant for the following k) seed source x fire x mowing of the grass sward; l) seed source x fire x frost; m) seed source x frost x mowing of the grass sward; and n) fire x frost x mowing of the grass sward. Wy = seed sourced from Wyford; Zsp = seeds sourced from Zandspruit; B = burnt; UB = unburnt; F = frost; N F = no frost; M = mowing of the grass sward; N M = no mowing.

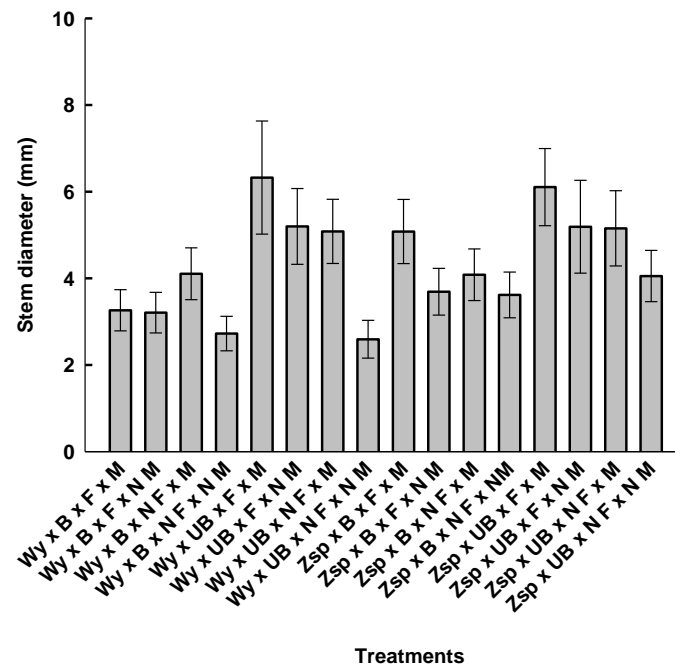


Figure 3E.8: Wyford site (mid-altitude): The effect of all treatments and combinations thereof on stem diameter (mean  $\pm$  SE). Treatments were not significant for o) seed source x fire x frost x mowing of the grass sward. Wy = seed sourced from Wyford; Zsp = seeds sourced from Zandspruit; B = burnt; UB = unburnt; F = frost; N F = no frost; M = mowing of the grass sward; N M = no mowing.

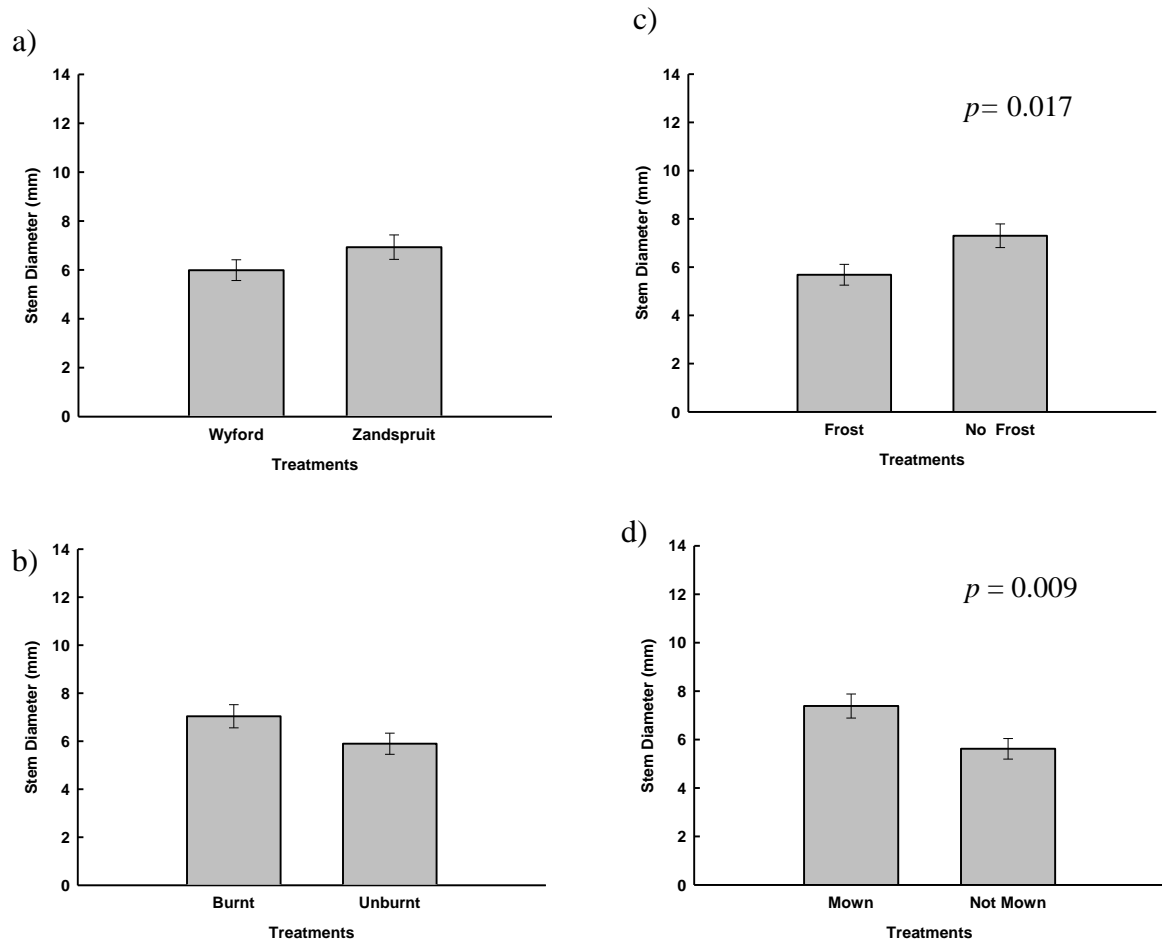


Figure 3E.9: Zandspruit site (low-altitude): The effect of all treatments and combinations thereof on stem diameter (mean  $\pm$  SE). Two of the four main treatments a) seed sourced from Wyford and Zandspruit, b) fire, c) frost and d) mowing of the grass sward were significant: c) frost; and d) mowing of the grass sward.

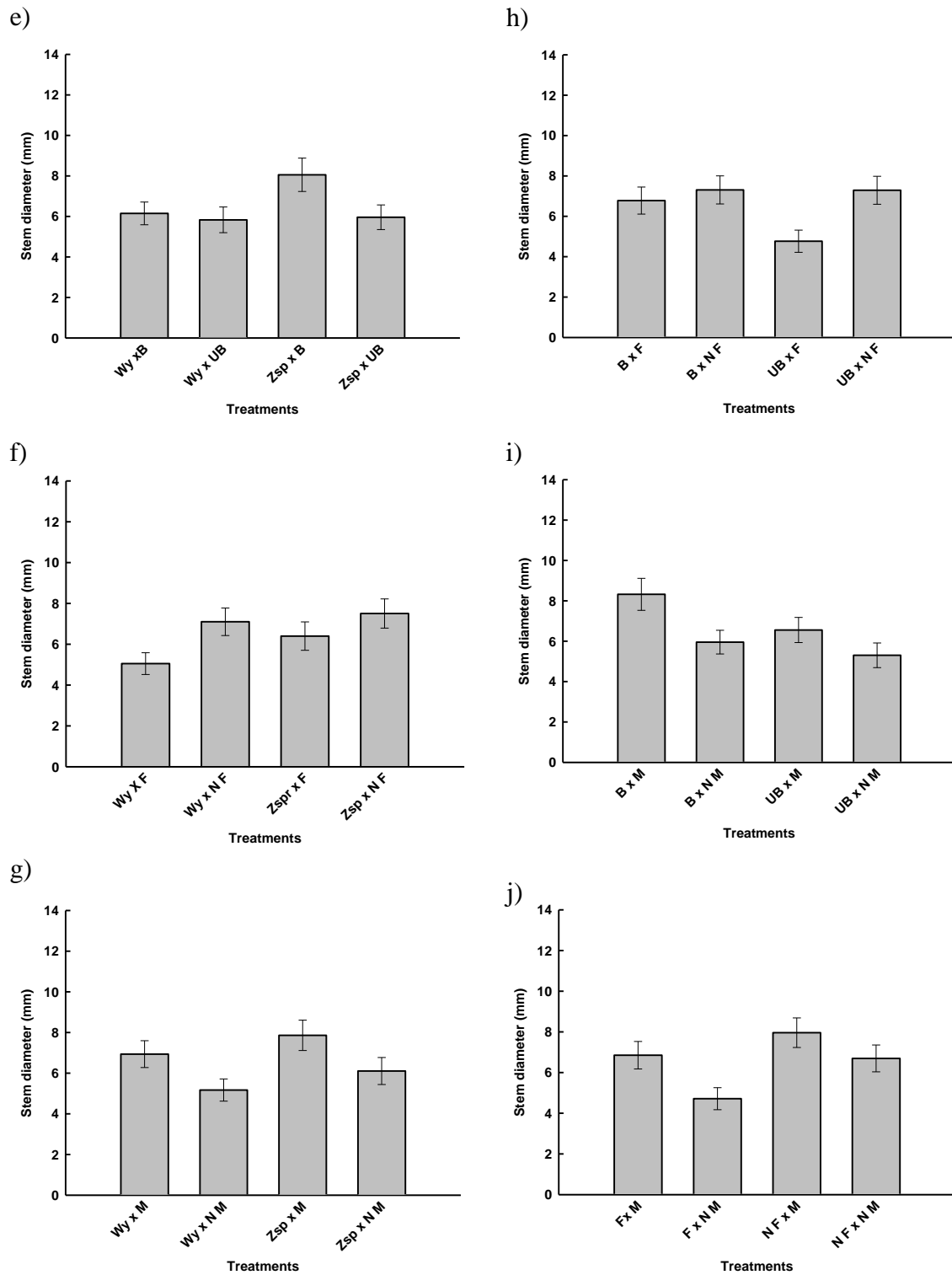


Figure 3E.10: Zandspruit site (low-altitude): The effect of all treatments and combinations thereof on stem diameter (mean  $\pm$  SE). None of the following treatments were significant: e) seed source x fire; f) seed source x frost; g) seed source x mowing of the grass sward; h) fire x frost; i) fire x mowing of the grass sward; and j) frost x mowing of the grass sward. Wy = seed sourced from Wyford; Zsp = seeds sourced from Zandspruit; B = burnt; UB = unburnt; F = frost; N F = no frost; M = mowing of the grass sward; N M = no mowing.

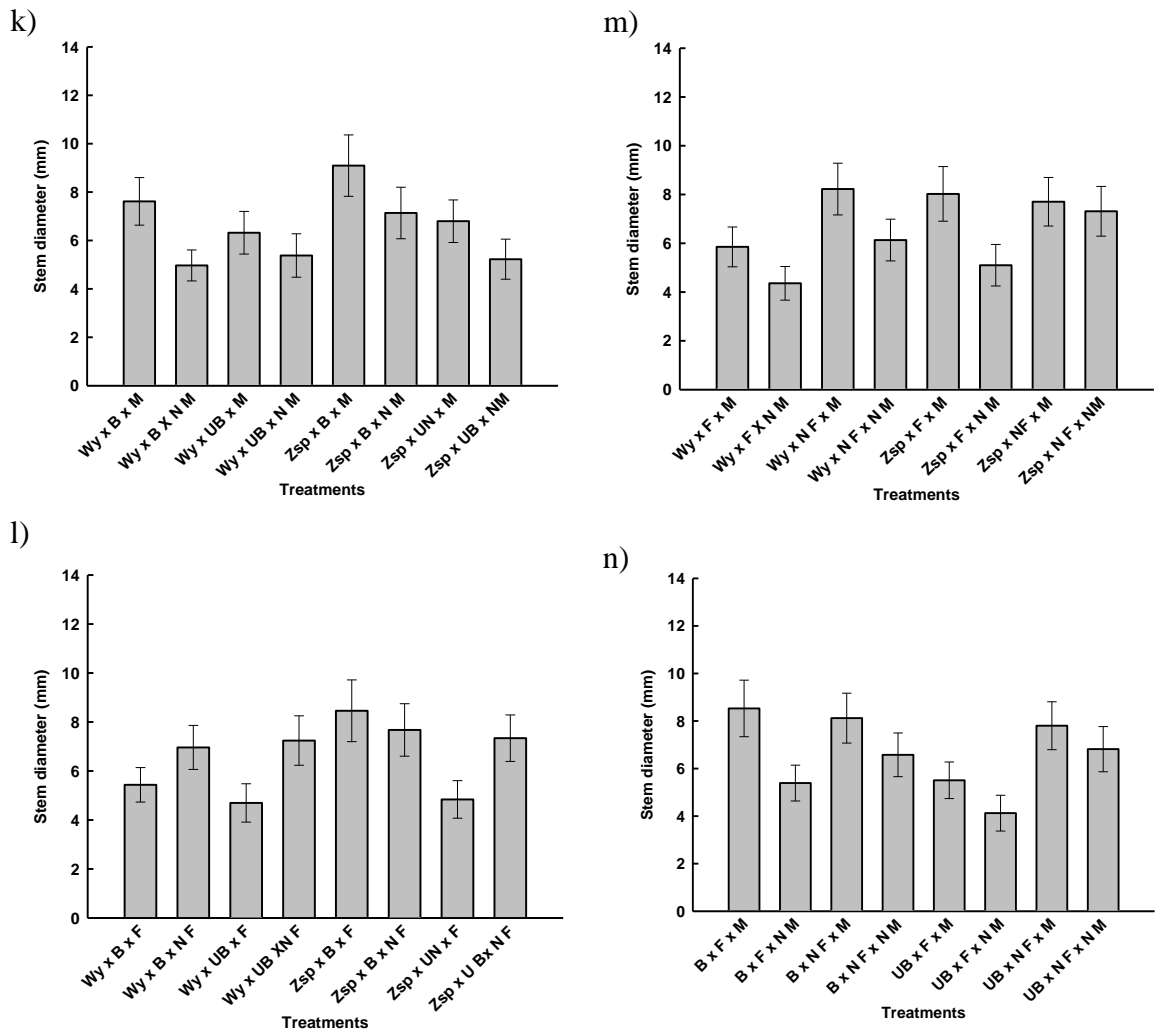


Figure 3E.11: Zandspruit site (low-altitude): The effect of all treatments and combinations thereof on stem diameter (mean  $\pm$  SE). None of the treatments were significant for the following k) seed source x fire x mowing of the grass sward; l) seed source x fire x frost; m) seed source x frost x mowing of the grass sward; and n) fire x frost x mowing of the grass sward. Wy = seed sourced from Wyford; Zsp = seeds sourced from Zandspruit; B = burnt; UB = unburnt; F = frost; N F = no frost; M = mowing of the grass sward; N M = no mowing.

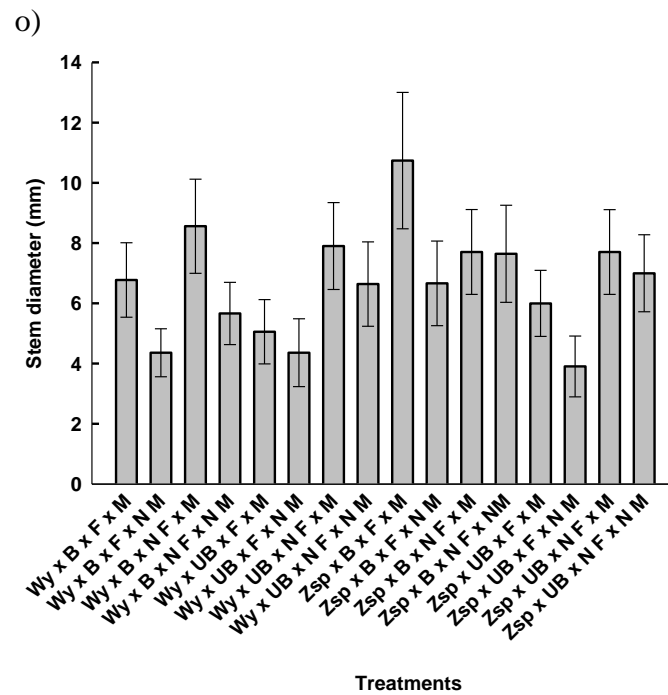


Figure 3E.12: Zandspruit site (low-altitude): The effect of all treatments and combinations thereof on stem diameter (mean  $\pm$  SE). Treatments were not significant for o) seed source x fire x frost x mowing of the grass sward. Wy = seed sourced from Wyford; Zsp = seeds sourced from Zandspruit; B = burnt; UB = unburnt; F = frost; N F = no frost; M = mowing of the grass sward; N M = no mowing.

## Appendix F: Recording ambient temperatures in the field

Complexities and uncertainties regarding ambient temperature recordings in the field

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### Abstract

In recording environmental parameters between study sites, one seeks either to compare trends between study sites or to find absolute values. The effect of solar radiation shield quality on the accuracy of the recorded data come into question during a study where ambient temperature was measured along an altitudinal gradient. It was found that homemade radiation shields of the same basic design and colour as commercial radiation shields, introduced a margin of error into the temperature data collected. While research budgets may not allow for the very best equipment to be purchased, these potential sources of error need to be recognised and accounted for when undertaking fieldwork.

Key words: Comparative temperature trends, hysteresis effect, solar radiation shield, temperature logger, ventilation shield



### 3F.1 Introduction

Recordings of environmental parameters serve two purposes. Either one seeks comparative trends or absolute values. Sometimes, a combination of both is required. While investigating encroachment of *Vachellia sieberiana* var. *woodii* (Burt Davy) (formerly *Acacia sieberiana* var. *woodii* (Burt Davy) in high altitude grasslands, ambient temperature was recorded at three sites along an altitudinal gradient up the Drakensberg escarpment in northern KwaZulu-Natal, South Africa. The accuracy (i.e. how close to true ambient temperature the equipment was able to measure); the precision (i.e. how consistently the equipment was able to give the same result under the same conditions); and the interchangeability (i.e. whether equipment could be substituted among sites) of the equipment were of importance. The results lead to the consideration of the effect that the type and quality of solar radiation shields had on the quality of the data recorded.

Ambient temperature and relative humidity are commonly measured in the field using a combined air temperature and relative humidity sensor. The sensor is placed inside a solar radiation shield to protect the sensor from direct, diffuse and reflected radiation, which would result in an elevated and misleading temperature measurement. The sensor is attached to a logger which records and stores the data collected by the sensor. Besides the quality of the sensor itself, there are various types of solar radiation shields available which may affect the accuracy and precision of the sensor measurements. There are two basic designs of solar radiation shields: aspirated and naturally ventilated. The aspirated solar radiation shields are generally accepted as the most accurate (Whiteman, Hubbe & Shaw 2000). A small fan draws an air current over the temperature sensor, thereby maintaining the sensor in an environment close to, if not equal to, ambient temperature. The most commonly used naturally ventilated solar radiation shields are the Stevenson Shield (also known as the Cotton Region Shelter), the Gill shield, the Maximum-Minimum Temperature System shield and the Automated Surface Observing System shield (Hubbard, Lin & Walter-Shea 2001) (Figure 3F.1a-d). Generally, solar radiation shields used in the field are naturally ventilated, due to the relatively high current drain of the electric fan of aspirated shields, which will greatly reduce the life of the batteries where no main electric power is available.

### 3F.2 Methods

The field equipment comprised of two HOBO Pro v2® Temperature/Relative Humidity data loggers with external temperature/relative humidity sensors, manufactured by Onset, USA. Two data loggers and sensors were placed at the coldest study site (1697 m ASL). One sensor was mounted within a seven-plate HOBO® solar radiation shield (model number RS3), based on the

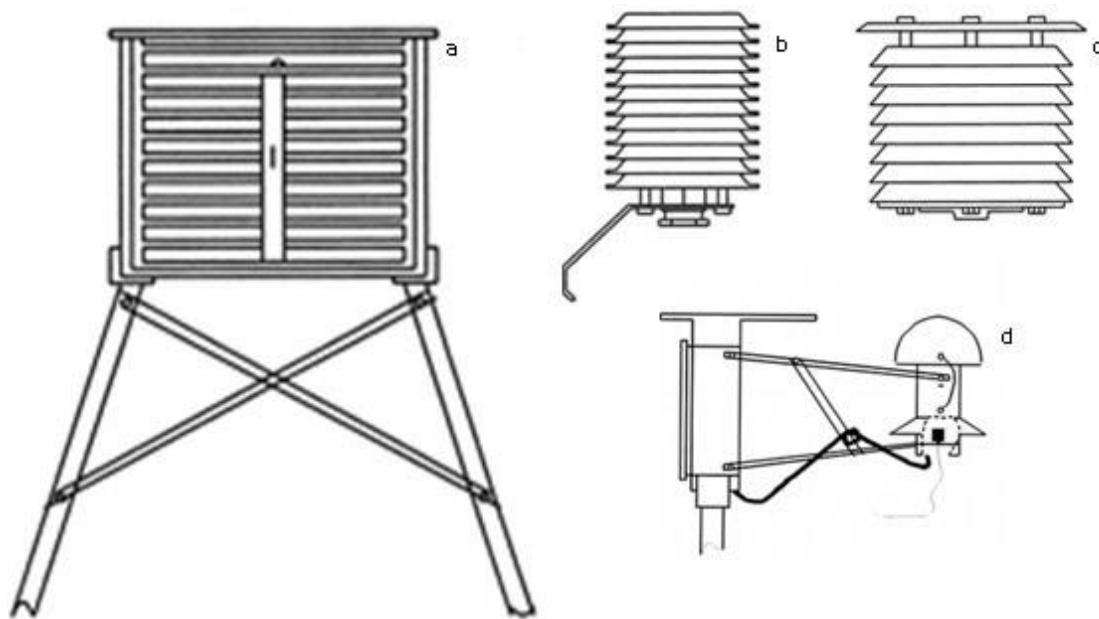


Figure 3F.1: (Not to scale) Diagrams of the four most commonly used, naturally aspirated solar radiation shields: a) Stevenson Shield; b) Gill shield; c) Maximum-Minimum Temperature System shield; and d) Automated Surface Observing System shield. (Adapted from Hubbard et al. (2001) and Lin et al. (2001))

Gill III design (Gill 1983). The second sensor was mounted within a homemade seven-plate radiation shield, also based on the Gill III design (Gill 1983). The homemade shield was made of inexpensive triangular plastic plates, measuring 170 mm x 170 mm. As with the plates of the HOBO® solar radiation shields, the plates of the homemade shield were 12 mm apart and painted white. The sensors within their respective solar radiation shields were placed within the open grass sward at tree seedling canopy height of 100 mm above ground level and were co-located, being secured to the same anchor point. Ambient temperature and relative humidity were logged on a five-minute time interval, (data logger clocks synchronized). The two loggers recorded ambient conditions for three days.

### 3F.3 Results and discussion

Ambient temperatures ranged between  $-7^{\circ}\text{C}$  and  $28^{\circ}\text{C}$ . When the recorded data of the two loggers were compared, it was found that there was up to a  $3^{\circ}\text{C}$  discrepancy between the two. In general, the sensor with the HOBO® solar radiation shield recorded lower temperatures than that of the homemade solar radiation shield, particularly at the extremes of the temperature range. As the lower end of the temperature range was of pivotal importance to this vegetation study, only temperatures of less than  $5^{\circ}\text{C}$  as recorded by the sensor with the HOBO® shield were plotted against the simultaneously recorded temperatures from the sensor with the homemade shield (Figure 3F.2). The graph indicated a hysteresis effect. Farrell (1999) described a hysteresis effect as when “a system responds

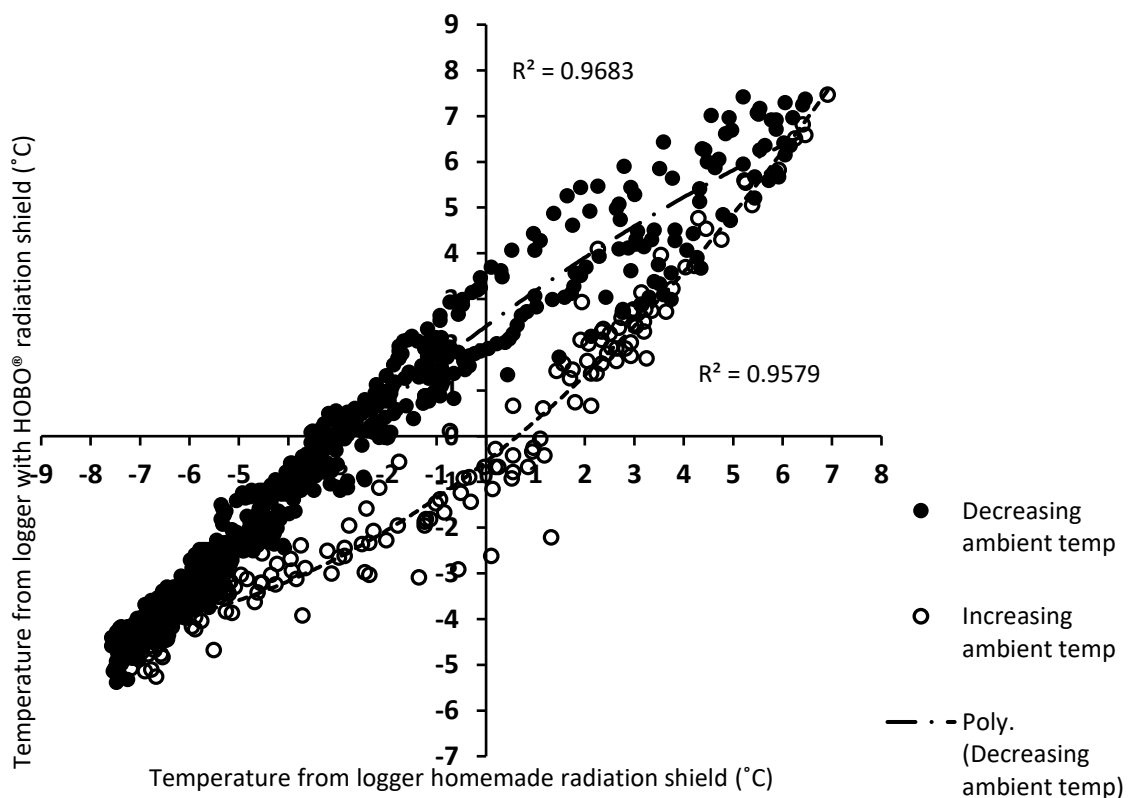


Figure 3F.2: Graph showing the hysteresis effect caused by the two different solar radiation shields used in a field experiment

differently to identical inputs depending on the direction in which the system is being driven.” (see also Haines (1930), BaČA (2008), Fauchald (2010)).

In this case, the hysteresis effect can be attributed to differences in the materials used for the solar radiation shields. The Hobo® solar shields were made of acrylonitrile styrene acrylate (ASA) which is a weather resistant thermoplastic. The type and characteristics of the plastic plates of the homemade solar radiation shield were unknown. Different materials will have different thermal properties, such as surface radiation (emissivity), absorption of radiation, conductivity and specific heat (Cengel & Boles 2015). These properties directly or indirectly affect heat transference. Emissivity off the solar radiation shield plates directly affects the degree of accuracy of the temperature data. The temperature probe within the shield need not be in contact with the solar radiation shield plates for heat transference to take place (Cengel & Boles 2015). Quantity of radiation absorbed by the solar radiation shield plates, characteristic conductivity and specific heat of the material of the plates will all affect emissivity (Cengel & Boles 2015) and, therefore, have an indirect effect on the accuracy of the measurements.

The shape of the solar radiation shield plates could also play a role in creating the hysteresis. The homemade solar radiation shield was larger in diameter than the Hobo® solar radiation shields. The plastic plates also had a more pronounced lip around the edge. Although the spacing between the plastic plates was the same as that between the Hobo® solar radiation shield plates, the size and greater curvature of the homemade solar radiation shield plates could have restricted airflow around the temperature probe, thus, further insulating the temperature probe from fluctuations in ambient air temperatures.

To ascertain accuracy and interchangeability of the sensors, four temperature/relative humidity sensors and loggers were concurrently tested in a controlled environment. The sensors included the two HOBO® sensors combined with the HOBO Pro v2® Temperature/Relative Humidity data loggers, which had been used in the field; a CS215 sensor (manufactured by Campbell Scientific, USA); and a MNP60 sensor (manufactured by Vaisala, Finland). The data from the latter two sensors were stored on a CR200 logger, manufactured by Campbell Scientific, USA. The results showed very little variability among all four sensors (Table 3F.1), which suggested a high level of interchangeability. This confirmed that the hysteresis effect was caused by the solar radiation shields and not the sensors. The results also showed that the comparatively less expensive HOBO® sensors were as accurate as the very expensive ones.

Table 3F.1: A comparison showing the degree of interchangeability and accuracy of the four sensors when measuring temperature (in °C) in three different temperature regimes. Once the sensors had stabilized within the controlled environment, temperatures were recorded for one hour at one- minute intervals.

	Difference between				
	HOBO® 1	HOBO® 2	HOBO® 1 & 2	CS215®	HMP62®
Freezer	-19.8	-19.8	0	-19.3	-19.1
Fridge	4.7	4.4	0.3	5	4.8
Ambient	23.9	23.6	0.2	23.6	23.9
Average	2.9	2.7	0.2	3.1	3.2

In seeking to measure ambient temperature in the field, potential sources of variability must be kept in mind, particularly if one is seeking comparative data. Besides quality and uniformity of the equipment, age also plays a significant role in accuracy, precision and repeatability of data collected. Although not investigated here, it has been noted that Lopardo *et al.* (2014) compared solar radiation shields of identical specifications, but of different ages. They found that there was an average difference of 0.46 °C in daily maximum temperature measured by sensors within new and five-year old solar radiation screens (Lopardo *et al.* 2014). Data gathering equipment needs to be prioritized in the research budget. These findings show the importance of careful calibration of air temperature sensors against accurate standards with an assessment of the influence of the solar radiation shield prior to equipment installation.

#### Acknowledgments

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**CHAPTER 4: FROST TOLERANCE IN *VACHELLIA SIEBERIANA* VAR. *WOODII* (FORMERLY *ACACIA SIEBERIANA* VAR. *WOODII*)**

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**Abstract**

High altitude grasslands are frequently within a climatic zone that would support trees, yet trees are absent. The answer as to what mechanism excludes trees from these grassy biomes continues to elude researchers. Very often low temperature is cited as a possible mechanism. *Vachellia sieberiana* var. *woodii* is a typical savanna tree which is absent from high altitudes. We investigated the effect of white frost and black frost on the survival rate of *V. sieberiana* saplings in a controlled environment. We also investigated whether *V. sieberiana* saplings, grown from seed collected from two different altitudes, might exhibit a preadaptation to tolerate frost. We found that *V. sieberiana* saplings were able to coppice readily after 100% topkill as a result of white frost and that they could survive black frost down to  $-6^{\circ}\text{C}$ . An interaction between frost and seed source suggests a preadaptation in the saplings grown from seed collected from the site that has a higher number of frost days which enables the sapling to compensate rapidly for frost damage. Two further preliminary investigations were carried out. Many frost tolerant plants increase metabolites within the cell sap to avoid freezing. Therefore, we tested whether *V. sieberiana* tolerated frost by increasing soluble sugars in the cell sap after chilling. We also investigated whether *V. sieberiana* seeds remained viable after freezing. *V. sieberiana* did not increase soluble sugars within the cell sap in response to stress. It may be that, as a deciduous tree, *V. sieberiana* avoids frost damage through dormancy in the winter months, rather than increasing metabolites within the cell sap. The seeds of *V. sieberiana* are resistant to freezing, maintaining viability at  $-9^{\circ}\text{C}$ , although the germination rate was reduced.

**Key words:** Black frost, preadaptation, soluble sugars, topkill, white frost.



#### 4.1 Introduction

It is generally held that climate dictates the distribution of the major biomes (Polis 1999; Bond 2005). However, the grassy biomes, including savannas, are an exception. They frequently occur within a climatic zone that would support a higher woody biomass and yet savannas are characterised by a co-dominance of trees and grasses (Bond 2005; Bond 2008). The mechanisms that determine that tree:grass ratio of a savanna have been long debated by ecologists. In arid and semi-arid savannas, climate appears to be the main factor. In their review of over 850 sites in Africa, Sankaran *et al.* (2005) demonstrated that tree cover increased linearly with mean annual precipitation (MAP), i.e. resources such as water are the limiting factors. Above MAP of ~650 mm the tree:grass ratio is determined by disturbance such as fire or herbivory (Sankaran *et al.* 2005; Sankaran *et al.* 2008). Obviously, it is a complex interaction between all determinants, be they “bottom-up” (resources) or “top-down” (disturbance) (Bond 2008; Sankaran *et al.* 2008).

Frost is a disturbance that is seldom considered as having a significant impact on the structure of savannas (Whitecross *et al.* 2012). If it is considered at all, it is generally in conjunction with herbivory and/or fire (Holdo 2005; Holdo 2007), although see Brando and Durigan (2005); Muller *et al.* (2016). Frost is regarded as the greatest weather hazard for crops and forests (André *et al.* 2010). Much has been written on the effect of chilling and freezing on crop cultivation (for example Jacobsen *et al.* 2005; Snyder and de Mel-Abreu 2005; Schultze 2006) and forestry (for example Greer and Warrington 1982; Pollock *et al.* 1986; Greer *et al.* 1989). Savage (2012) defined frost as “the condition for which the surface and earthbound objects have a temperature at or below 0°C, often resulting in ice on leaves and soil if the temperature of the surface, or air near the surface, is less than or equal to the dewpoint temperature.” Frosts are generally the result of radiative cooling and require calm, clear nights (Schultze 2006; Savage 2012). When dew point temperature is higher than minimum temperature, a white or hoar frost forms on the surface of an object, but, when dew point temperature is lower than the minimum temperature, there is no surface ice and ice crystals form inside the plant tissue, resulting in black frost (Schultze 2006; Savage 2012). In nature, freeze injury occurs when ice formation is extracellular (within the intercellular spaces) (Levitt 1980). The damage is the result of water stress and desiccation of the plant cells (Snyder and de Mel-Abreu

2005) as a consequence of cell water evaporating and passing out of the cells into the intercellular spaces.

Plants resist cold temperatures by avoidance or tolerance (Levitt 1980; Osmond *et al.* 1987). Avoidance may take the form of dormancy, desiccation, the development of bulky organs, or an accumulation of solutes in the cell sap (Osmond *et al.* 1987; Schultze 2006; Taiz and Zieger 2006). Plants that are tolerant to low temperatures are able to maintain metabolic functions through a process known as hardening (Taiz and Zieger 2006). This is accomplished by changes in the cell biology, such as an increase in unsaturated fatty acid chains in the cell membranes (Beck *et al.* 2004; Taiz and Zieger 2006).

As frost acts as an agent of disturbance within savanna and grassland systems, we investigated the impact of frost on a common southern African savanna tree, *Vachellia sieberiana* var. *woodii*. We conducted controlled frost experiments on saplings grown from seed in the arboretum of the University of KwaZulu-Natal as well as two preliminary investigations into the possible mechanism *V. sieberiana* uses to avoid frost damage and the effect of freezing on seed viability. Our hypotheses were:

1. The greater the degree of top-kill as a result of white frost, the lower the overall plant biomass after the end of the following growing season.
2. Black frost would kill *V. sieberiana* saplings.
3. Saplings grown from seeds from the colder site would survive cold temperatures better than those from the warmer site.
4. *V. sieberiana* saplings would avoid freeze damage by increasing soluble sugars within their cell sap.
5. Freezing the seeds of *V. sieberiana* would reduce seed viability.

## **4.2 Materials and methods**

### **4.2.1 Propagation**

Ripe seed pods were collected from *V. sieberiana* from the district of Van Reenen, north-western KwaZulu-Natal, from two farms: Zandspruit (S28° 26' 30.8"; E29° 32' 49.4") at an altitude of 1 165 m above sea level (asl) and Wyford (S28° 24' 20"; E29° 25' 30.7") at 1 350 m asl during the months of June and July. The pods were harvested from all trees bearing pods, by knocking the pods out of the trees and collecting the fallen pods. Where pods had already dropped to the ground, these were also collected. The pods from the two

sites were kept separate, the seeds were removed and damaged seeds discarded. Damage to the seeds was largely by bruchid beetles and amounted to 59% of the total number of seeds. The remainder were, potentially, viable. At the start of spring, the seeds were scarified by clipping the end opposite the hilum and soaked overnight before being planted out into 16 seed trays in a 50:50 compost:sand mix. Each tray held 128 seedlings. Once the seedlings had reached 7.5 cm in height, they were transplanted into 5-liter plastic bags of river sand and kept under shade cloth in the arboretum at the University of KwaZulu-Natal, Pietermaritzburg, for two hours twice a week between 9h00 and 11h00, using an automatic sprinkler system.

#### 4.2.2 Frost trials

In July, mid-winter when *Vachellia sieberiana* in the field would have been subjected to frosty conditions, 88 of the most robust saplings (44 saplings from the population from Zandspruit and 44 saplings from the population from Wyford) were selected. These were then divided into two groups: 40 saplings (20 saplings from the population from Zandspruit and 20 saplings from the population from Wyford) in one group and 48 saplings (24 saplings from the population from Zandspruit and 24 saplings from the population from Wyford) in the second group. The first group of saplings was used for testing the effects of white frost, the second for testing the effects of black frost. The selection of saplings for treatment was entirely random, assigning computer generated random numbers to each sapling. In the field would have been subjected to frosty conditions, 88 of the most robust saplings (44 saplings from the population from Zandspruit and 44 saplings from the population from Wyford) were selected. These were then divided into two groups: 40 saplings (20 saplings from the population from Zandspruit and 20 saplings from the population from Wyford) in one group and 48 saplings (24 saplings from the population from Zandspruit and 24 saplings from the population from Wyford) in the second group. The first group of saplings was used for testing the effects of white frost, the second for testing the effects of black frost. The selection of saplings for treatment was entirely random, assigning computer generated random numbers to each sapling. For all the statistical analyses we used IBM SPSS Statistics® software, version 25.

#### 4.2.2.1 White frost

We simulated white frost by pouring liquid nitrogen over each sapling, except the controls. The proportions of the saplings exposed to liquid nitrogen were as follows: the apical meristem; 25%; 50%; and 75%. There were, therefore, two levels of treatment: seed source (saplings grown from seeds collected from Zandspruit and from Wyford) and percentage frost. Each treatment was replicated four times. At the end of the following summer (late February), which gave the saplings a full growing season in which to recover, the saplings were harvested. The roots and shoots were oven dried separately at 60°C for 48 hours and weighed to ascertain how much biomass the saplings could regain after different amounts of frost damage within a given time and whether there was a difference in the response between the saplings from different seed sources. We analyzed root and shoot biomass data using Generalised Linear Models (GLMs). The best goodness of fit was the log linked gamma distribution. Where the analysis revealed significant effects ( $p \leq 0.05$ ), Fisher's Least Significant Difference was used to separate the means.

#### 4.2.2.2 Black frost

We simulated black frost using a technique adapted from Pollock et al. (1986). There were two levels of treatment: seed source (saplings grown from seeds collected from Zandspruit and from Wyford) and temperature. Each treatment was replicated four times. This meant that for each target temperature there were four saplings grown from seed collected from Zandspruit and four saplings from seed from Wyford. For each treatment, saplings were placed into a controlled environment unit (CEU) (see also Ceulemans *et al.* 2017) at ambient temperature. Temperature in the CEU decreased until the target temperature (e.g. 0°C) was reached. The target temperature was maintained for eight hours. Thereafter the temperature within the CEU returned to 12°C (average daily temperature two hours after sunrise). The saplings were removed from the CEU and replaced in the arboretum for observation. The trials were conducted overnight as this was the period when saplings in the field would experience minimum temperatures. The procedure was repeated for the remaining groups of saplings, but at the different target temperatures, which were: 0°C, -3°C, -6°C, -9°C and -12°C. The control groups were maintained in the arboretum at ambient temperature. At the end of the following summer, those saplings that had survived were harvested. Roots and shoots were separated, oven dried at 60°C for 48 hours and then weighed. We analysed the results using GLMs, the

best fit for both root and shoot biomass being a normal distribution with an identity linked function. A Fisher's Least Significant Difference post hoc test was used to separate the means ( $p \leq 0.05$ ) where results were significantly different. Root and shoot biomass were also expressed as a root:shoot ratio and the differences compared using GLMs. Best fit was provided by gamma identity with a log function. We also calculated the temperature at which  $p = 0.5$ , i.e. the temperature at which one could expect 50% mortality. The calculation was made using the following equation:

$$temperature = negative\ intercept / slope$$

We also used a binomial GLM using the logit function to determine the predicted mean at which  $p = 0.5$  to demonstrate the result graphically. We transformed the data by making one value in the group subjected to  $-3^{\circ}\text{C}$  equal to 0 (see Kiepiel and Johnson, 2014). The following winter, further trials were conducted to test the above calculation. The same procedure was followed, as above, but with the target temperatures being  $-6^{\circ}\text{C}$ ,  $-7^{\circ}\text{C}$ ,  $-8^{\circ}\text{C}$  and  $-9^{\circ}\text{C}$ .

#### 4.2.3 Frost avoidance - testing for soluble sugars within the cell sap

We chose to test for soluble sugars as a reaction to stress in *V. sieberiana* as work done Kebbas et al. (2015) showed that *Acacia* (= *Vachellia*) *tortilis* subsp. *raddiana* increased sugars within the cell sap in response to water stress (see also Santarius 1973). In early April, when the winter minimum temperatures in the field would be approaching  $0^{\circ}\text{C}$ , 12 bagged saplings from each population (Wyford and Zandspruit) were selected. From each group of 12, six remained in the arboretum at ambient temperature. These were the controls. The remaining six saplings from each group, together, were to be chilled to bring about cold hardening. They were placed in the CEU at ambient temperature under ultraviolet lights. The lights were on a time switch to mimic a day/night cycle of 14 hours daylight and 10 hours night. Temperature in the CEU was then reduced to  $0^{\circ}\text{C}$  and the saplings left within the CEU for a week for hardening to occur. Thereafter, temperature within the CEU returned to ambient. We harvested approximately 100 g of leaves from both the control saplings and the chilled saplings. The leaves from each sapling were kept separate and oven dried for 48 hours at  $60^{\circ}\text{C}$ . Thereafter, the leaves were ground to a fine powder with pestle and mortar. The procedure followed for extracting soluble sugars was adapted from Chow and Landhäusser (2004). From each sample, a 50-mg subsample of

leaf material was extracted three times by boiling the samples in 5 ml of 80% ethanol in glass tubes capped with glass marbles in a 95°C water bath for 10 minutes. After each extraction, the tubes were centrifuged at 2 500 rpm for 5 minutes. Each time the supernatant was taken off and a further 5 ml 80% ethanol added. The supernatant from each subsample was pooled and made up to 25 ml with distilled water. To test for soluble sugars, we followed the procedure described by Miller (1959), using the modified reagent which comprised 1% dinitrosalicylic acid, 0.2% phenol, 0.05% sodium sulphite and 1% sodium hydroxide. Three ml reagent and 3 ml supernatant were placed in a test tube and heated for 5 minutes in a water bath of boiling water. It was then cooled under running water to room temperature. One ml of 40% solution of Rochelle Salt was added after the development of colour, but before the mixture cooled, to stabilise the colour. Absorbance was read at 575 nm.

Absorbance was converted to mg glucose per g dry mass of plant material using the following formula:

$$x = (100 * (a/0.0983) * 0.01)/0.02$$

Where  $x$  = mg glucose per g dry plant mass and  $a$  = absorption (nm).

We performed a univariate analysis of variance on the results, having first log transformed the data to ensure that the assumptions were met.

#### 4.2.4 Effect of freezing on seed viability

We placed five separate batches, each of 50 *V. sieberiana* seeds, in the CEU at ambient temperature. Temperature was reduced to the target temperature, which was 0°C, -3°C, -6°C and -9°C respectively and held for two hours before returning to ambient temperature and the seeds removed. The fifth batch, the control group, was kept at ambient temperature. Thereafter, we prepared the seeds for planting by first scarifying them, then soaking them overnight. The next day we planted the seeds out into seed trays in a 50:50 sand:compost mix and watered regularly to avoid water stress. Germination success was analysed with a Generalised Linear Model using a binomial distribution with a probit model. Where results were significant a sequential bonferroni post hoc test was used.

## 4.3 Results

### 4.3.1 Frost trials

#### 4.3.1.1 White frost

Topkill of the saplings was the same as the proportion treated. For example, if the apical meristem was frosted, then the amount of topkill was just the apical meristem. There was an overall significant effect of simulated white frost on dry shoot and root biomass ( $p < 0.05$ ). There was no overall significant difference between the dry biomass of roots and shoots of the saplings grown from seeds collected from Wyford and from Zandspruit respectively, but there was an interactive effect between frost and the saplings from the two population groups (Table 4.1).

Table 4.1: The effect of simulated white frost and seed source on shoot and root biomass on *Vachellia sieberiana* saplings, using a generalized linear model with a log linked gamma distribution. Saplings were grown from seeds collected from two different sites at different altitudes (seed source): Wyford (1 350 m asl) and Zandspruit (1 165 m asl). p-values in bold with \*\* are significant

	Roots			Shoots		
	Likelihood Ratio Chi-Square	Chi-df	Sig.	Likelihood Ratio Chi-Square	Chi-df	Sig.
% Topkill	22.782	4	0.000**	26.381	4	0.000**
Seed source	0.417	1	0.519	1.275	1	0.259
% Topkill*Seed source	13.129	4	0.011**	9.777	4	0.044**

Dry shoot biomass of the control saplings was significantly higher than those that experienced 25%, 50% and 75% topkill, but not significantly different to those that had only the apical meristem exposed to frost, i.e. as the proportion of the sapling exposed to frost increased, so shoot biomass decreased. However, below 25% topkill, the differences were not significant (Figure 4.1a). Dry root biomass of saplings followed a similar trend (Figure 4.1b). The interaction between frost and seed source, although significant, on the shoot biomass differed between saplings from the different population groups (Figure 4.1c). The shoot biomass of the control Wyford saplings and those which had only the apical meristems frosted was greater than those which had experienced 25%, 50% and

75% topkill. Within the Zandspruit seedlings there was no distinct pattern. The root biomass of the saplings grown from seed from Wyford decreased linearly in response to

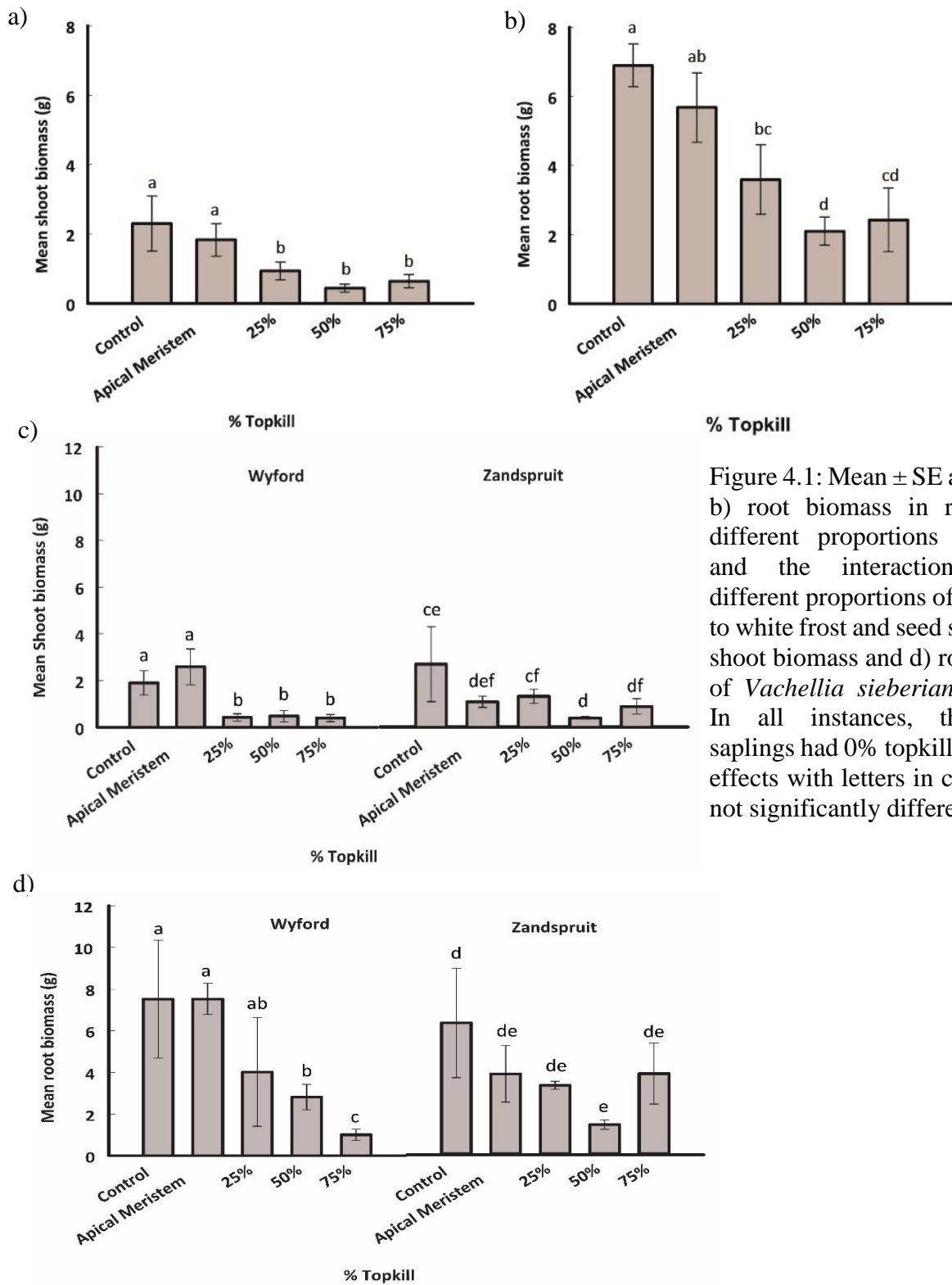


Figure 4.1: Mean  $\pm$  SE a) shoot and b) root biomass in response to different proportions of topkill; and the interaction between different proportions of topkill due to white frost and seed source on c) shoot biomass and d) root biomass of *Vachellia sieberiana* saplings. In all instances, the control saplings had 0% topkill. Treatment effects with letters in common are not significantly different.



increasing amounts of topkill, while the saplings grown from Zandspruit seeds showed no clear pattern in response to different levels of topkill (Figure 4.1d).

#### 4.3.1.2 Black frost

The saplings that experienced black frost at 0°C, -3°C and -6°C all survived, despite varying amounts of topkill, while those exposed to -9°C and -12°C died. At 0°C, one sapling experienced 10% topkill and another 20% topkill. The remaining two saplings were not damaged. At -3°C there was 10% topkill on three of the saplings and 25% topkill on one sapling, all of which coppiced from the ends of the surviving stems. At -6°C there was 100% topkill of the saplings and new growth coppiced from the base of the stems. By the end of the following growing season, all surviving saplings had sprouted from stems and side shoots, except for those that had been exposed to -6°C which coppiced from the base of the stem. A generalized linear model of the dry biomass of roots and shoots showed a significant effect of temperature on both roots and shoots. The source of the seeds from which the saplings were grown was also significant: saplings grown from seeds collected from Zandspruit (mean =  $29.14 \pm 8.37$  mm) had a higher biomass than those grown from seeds collected from Wyford (mean =  $12.95 \pm 11.49$  mm). There was no interaction between treatments (Table 4.2).

Table 4.2: The effects of black frost at decreasing temperatures and seed source on shoot and root biomass of *Vachellia sieberiana* saplings, using a generalized linear model with the identity linked function. Saplings were grown from seeds collected from two different sites at different altitudes (seed source): Wyford (1 350 m asl) and Zandspruit (1 165 m asl). Shaded p-values with \*\* are significant

	Shoots			Roots		
	Likelihood Ratio			Likelihood Ratio		
	Chi-Square	df	Sig.	Chi-Square	df	Sig.
Temperature	15.193	3	0.002**	11.856	3.000	0.008**
Seed Source	18.043	1	0.000**	20.322	1.000	0.000**
Temperature * Seed Source	2.749	3	0.432	1.033	3.000	0.793

The difference between the control saplings and those exposed to -3°C and -6°C was significant ( $p < 0.05$ ; Figure 4.2a and b). The calculated temperature at which 50% of the

*V. sieberiana* saplings could be expected to die was  $-6.31^{\circ}\text{C}$  (Figure 4.3). In the subsequent trials all the saplings, bar one which survived  $-8^{\circ}\text{C}$ , died between  $-6^{\circ}\text{C}$  and  $-7^{\circ}\text{C}$ , which confirmed our calculations.

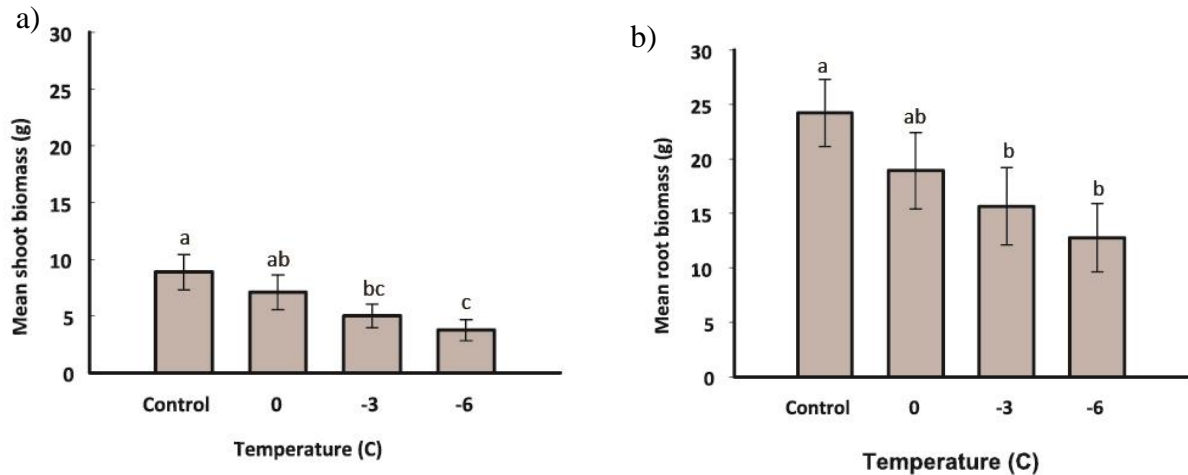


Figure 4.2: Mean  $\pm$  SE biomass of a) shoot, b) root biomass of *Vachellia sieberiana* saplings in response to black frost at different temperatures. There was no topkill in the control saplings. Treatment effects with letters in common are not significantly different.

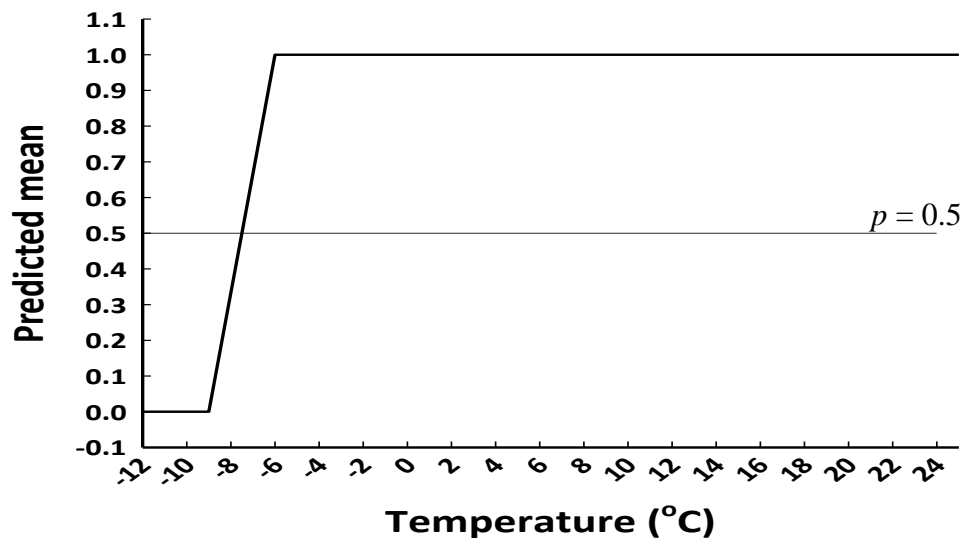


Figure 4.3: The temperature at which 50% (i.e.  $p = 0.5$ ) of the *Vachellia sieberiana* saplings die when exposed to black frost

### 4.3.2 Frost avoidance - testing for soluble sugars within the cell sap

Univariate analysis showed no significant difference in glucose content between saplings that were chilled and the controls. Nor was there any significant difference in glucose content between saplings that had been grown from seeds from collected from different altitudes.

### 4.3.3 Effect of freezing on seed viability

Freezing had a significant effect on germination success ( $\chi^2 = 14.65$ ;  $df = 4$ ;  $p = 0.005$ ). The control seeds were the first to emerge from the substrate 12 days after planting, followed by the seeds that had been chilled and frozen at  $0^\circ\text{C}$  and  $-3^\circ\text{C}$  respectively. These seeds emerged on the 13<sup>th</sup> day after planting. The seeds that had been frozen at  $-6^\circ\text{C}$  and  $-9^\circ\text{C}$  emerged on day 17 and day 22 respectively. Germination success was between 70% and 77% for the all the seeds, except for those that were frozen at  $-9^\circ\text{C}$ , of which 44% emerged (Figure 4.4).

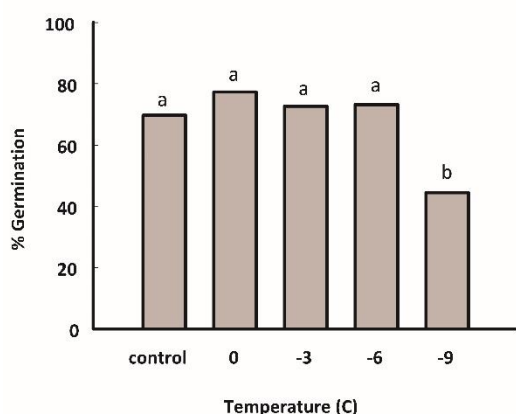


Figure 4.4: Germination success in percentages of *Vachellia sieberiana* seeds after freezing. Treatment effects with letters in common are not significantly different.

## 4.4 Discussion

### 4.4.1 White frost

Topkill of the apical meristem of *V. sieberiana* saplings by frost had no significant effect on the saplings' ability to recover. By the end of the summer following topkill, shoot biomass had compensated for the loss of tissue. Any further loss of plant tissue due to topkill ( $\geq 25\%$ ) resulted in the plants being unable to adequately compensate for the loss before the next winter. Consequently, these saplings would remain vulnerable to fire, herbivory and to further frost, preventing the saplings from reaching reproductive

maturity. Although the sapling roots were protected from any potential damage by the frost treatment by the substrate, they showed a similar pattern in reduction in biomass as the shoots. This was most likely to be the result of the reduction in above-ground photosynthetic material. There was an interaction between treatments (seed source and degree of topkill). Although differences were significant, there was no clear pattern in the shoot and root biomass of the saplings grown from seed from Zandspruit in response to varying degrees of topkill. However, a clear pattern was exhibited by the saplings grown from seed from Wyford in response to different levels of frost, this suggests that the saplings grown from the Zandspruit seeds are better adapted to withstand frost which is not due to a hardening process of the sapling itself, but rather some inherent trait within the seed. Other *Vachellia* and *Acacia* species have demonstrated a degree of frost tolerance that is associated with the origin of the seeds. Pollock *et al.* (1986) found that *Acacia* seedlings grown from seed obtained from high altitude sources (cold) were more frost tolerant than those of the same species obtained from low altitude sources (milder). In controlled field trials, Wakeling *et al.* (2012) noted that *Acacia* (= *Vachellia*) *karroo* saplings, obtained from a high altitude where temperatures frequently fell below 0°C in winter, were better able to withstand lower temperatures than the same species obtained from lower, milder altitudes. Although Wakeling *et al.* (2012) do not state whether the two, different population of saplings were grown from seed or were transplanted, it is apparent that both species exhibited a preadaptation to cold and that *V. sieberiana* exhibits a similar ability.

#### 4.4.2 Black frost

*Vachellia sieberiana* saplings did not survive prolonged exposure to temperatures < -6°C under controlled conditions. The critical temperature from which the saplings did not recover was between -6°C and -7°C. Although there was 100% topkill at -6°C, the roots of the saplings survived and the saplings readily coppiced from the base of the stem. Below -6°C the roots did not survive. What is pertinent is the speed at which *V. sieberiana* can recover and, ultimately, become reproductively viable. As with white frost, the greater the damage to the saplings through black frost, the longer it took for the saplings to regain the lost above-ground growth, making them more vulnerable to fire and herbivory than those saplings that escape frost damage. The shoot and root biomass of

saplings grown from seeds collected at Zandspruit was significantly greater than the biomass of saplings grown from seeds collected at Wyford. As shown with the white frost trials, it is possible that the *V. sieberiana* saplings demonstrated some preadaptation to longer winters of Zandspruit, with more frost days. In our trials, while Zandspruit was at a lower altitude than Wyford, Zandspruit had almost twice as many potential frost days than Wyford, although minimum temperatures at the two sites were similar. The preadaptation in *V. sieberiana* may be the result of number of frost occurrences rather than temperature. This is borne out by there being no significant difference in *V. sieberiana* shoot biomass below  $-3^{\circ}\text{C}$  nor in the root biomass below  $0^{\circ}\text{C}$ .

#### **4.4.3 Frost avoidance**

There are various strategies that plants utilise to avoid frost damage (Osmond *et al.* 1987; Schultze 2006). An increase in soluble sugars within the cell sap is one of these and at least one species of *Vachellia* employs this method in response to water stress (Kebbas *et al.* 2015), which is similar to the stress caused by freezing. However, *V. sieberiana* does not increase soluble sugars in response to chilling. Being described variously as a deciduous or semi-deciduous tree, it is probable that *V. sieberiana* relies on dormancy to survive the cold winter months. As *V. sieberiana* also possesses an intrinsic resistance to fire through a thick bark (Kruger 2005), the stems and trunk may also be resistant to frost, the bark being an effective insulator, not only against heat but also against cold.

#### **4.4.4 Effect of freezing on seed viability**

Only once temperatures reached  $-9^{\circ}\text{C}$ , did we observe a significant reduction in germination success of *V. sieberiana* seeds, which indicated that very low temperatures may affect recruitment rate. *V. sieberiana* seeds may be stored for several years without losing viability (per. obs.) because of their very low moisture content. It seems that the seeds are, to a limited extent, protected from frost damage through desiccation, one of the strategies for avoiding cell damage through freezing (Osmond *et al.* 1987; Schultze 2006).

### **4.5 Conclusion**

Frost does have the potential to limit the establishment of *V. sieberiana* at high altitudes, but only where temperatures regularly drop to below  $-6^{\circ}$ . It is, therefore, probable that low temperatures and frost have excluded *V. sieberiana* from mesic high-altitude grasslands by creating a bottleneck at recruitment level. However, in most environments

it is unlikely to be a primary agent of disturbance. Because of the ability of *V. sieberiana* to coppice, even after 100% topkill, frost, generally, will maintain *V. sieberiana* saplings within the frost zone, making them vulnerable to further damage by fire and herbivory. In this way, frost will play a role in maintaining the tree;grass equilibrium as, with constant regular damage to side shoots and meristems, flowering and seed-set could be retarded. This has been observed by P Swan (farmer: pers. comm<sup>9</sup>; 2017), where an introduced *V. sieberiana* tree has not flowered in 11 years because of annual frost damage to lateral branches. In this way, any *V. sieberiana* saplings that may have been introduced into a high-altitude grassland through an agent of seed dispersal and have managed to survive the first few winters, could be prevented from flowering and setting seed. Consequently, any changes in climate as the result of global warming will impact the distribution of *V. sieberiana*, making the high-altitude grasslands vulnerable to encroachment by this species.

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<sup>9</sup> Mr P Swan: Chartwell Farms, Seven Oaks, KwaZulu-Natal

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## CHAPTER 5: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER RESEARCH

### 5.1 Aerial photographs

The historic photograph of Van Reenen's Pass, taken on the farm Wyford, provides a single snapshot of what the vegetation in that area may have been like – extensive grasslands with forests in sheltered valleys. However, a single photograph provides a narrow, biased view of the landscape (Kull 2005) and, when a repeat is taken of the same landscape, one may have the impression that the change, if it has occurred, has been linear (Hongslo *et al.* 2009). Instead, the change may have been cyclical with the landscape oscillating between grassland and closed savanna as a consequence of climatic changes or changes in land-use (Archer 1989). These shortcomings may be overcome by remote sensing, aerial photographs and satellite imagery, which fill the gap between the date of the historic ground photo and the present (Hongslo *et al.* 2009). While acknowledging that remote sensing does not detect small saplings and 'gullivers' (small suppressed trees, see Skowno *et al.* (1999)) within the grass layer, aerial photographs and satellite imagery have shown a linear increase in the density of mature *Vachellia sieberiana* trees at Zandspruit (1 165 m above sea level (ASL)). At Wyford (1 350 m ASL) the trend is obscured by invasion of *Acacia dealbata* and their subsequent felling and regrowth. However, despite this, one can discern individual *V. sieberiana* trees (which prefer open vegetation and full sun as opposed to *A. dealbata* which will grow in dense thickets) in the earliest aerial photograph and their gradual increase in density over time. Taken in conjunction with the historic ground photograph, it appears that *V. sieberiana* treeline is indeed moving up the altitudinal gradient. At Waterfall (1 697m ASL), ground inspection confirmed what the remotely sensed photographs suggested: that there were no *V. sieberiana* trees at this high-altitude site. The changes in woody cover were due to changes in density of introduced woody species as well as a slow increase in *Leucosidea sericea* shrubs.

### 5.2 The effect of fire, frost and simulated grazing (mowing)

Much has been written about what might control the balance between trees and grasses within a savanna system (for example Skarpe 1992; Scholes and Archer 1997; Sankaran *et al.* 2008), as well as what might limit trees within grasslands which, climatically, could

support woodlands or forests (Bond 2008; Wakeling *et al.* 2012). With regard to the tree:grass dynamic within savannas various hypotheses have been proposed. There is the “top-down” approach that cites disturbance as the driver in the tree:grass dynamic. Disturbance includes drivers such as climate, fire and herbivory. The “bottom-up” approach evokes competition between grasses and trees for resources such as soil moisture, nutrients, light and physical space. Very often there may be an overarching driver, with other drivers playing a modulatory role. The field trials at Zandspruit, Wyford and Waterfall suggested that there was no single driver that affected the distribution of *V. sieberiana* at all three sites, but rather an interplay among several, with a possible overarching driver.

### 5.2.1 Fire

Fire is a “top-down” disturbance that has the potential to maintain the open nature of savannas and grasslands (Bond *et al.* 2005; Gordijn and Ward 2014). However, the effectiveness of fire depends on the fire return frequency, grass fuel load and woody-plant species composition (Gordijn and Ward 2014). It has been noted that tree density is often unresponsive to fire (Higgins *et al.* 2007; Gordijn and Ward 2014) and that fire creates a storage effect (Higgins *et al.* 2000) with ‘gullivers’ trapped within the fire zone until conditions are conducive for them to escape. Therefore, while fire may suppress sapling growth, it may not always be effective in preventing an increase in tree density, particularly if seeds of the encroaching trees are brought in by seed dispersal agents.

Like most savanna trees, *V. sieberiana* is well adapted to survive fire and the transplanted saplings at the study sites coppiced readily after being damaged by fire. At the Waterfall (high-altitude) site, the saplings experienced 100% topkill, but the stem diameters of the burnt saplings were not significantly different to the unburnt ones at the end of the growing season after the trial. This was unexpected, as the fuel load was sufficient to produce an extremely hot fire ( $> 6\ 000$  kJ/m/s) (see Trollope and Potgieter (1985)). Waterfall was the coldest site, with the soils lowest in nutrients. The saplings at this site, while they survived, did not thrive. As growth of plants of the tropics and subtropics may be inhibited by temperatures below 12.5 °C (Snyder and de Mel-Abreu 2005), the relatively short (seven months as opposed to nine months at each of the two lower sites),

cool summers at the high-altitude site, combined with poorer soil nutrients, could have overridden the effect of fire.

At Wyford (mid-altitude), the fire was, again, extremely hot ( $> 8\,000$  kJ/m/s) and all the burnt saplings experienced 100% topkill. The stem diameters of the burnt *V. sieberiana* saplings were significantly less than those of the unburnt saplings. This was as expected. These saplings, potentially, would be trapped within the fire zone and, depending of the fire return frequency, would remain as ‘gullivers’ in a demographic bottleneck. A comparison of pre- and post-burn sapling heights at this mid-altitude site confirms very little change in sapling height after one growing season after treatment. As long as there was no further recruitment of *V. sieberiana* seeds, it would be possible to maintain current tree density using judicial burning at Wyford.

At Zandspruit (The low-altitude site), there was no significant response in stem diameter to fire treatment and topkill was only partial, despite the fire potentially being the most intense ( $> 11\,500$  kJ/m/s). The fuel load here comprised predominantly of *Cymbopogon validus*, which, at time of burning stood approximately 2 m high. During burning, only the basal leaves of *C. validus* had been burnt, leaving the tall stems and the inflorescence only scorched and still standing. Consequently, the fuel load comprised only of the shorter, less abundant grass species, resulting in a much cooler fire than computed. As a result, the saplings were relatively unaffected by the fire. This is also borne out by the comparison of pre- and post-treatment stem heights. Saplings were markedly taller in most cases at the end of the trials, but were, potentially still within the fire trap because of the overall height of the sward. This, however, was deceptive as *C. validus* did not provide the bulk of the fuel load. Not only, then, is fuel load important for the effect of fire on woody plants, but also the grass species needs to be taken into consideration when applying fire as a method to control woody encroachment. However, the correct use of fire could change the grass species composition of the sward, from the tall *C. validus* to a shorter sward (Kirkman *et al.* 2014), thus making fire a more effective in controlling the spread of *V. sieberiana*.

## 5.2.2 Frost

### 5.2.2.1 Field trials

It is generally accepted that tree-lines are determined by temperature (see for example Körner (1998); Körner and Paulsen (2004)) and that tree-lines are moving into higher altitudes as a result of increased global temperatures (Grace *et al.* 2002; Gehrig-Fasel *et al.* 2007; Harsch *et al.* 2009). These temperature-mediated tree-lines occur in Alpine regions, but, as Bond *et al.* (2003) point out, none of the mesic grasslands in South Africa occur above the global tree-line and are, therefore, not determined by a thermal boundary. Frost, however, can still have a modifying effect on vegetation structure, often in conjunction fire or browsing (Holdo 2005; Holdo 2007), although there were no interactions between frost and fire at any of the study sites.

At Waterfall (high-altitude), frost did not have a significant effect on stem diameter of the transplanted saplings. This could be because the lower temperatures throughout the year had a negative effect on growth rates (see Snyder and de Mel-Abreu (2005)) and, combined with the low phosphorus level in the soils, overrode any potential effect of frost on relative growth rates. Archival climate data for Van Reenen indicate that anticipated minimum winter temperatures for the area at the top of the escarpment are around  $-8\text{ }^{\circ}\text{C}$  (Meteoblue 2018). Bearing in mind that weather stations are mounted 1.25 m to 2 m above ground level and that temperatures at ground level could be as much as  $5\text{ }^{\circ}\text{C}$  less than those measured by the weather station (Savage 2012), conditions at ground level could be much colder than reported. Consequently, in recording temperatures in the field trials, the sensors used were placed at approximately 10 cm above ground level, at tree sapling canopy height. Despite this, minimum temperatures during the trial period at Waterfall, the coldest site, did not drop below  $-5.95\text{ }^{\circ}\text{C}$ . It appears, therefore, that the winter during which the trials were run was a mild winter and this may be why the transplanted *V. sieberiana* saplings survived the frost. Considering that *V. sieberiana* saplings died at  $-7\text{ }^{\circ}\text{C}$  in controlled experiments (Russell and Tedder, 2018, unpublished data), it is possible that, under normal winter condition, the saplings would not survive at the high-altitude site at Waterfall, when minimum temperatures at ground level could potentially reach  $-13\text{ }^{\circ}\text{C}$ .

At both Wyford (mid-altitude) and at Zandspruit (low-altitude) there was a significant response in stem diameter of the *V. sieberiana* saplings to frost. Unexpectedly, the stem diameters of the saplings at Wyford increased in response to frost, whereas at Zandspruit, as anticipated, the stem diameters decreased in response to frost. While minimum winter temperatures at Wyford and Zandspruit were similar ( $-3.25^{\circ}\text{C}$  and  $-3.58^{\circ}\text{C}$  respectively), Zandspruit had more potential frost days than Wyford (29 days as opposed to 15 days at Wyford). Therefore, while minimum temperatures would not have induced stem mortality, the moderate frost damage at Wyford may have had the same effect as moderate browsing, where resources are reallocated into stems and shoots as over-compensation for the browse damage (Stuart-Hill and Tainton 1988; Fornara and Du Toit 2007; Riginos and Young 2007). However, at the Zandspruit site, because of a greater number of frost days, the damage to the saplings would have compounded over time and, consequently, reallocation of resources would have been inadequate to compensate. Once again, the relatively mild winter, as discussed above, may have meant that top-kill due to frost was not as severe as it might have been.

#### *5.2.2.2 Controlled environment trials*

##### **5.2.2.2.1** White frost

The controlled frost trials showed that the extent of topkill affected the saplings' ability to compensate for lost biomass within a given time. The greater the degree of topkill, the lower the sapling biomass, up to a point, and frost, therefore, has the potential to maintain the saplings within the fire zone. Although the differences between shoot biomass of the two population groups (one group grown from seed collected from Wyford (which experienced fewer frost days than the Zandspruit site), the other from seed collected from Zandspruit) were significant, there was a distinct pattern in the frost induced differences in shoot biomass in the Wyford saplings, whereas there was no distinct pattern in the Zandspruit saplings. The saplings from the Wyford population group were able to compensate adequately after the loss of the apical meristem so that there was no difference in biomass between these saplings and the controls, but where topkill was  $\geq 25\%$  there was no significant difference in regrowth, nor was the regrowth adequate to compensate for biomass loss. The population group from Zandspruit showed no such regular pattern. The root biomass of the Wyford population group steadily decreased with increasing amounts of topkill, suggesting that while frost had no direct effect on the roots,

the loss of above-ground photosynthetic material resulted in a reduction in root biomass. The root biomass from the Zandspruit population group, like the shoots, showed no regular pattern, although it mimicked the pattern of the shoot biomass. Again, this suggests that root biomass was affected by the loss of photosynthetic material and not directly by frost. The field trials at Wyford suggested that, with light frost damage, the saplings may overcompensate as some trees do in response to browsing (see for example Fornara and Du Toit 2007)). However, this was not evident in the controlled trials, even when just the apical meristems were frosted. Saplings in the field at Wyford would have been subjected to a very light frosting, which may not have been as damaging to the apical meristem as in the controlled frost trials.

#### 5.2.2.2.2 Black frost

As with white frost, the *V. sieberiana* saplings resprouted readily after topkill from stems and side shoots. At between -3 °C and -6 °C the saplings grown from seed from both Zandspruit and Wyford experienced 100% topkill, but coppiced from the base of the stems and, although the saplings from Zandspruit were significantly larger than those from Wyford, temperature equally affected them. The calculated temperature at which mortality equalled 50% ( $p = 0.5$ ) was -6.31°C and only one sapling survived -8°C. It suggests that *V. sieberiana* could be excluded from very cold sites because of sensitivity to low temperatures and the resultant intercellular freezing within the plant tissue. However, black frost above -6 °C merely reduces plant biomass and slows down growth rates, as with white frost, trapping the saplings within the frost or fire zone as ‘gullivers’. Should there be more frequent milder winters one could anticipate the *V. sieberiana* tree-line to shift into the higher altitudes, as suggested by Venter *et al.* (2018).

#### 5.2.2.3 Preadaptation to low temperatures

In the field, there was no difference between saplings grown from seeds collected at Zandspruit, compared with those grown from seed collected from Wyford. However, in the controlled frost trials, there was a significant difference between them as, in the field, the frost would have been lighter than the controlled trials. The differences were particularly notable in the white frost trials where there was a significant interaction between the extent of topkill and the seed source (Wyford or Zandspruit). The shoot biomass of the saplings from the Wyford population showed a significant reduction in

biomass at 25% topkill and greater. In contrast, the shoot biomass of the saplings grown from Zandspruit seeds, while there were some significant differences, showed an irregular pattern. This suggests that the saplings from the Zandspruit population group were better able to recover after freezing than their counterparts from Wyford. It also suggests that the ability to be more tolerant of cold is inherent in the Zandspruit population having been grown from seed i.e. there is an “ecotypic variability” (see Greer *et al.*, 1991) and is not a hardening process that may be lost once ambient temperatures start to increase with the onset of Spring (see for example Menzies *et al.*, 1981). The black frost trials also indicated that the saplings from the Zandspruit population were better able to recover from freezing than those from the Wyford population as the biomass of those saplings grown from seeds collected from Zandspruit was greater than those grown from seeds from Wyford. However, relative growth rates were the same, as was the response to black frost with a linear decrease in biomass with decreasing temperature.

#### 5.2.2.4 Frost avoidance and tolerance

There appears to be no generally accepted definition as to what constitutes the two different types of strategies and very often the two overlap. What is clear is that plants have diverse adaptive mechanisms to avoid or tolerate freezing. Characteristics such as the development of bulky organs, such as a thick bark with high heat capacity; deciduousness; a dense canopy that protects frost-tender shoots within the canopy; or the selection of a micro-site, such as in a rocky crevice, that maintains warmth throughout the night (Snyder and de Mel-Abreu 2005) could be regarded as avoidance strategies. Frost tolerance may include physiological changes within the plant tissue. For example, plants may increase solute concentration within the cell protoplasm (Levitt 1980); they may increase the fluidity of the plasma membranes by increasing the proportion of unsaturated fatty-acids of the membrane lipids (Levitt 1980; Taiz and Zieger 2006); or they may shrink the protoplasm within the cells to increase tolerance to desiccation caused by intracellular freezing (Snyder and de Mel-Abreu 2005). Although much work has been done on how agricultural and forestry crops cope with chilling and freezing, very little has been done on vegetation that has little direct economic value, except to classify whether it is frost resistant or not. Therefore, a preliminary investigation was done into how *V. sieberiana* resisted damage. Unlike *Acacia* (= *Vachellia*) *tortilis* that increased the concentration of soluble sugars within the cell sap in response to water stress (or



desiccation) (Kebbas *et al.* 2015), *V. sieberiana* did not respond to chilling by doing likewise. This may mean that *V. sieberiana* employs metabolites, such as proteins or tannins, other than soluble sugars, to provide it with a degree of tolerance to freezing. As *V. sieberiana* leaves and pods contain cyanic acids and tannins, it would be instructive to investigate whether either of these compounds increase in response to chilling. As a deciduous tree, *V. sieberiana* may merely avoid freezing through dormancy and its thick, corky bark may provide it with protection from freezing. However, testing for avoidance within a controlled environment is difficult (Wisniewski *et al.* 2014).

#### 5.2.2.5 Seed viability after freezing

The seeds of *V. sieberiana* are quiescent, in other words, germination is delayed until the environmental conditions are right. This is achieved through desiccation, one of the mechanisms employed to avoid freeze damage. It was not surprising, therefore, that *V. sieberiana* seeds were able to remain viable after freezing and that seed viability was only partially reduced once temperatures reached -9 °C. There would come a point below which the seeds would die. It would be another area worth investigating.

#### 5.2.3 Simulated grazing

Heavy grazing is a disturbance that is frequently cited as a cause of bush encroachment (see for example (Archer *et al.* 1995; Scholes and Archer 1997; Van Langevelde *et al.* 2003) as the reduction in the grass layer results in the reduction in competition for soil moisture (Davis *et al.* 1998; Van Langevelde *et al.* 2003; February *et al.* 2013), nutrients (Tedder *et al.* 2012; Cramer and Bond 2013) and light (Cramer *et al.* 2007; Hagenah *et al.* 2009; Vadigi and Ward 2013). Mean annual precipitation at Zandspruit, Wyford and Waterfall is > 700 mm. Therefore, water is not limiting. Consequently, the saplings and grasses would have competed for light, not soil moisture. As *V. sieberiana* is shade intolerant (Vadigi and Ward 2013), removal of competition for light through mowing had a positive effect on the transplanted saplings at both Wyford (mid-altitude) and Zandspruit (low-latitude). The annual mowing of the veld grasses would, therefore, encourage the establishment of *V. sieberiana*, even if they remained at the 'gulliver' level of development. Herbivores introduce further seeds into the area which germinate at the first rains, when the grasses are still short, and, being fast growing, the saplings soon become established. Should there be an interruption in the mowing programme, the *V.*

*sieberiana* ‘gullivers’ quickly become large trees, capable of producing flowers and seeds. Heavy grazing would have the same effect as mowing regarding competition between *V. sieberiana* saplings and grass for light. At Waterfall, mowing of the grass layer appeared to have no effect on the transplanted *V. sieberiana* saplings. At this site the grass layer was naturally short and sparse and, even in the unmown plots, the competition for light was insignificant.

### **5.3 The effect of soil nutrients**

The transplanted *V. sieberiana* saplings, at the end of the field trials, had the smallest stem diameter at Waterfall (high-altitude site) and the largest stem diameter at Zandspruit (low-altitude site). Soil analysis of all three sites indicated that there was a nutrient gradient along the altitudinal gradient. Waterfall soils had the lowest levels of phosphorus and nitrogen while Zandspruit had the highest. Preliminary trials done by N Makhaye and A Magadlela (2017, unpublished data) suggested that the low levels of phosphorus at the Waterfall site could well have affected the growth rates of the *V. sieberiana* saplings (see also Wakeling *et al.* 2010). However, other, well-grown, tree species were present at the Waterfall site. It could be that these species had a lower requirement for phosphorus than *V. sieberiana* (see for example Attiwill (1980)). An in-depth investigation into the role of soil nutrients in the distribution of *V. sieberiana* was not within the scope of this project, but the role of nutrients in the distribution of certain savanna trees has been investigated by Wakeling *et al.* (2010). They found that, while low soil nutrients did not exclude savanna trees from high-altitude grasslands, soil nutrients could play a modulating role (Wakeling *et al.* 2010).

### **5.4 The effect of root gaps**

Trees and grasses may compete for root gaps, viz. the physical space available for the roots of the saplings and grasses to co-exist (Milchunas and Lauenroth 1989; Jurena and Archer 2003; February and Lewis 2016). Wakeling *et al.* (2015) suggested that trees were excluded from high altitude grasslands because the grasses formed dense mats of roots. These mats prevented tree seedlings from taking root (Wakeling *et al.* 2015). At the Waterfall, Wyford and Zandspruit study sites there was no correlation between grass root biomass and tree density and physical inspection of the soil layer did not reveal dense root mats at any of the sites. The grasslands within which Wakeling *et al.* (2015) were

working had a different grass species composition to those species in the Van Reenen's Pass area. This may explain the differences in the results.

### 5.5 Conclusion

*Vachellia sieberiana* is certainly increasing in density in the lower reaches of the Drakensberg escarpment in the vicinity of Van Reenen's Pass. While it has not yet reached the top of the escarpment, this may only be a matter of time if the predictions with regard to global warming are correct. Woody encroachment has serious ecological implications, affected not only grass sward composition and soils, but also the hydrology of the areas affected and beyond. This will have long term negative effects on primary productivity and the sustainability of animal husbandry in these areas, as well as the degradation in volume and quality of water, both for animal and human consumption.

Aerial photographs give a clear indication that *V. sieberiana* is gradually encroaching into the grasslands of the Drakensberg escarpment in the vicinity of Van Reenen's Pass. There was a distinct effect of altitude on the distribution of *V. sieberiana*. The transplanted saplings at Waterfall, at the top of the escarpment had the smallest stem diameter with those saplings at the foot of the pass being the most robust. Until the present time, temperature and frost appear to have played a major role excluding *V. sieberiana* from the high-altitude site, with other drivers playing a modulatory role. However, this could change with global warming, as seen during the mild winter when the field trials were undertaken. During this period, temperatures did not drop low enough to create a killing frost. The effect of global warming on tree-lines has been well demonstrated by the alpine tree-lines moving into higher altitudes where low temperatures previously excluded them. Increases in concentrations of atmospheric carbon dioxide will also favour the vigour of *V. sieberiana*, having a C<sub>3</sub> photosynthetic pathway. This will allow the trees to recover more rapidly after topkill resulting from frost than previously, enabling them to escape from the frost zone to become reproductively viable. Soil nutrients also affected the vigour of *V. sieberiana* with the poorer soils at the top of the escarpment and the richest soils at the foot of the pass, i.e. there was a catenal gradient in soil nutrients, as demonstrated in preliminary pot trials. The nutrient particularly affecting the vigour of *V. sieberiana* was most likely phosphorus.

At the lower altitudes, where *V. sieberiana* is already well established, it becomes a question of how best to manage the trees. Depending on the species composition of the grass sward, fire can maintain the current density of *V. sieberiana* by creating a demographic bottleneck, resulting in the trees being trapped within the fire zone as non-reproductive ‘gullivers’. With the increases in concentrations of atmospheric carbon dioxide increasing the vigour of *V. sieberiana*, these trees with their ability to rapidly coppice after topkill will be able to escape the bottleneck created by fire. Grazing of the grass sward removes competition for light, benefitting the saplings, and reducing the fuel load for fires. Consequently, the fire return period would need to be carefully monitored. However, these areas are commercial rangelands for cattle and the landowners, having been victims of devastating wild-fires in the past, are reluctant to employ fire to effectively control bush encroachment. Cool, frequent fires are favoured in this area, which will exacerbate the problem of encroachment. Allowing large numbers of browsers into the rangelands in conjunction with burning may be an alternative option to control encroachment. However, considering the resilience of *V. sieberiana*, tree felling and spot poisoning may be the best option to remove unwanted trees, leaving only a few in the savannoid areas to maintain the natural structure of the grassland. This could be followed-up with regular and judicious use of fire combined with browsing. This could well change the composition of the grass sward to more flammable grass, in the case where species like *Cymbopogon* dominate, making fire more effective.

## 5.6 Challenges

The biggest challenge was the relatively short duration of the project combined with a severe drought in the area that coincided with the setting up of the field trials. Even though the drought was followed by two extremely wet seasons, the transplanted saplings suffered a severe set-back in establishing themselves. A further challenge was working on privately owned, commercial rangeland. Landowners were understandably wary of burning late in the season, which would have been optimal for observing the effect of fire on the *V. sieberiana* saplings. Consequently, the burning treatments had to coincide with the burning of firebreaks, which were early in the winter season. Despite this, extremely hot fires were achieved, but at a stage with the saplings were going into a period of dormancy.

### 5.7 Future directions

If global warming and rising atmospheric carbon dioxide concentrations are, indeed, driving bush encroachment and invasion, effective and sustainable management of the grasslands needs careful evaluation. Fire has been shown to be the most significant agent in maintaining mesic and moist grasslands and savannas in the past. *V. sieberiana* has shown itself to be extremely resilient to fire and fire alone is unlikely to have an effect on this species. A long term, large scale project which investigates the combined effect of fire and browsing on *V. sieberiana*, as has been done on other *Vachellia* species, would assist in planning a management strategy to control encroachment.

It has been demonstrated that light browsing of certain savanna trees results in a reallocation of carbon from roots to shoots. This may result in over-compensatory elongation of the shoots. *V. sieberiana* may conceivably respond similarly. Further trials to quantify the effect of browsing on shoot elongation of *V. sieberiana* would further aid management of these trees.

Soil phosphorus seems to affect growth rates of *V. sieberiana* saplings. A survey of soil nutrients in high-altitude grasslands, where *V. sieberiana* is absent may help elucidate what limits the distribution of *V. sieberiana*. Controlled pot trials to verify the limiting nutrients should be undertaken, as well. Further investigation into the role of root gaps should be undertaken. Certain grass species do create dense roots mats. Do these species generally predominate in grasslands that preclude savanna trees?

### 5.8 Final comments

Despite the challenges, this project has provided a rare opportunity to conduct field trials on the effect of fire, frost and simulated grazing on *V. sieberiana* across a steep altitudinal gradient within one climatic zone. While much work has been done on frost hardiness of many agricultural and forestry plant species, there are very few studies on the effect of frost on naturally occurring South African plants and their distribution. While *V. sieberiana* has been described as “bullet proof”, some of its vulnerabilities have been high-lighted. Although there has been some work done on temperature and tree-lines in South Africa, this project pinpoints the temperature below which *V. sieberiana* will die in a controlled environment. In the field there may well be mitigating factors which will alleviate the effect of temperature and frost on *V. sieberiana*, but there will come a point

where this species will not survive in the higher altitudes as a result of temperature. It is also probable that *V. sieberiana* is sensitive to a deficiency in soil phosphorus. If high-altitude grasslands are low in phosphorus, it is likely that *V. sieberiana* will not establish itself. This project also underscores the potential to inadvertently exacerbate encroachment by *V. sieberiana* if management does not consider that this species has the potential to over-compensate shoot elongation in response to light damage, be it fire, frost or browsing.

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