Germination Potential of Seeds Harvested at the Worcester Veld Reserve

By

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In accordance with Rule G4.6.3, I hereby declare that this dissertation is my own work and that it has not previously been submitted for assessment to another university or for another qualification.

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DATE: 15 March 2019

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ABSTRACT

The rangelands of the Succulent Karoo and Nama Karoo biomes are in various states of degradation. The injudicious stocking of overly high numbers of domestic livestock is considered to be the anthropogenic cause of this degradation. The palatable plants of these rangelands have been greatly reduced in number or extirpated from some areas.

In order to return palatable plants to degraded rangelands and improve rangeland productivity, many restoration projects have been attempted that involve reseeding. The sowing of seeds into degraded rangelands, or other disturbed areas, has often provided disappointing germination results. This study will attempt to determine what some of the main aspects are that affect Karoo seed germination, both positively and negatively. The specific objectives of the study are to determine the causes of pre-sowing seed mortality, the effect of drying on seed germination, the effect of planting depth on seed germination and the effect of storage time on seed germination.

The seeds of four palatable Karoo plant species harvested at the Worcester Veld Reserve were selected to be subjected to germination trails and viability testing. This study uses germination trials under controlled light and temperature conditions, as well as 2, 3, 5-triphenyl tetrazolium chloride tests to determine whether the four species of Karoo seeds harvested at the Worcester Veld Reserve for rangeland reseeding projects are viable and germinable. The practice of drying seeds after harvesting is tested to determine the effect of drying on seed germination. The germination of seed was also tested over 17 intervals of 3 weeks to determine the effect of storage time on seed germinability. Germination trials were also conducted in a nursery to determine whether there was a difference in seedling emergence between seeds planted at 10 mm depth compared to seeds planted on the surface with a partial covering of sand.

The seed viability of all four species studied was found to be below 50%. Drying only significantly improved the germination of *Osteospermum sinuatum* seeds. The seeds of *O. sinuatum* and *Eriocephalus africanus* germinated reasonably well throughout the 17 time intervals, while the seeds of *Chaetobromus involucratus* and *Gorteria integrifolia* germinated poorly during the first six months after harvesting, after which germination improved markedly. Seedling emergence of all four species studied was significantly higher when planted at the substrate surface, compared to seeds planted at 10 mm depth.

The findings of this study show that while the viability of the seeds harvested for rangeland reseeding projects are quite low, the appropriate pre-treatment, seed age and planting depth will allow a significant increase in germination. This increase in germination should provide a greater chance of establishing palatable plants in rangeland reseeding projects.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Chapter 1: Introduction

Background to the Karoo

The Karoo is a semi-arid region in the central and south-western plateau of South Africa, spanning an area of approximately 359 000 km^2 (Mucina & Rutherford 2006). It comprises distinct biomes – the Succulent Karoo and the Nama Karoo, both occurring on lowlands and plains, with the more mountainous regions supporting the Renosterveld and Fynbos biomes (Figure 1.1). The Succulent Karoo biome is found in the Namaqualand, Roggeveld and Tanqua regions as well as in the Little Karoo and the valleys between the Cape Fold Mountains (Mucina *et al.* 2006a; Vlok & Schutte-Vlok 2015). The Succulent Karoo biome receives low but relatively reliable rainfall, with winter rainfall predominating in the west and a bimodal winter/summer rainfall regime in the eastern Little Karoo (Mucina *et al.* 2006a; Vlok & Schutte-Vlok 2015). The region has very high floral diversity and supports an unusually large diversity of leaf-succulent shrubs, particularly the 'vygies' and other members of the Aizoaceae, many species of Crassulaceae, as well as many Asteraceous shrubs (Mucina *et al.* 2006a; Vlok & Schutte-Vlok 2015). The Nama-Karoo biome occurs towards the south western interior of South Africa, where the rainfall is more variable, and falls mostly in summer. Asteraceae, Poaceae and Fabaceae are the dominant plant families of the Nama Karoo (Mucina *et al.* 2006b). Livestock farming, mostly sheep and goat ranching, are the main form of agriculture in the karoo because of the low rainfall and frequent droughts (Hoffman *et al.* 1999; Esler *et al.* 2006). Warm desert ecosystems, such as areas of the Nama Karoo, are coming under increasing pressure from human population growth and habitat degradation (Cowling *et al.* 1999; Hoffman *et al.* 1999; Hoffman & Ashwell 2001). Rangeland degradation due to over utilization by domestic livestock is a prevalent phenomenon throughout both the Succulent and Nama Karoo (Cupido 2005; Esler *et al.* 2006; Hoffman & Ashwell 2001).

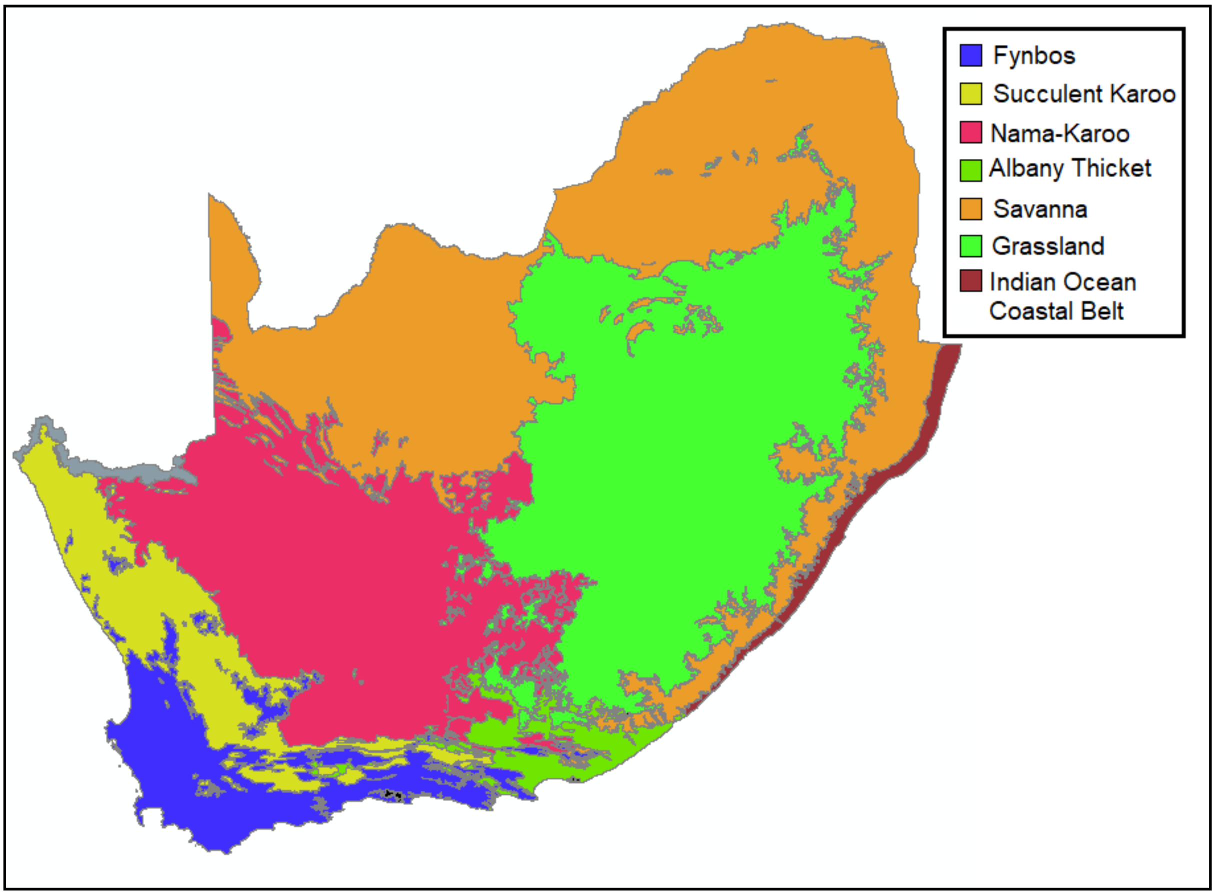

Figure 1.1: Biomes of South Africa. Adapted from Mucina & Rutherford (2006).

Land degradation

Karoo rangelands often have degraded patches with little or no vegetation cover, a dominance of unpalatable plant species and signs of erosion (Esler *et al.* 2006). These bare, degraded patches are caused by trampling, as livestock tend to congregate around watering points, shaded areas and feeding stations (Dean & Macdonald 1992; Todd 2006). Some Karoo vegetation types seem to be less resilient to the effects of over-utilization by herbivores, especially those that are both very arid and have more succulent plants than adjacent areas (Dean & Macdonald 1992; Saayman *et al.* 2016). The species composition of rangeland vegetation can be strongly linked to prior management of herbivorous livestock, while vegetation cover is more strongly linked to rainfall (Seymour *et al.* 2010; Van der Merwe *et al.* 2018). Poor management decisions have allowed long-term selective herbivory and the subsequent reduction in numbers or loss of palatable species from Karoo rangelands (Milton 1993; Esler *et al.* 2006). Herbivory by livestock and game selectively targets the reproductive structures of palatable shrubs, while those of unpalatable species are often ignored (Milton 1993). Some palatable, perennial Karoo shrubs produce seeds with very little or no dormancy (Esler 1993). If these shrubs are targeted by selective herbivory, they are unlikely to reproduce, since their reproductive structures are selectively consumed. This selective herbivory, if allowed to continue over several flowering seasons, leads to the increased production and accumulation in the seed bank of the seed from poisonous and unpalatable plants, since their reproductive structures are largely ignored (Milton & Dean 1990; Milton 1993; Esler *et al.* 2006). As the palatable plants in such a rangeland die, they are likely to be replaced by unpalatable or poisonous species. This leads to a situation where unpalatable species dominate the rangeland, which lowers its capacity to support livestock or game and reduces plant species diversity (Esler *et al.* 2006).

The decrease in palatable species, vegetation cover and subsequent decrease in rangeland productivity has placed more pressure on the remaining vegetation and has forced farmers to adopt lower stocking rates (Dean & Macdonald 1992). Hoffman and Todd (2000) found a consensus among extension officers of the Department of Agriculture that veld degradation in commercial farming areas has been reversed in some areas by education and state intervention programs. It was further suggested that the focus of state intervention programs will shift to supporting communal farming areas and move away from supporting commercial farmers. This may lead to further land degradation as commercial farmers receive less support for rangeland management. Commercial farmers in the Central Karoo are adopting lower stocking rates and these farms are experiencing a decline in economic productivity (Conradie *et al.* 2013).

Degraded rangelands suffer a reduction in plant productivity and biodiversity (Esler *et al.* 2006; Kinyua *et al.* 2009). Restoration of degraded rangelands is important, in the light of a global increase in the demand for meat and the effects of climate change on rangeland productivity (Seymour *et al.* 2010). Passive restoration methods, such as the temporary withdrawal of livestock or game (resting), tend to be ineffective and time-consuming, even with the presence of good rainfall (Milton & Dean 1995; Simons & Allsopp 2007; Kinyua *et al.* 2009; Seymour *et al.* 2010). The practice of resting the vegetation is only effective if seeds of palatable species are present in the soil seed bank or surrounding landscape within the dispersal potential of the desired species (Seymour *et al.* 2010), combined with the presence of suitable germination microsites and rainfall (Esler 1999; Van den Berg & Kellner 2005; Carrick & Krüger 2007; Bosco *et al.* 2018). The vegetation of abandoned cultivated lands in Utah has been shown to take more than a century to recover with no human intervention (Morris *et al.* 2011). Van der Merwe and Van Rooyen (2011) have shown that disturbed Renosterveld requires more than 30 years for the species diversity to recover after disturbance, notably by historic over-utilization by livestock. Similarly, it is estimated that the Succulent Karoo, dominated by plant species with short-lived seeds and short-distance seed dispersal (Esler 1993), will take many decades to recover (Dean & Macdonald 1992; Wiegand & Milton 1996; Seymour *et al.* 2010). Natural dispersal of palatable plant seed is slow and unreliable, therefore palatable species are often unable to establish during periods of "rest" when livestock are removed (Weiersbye & Witkowski 2002). Rainfall also acts as a limiting factor to seedling establishment (Milton 1995a), since the periods of livestock withdrawal must coincide with adequate rainfall in order to facilitate seedling survival (Saayman *et al.* 2017).

Seedbanks in rangelands

Since palatable species are often completely absent from degraded lands due to selective herbivory, there may be no seeds of these species present to allow recruitment of their seedlings (Jones & Esler 2004). Seedbanks in degraded rangelands often have low similarity with the standing vegetation (Saaed *et al.* 2018). Late succession, palatable shrubs tend to have seeds with little or no dormancy that do not persist in the soil seedbank beyond the lifespan of the shrub (Esler 1993; Carrick & Krüger 2007). The seeds of most standing, perennial vegetation in a given Karoo rangeland are only transiently present in the seedbank (Esler 1993; Carrick & Krüger 2007; Saaed *et al.* 2018). The seeds of perennial Karoo shrubs are often short-lived and the seedbank may need to be replenished annually to allow these species to persist (Esler 1993). Shorter lived, ephemeral plants often have smaller seeds with inherent chemical and mechanical dormancy that can persist for long periods of time in the seedbank, while longer lived species tend to have larger seeds that have little or no dormancy and do not remain viable in the seedbank over long periods of time (Esler 1993; De Villiers *et al.* 2003; Carrick & Krüger 2007; Probert *et al.* 2009; Saaed *et al.* 2018).

The species composition of rangeland vegetation could theoretically be determined by the availability of seeds of each species. Assuming equal germination and seedling survival probabilities for all plant species within a community, the species that produces more seeds will eventually dominate the rangeland (Milton & Dean 1991). Continuous, selective herbivory reduces the reproductive capacity of palatable plants, thus giving more unpalatable and defended plants a reproductive advantage (Milton & Dean 1991). This continuous herbivory thus has the effect of slowly increasing the relative abundance of unpalatable plants.

Degraded areas dominated by unpalatable plants with significantly low vegetation cover, requires rangeland restoration that depends on the reintroduction of palatable, indigenous species (Jones & Esler 2004; Esler *et al.* 2006; Mallik & Karim 2008; Seymour *et al.* 2010; Saayman *et al.* 2017). Reintroducing palatable species to over-utilized rangelands can improve species diversity, increasing resilience against the effects of droughts and climate change (Seymour *et al.* 2010). Conversely, a lack of seed diversity in the seedbank retards recovery of disturbed land (Pywell *et al.* 2002).

The reintroduction of plants to degraded areas can be achieved by reintroducing their seeds (Pywell *et al.* 2002; Mahood 2003; Seabloom *et al.* 2003; Esler *et al.* 2006; Kinyua *et al.* 2009; Van Eeden 2010; Merritt & Kingsley 2011). While the introduction of perennial, palatable grasses and shrubs could greatly increase the productivity and stocking capacity of degraded lands (Barnard 1987), sowing these areas with such seed has often yielded disappointing results due to failed germination or poor seedling establishment (Commander *et al.* 2009a; Saayman *et al.* 2017). Poor seedling establishment could possibly be caused by external effects such as insufficient rainfall, unsuitably timed sowing of seed in relation to the timing of rainfall, herbivory of seedlings, or competition from established plants (Matthee 2015; Saayman *et al.* 2017). Sown seeds may be non-viable due to being stored too long before sowing, being exposed to extreme desiccation, malformation or damage by seed predators or pathogens (Kerley 1991, Milton 1995b, Weiersbye & Witkowski 2002).

Reseeding as an approach to returning species to degraded rangeland

Reseeding is commonly used to return vegetation to land left denuded after heavy disturbance, such as mining, ploughing or warfare (Merritt & Kingsley 2011; Merino-Martín *et al.* 2017). Achieving any form of vegetation cover on these denuded lands is often marred with difficulties, since seeds and seedlings in this setting are usually exposed and thus vulnerable to desiccation, wind erosion, temperature fluctuations and predation. If adequate microsites for seedling germination and establishment are provided, for example mulching or brush-packing, as well as persistent favourable rainfall (Simons & Allsopp 2007; Saayman *et al.* 2017 Saaed *et al.* 2018), the seeding of denuded lands may provide satisfactory results.

Introducing palatable plants into degraded rangelands offers additional challenges. These disturbed lands are rarely devoid of vegetation, and are often dominated by perennial unpalatable or poisonous plants (Saayman *et al.* 2017). The seed banks of these rangelands may furthermore be dominated by undesired vegetation and dispersal of palatable plants from adjacent areas are generally slow (Weiersbye & Witkowski 2002; Saayman *et al.* 2017). If seeds of palatable plants are introduced into such a rangeland, the forthcoming seedlings may have to compete with the established unpalatable plants, as well as the seedlings of unpalatable plants (Moretto & Distel, 1997).

Established vegetation, regardless of palatability or toxicity, does provide favourable germination microsites, which is absent in denuded lands (Carrick, 2003; Simons & Allsopp 2007; Merino-Martín *et al.* 2017). In order to achieve a meaningful number of established palatable plants in a degraded rangeland, a large enough volume of seeds needs to be introduced, with the possible addition of manually disturbing the standing, unpalatable vegetation through methods such as brush cutting or manual plant removal (Merrit & Kingsley 2011; Erickson 2015; Merino-Martín *et al.* 2017). Seedling establishment is also highly dependent on rainfall, and restoration efforts often fail due to poorly timed or absent rainfall events (Milton 1995a; Saayman *et al.* 2017).

Dormancy and germinability of Karoo seeds

Plants adapted to arid environments often produce seed with inherent chemical or mechanical dormancy, or possibly a combination of both (Weiersbye & Witkowski, 2002; Commander *et al.* 2009a; Erickson 2015). Seed dormancy is especially prevalent in small seeded, ephemeral and pioneer species such as *Mesembryanthemum crystallinum*, *Drosanthemum hispidum* and *Galenia* spp. (De Villiers *et al.* 2003; Dreber & Esler, 2011). Small, dormant seed can survive for long periods of time in the seedbank in order to respond to favourable conditions for germination. Seed dormancy is often lost over time with older seeds germinating more readily (Commander *et al.* 2009b). Longer-lived shrubs often produce larger seed that have little or no dormancy and are transiently present in the seedbank (Milton & Hoffman 1994; Saaed *et al.* 2018). In order to lift dormancy, seed can be exposed to physical or chemical treatments such as heat treatment, drying, chilling, scarification, exposure to acid, fire, smoke, or changes in daylight-length or light intensity, amongst others (Pywell *et al.* 2002; Weiersbye & Witkowski 2002; Kulkarni *et al.* 2006; Commander *et al.* 2009b; Ghebrehiwot *et al.* 2012; Waller *et al.* 2015). Many species have specific cues for germination and may thus require specific treatments to lift dormancy (Weiersbye & Witkowski 2002; Waller 2015). Many seeds harvested for re-seeding are also non-viable due to damage from seed predators such as rodents (Kerley 1991) or insects (Milton 1995b), or due to infection of the seed by pathogens (Weiersbye & Witkowski 2002).

Dreber (2010) found that nearly 90% of seed germinated from Karoo seedbank samples were found in the top 40 mm of the soil. It would thus seem that viable seeds exist in a thin layer below the soil surface (Esler 1993). Soil preparation prior to introduction of palatable seed is often difficult in the Karoo, due to prohibitive costs and legislation limiting the tilling of virgin land (Conservation of Agricultural Resources Act No. 43 of 1983 as amended 1996; National Environmental Management Act no. 107 of 1998 as amended 2014). In a study of eight species of palatable, Karoo plants, Barnard (1987) found that their seeds germinate better when planted at a depth of 10 mm or 20 mm, instead of being placed on the soil surface. It is possible that the surface-planted seeds were dislodged by watering and were thus unable to germinate.

Seed preparation and storage

Seeds are classified as either orthodox or recalcitrant, depending on their germination characteristics. Recalcitrant seeds remain viable for a very short period of time after ripening, and do not survive desiccation. Orthodox seeds tend to remain viable for long periods of time after maturing, sometimes several decades, and can remain viable after desiccating to moisture levels as low as 7% (Probert *et al.* 2009). Orthodox seeds can often be stored for extended periods of time while remaining viable (Probert *et al.* 2009). Low storage temperature and low seed moisture content can prolong viability of orthodox seed (Ellis *et al.* 1988; Ellis & Hong 2006; Probert *et al.* 2009). Models predicting longevity of orthodox seeds suggest that some species may remain viable for several thousand years, while others may lose viability within the span of a single year (Probert *et al.* 2009). Ellis and Hong (2006) showed that alfalfa and red clover seeds were able to remain viable after being desiccated to a moisture content of 2% and stored in a hermetically sealed environment at -20°C for nearly 15 years. Seeds with higher moisture contents (15.2%) stored at such low temperatures suffer a decline in viability as the moisture inside the seed freezes and causes damage to the embryo (Ellis & Hong 2006). Vertucci and Roos (1990) argued that the relative humidity at which seeds are equilibrated is more important than the moisture content of the seed and that the ideal relative humidity should be between 19% and 27%. Desiccating seeds to such low moisture contents and providing sealed storage conditions with controlled temperature and relative humidity is often impossible for small scale producers of seed for Karoo rangeland rehabilitation.

It seems to be the general rule, rather than the exception, that Karoo seeds have some form of innate dormancy or an after-ripening period which prevents freshly harvested seed from germinating readily. Freshly harvested seed requires seed treatments, such as drying, before the seeds will readily germinate (Henrici 1935a). It is assumed that the current drying treatment of the seed harvested at the Worcester Veld Reserve improves germinability and that the seed remains viable for use in rangeland improvement. It should be noted that germinability refers to the ability of seed to germinate when it is sown. This differs from viability, which refers to the seed being intact and alive. Seeds that are viable may not necessarily germinate when sown, since their germination is prevented by chemical or mechanical dormancy.

Study objectives

Since many seed based Karoo restoration projects provide disappointing results, this study will attempt to determine whether these failures are due to the inability of seed to germinate when sown, and to explore some factors that may lead to poor germination results.

The objectives of the study are to determine the causes of pre-sowing seed mortality, the effect of drying on seed germination, the effect of planting depth on seed germination and the effect of storage time on seed germination. The study will use seeds of four Karoo plant species used in Karoo rangeland restoration.

This study is intended to inform Karoo rangeland restoration regarding the reintroduction of seeds of palatable plant species, particularly in terms of optimal seed pre-treatment and planting depth.

Arrangement of thesis contents

The thesis is arranged to provide a general introduction and review of the topics covered in this study (Chapter 1), a description of the study area and study species (Chapter 2), followed by four research chapters that address the following: Seed viability (Chapter 3), the effects of drying on seed germination (Chapter 4), the effect of storage time on seed germination (Chapter 5) and the effect of planting depth on seed germination (Chapter 6). Chapters 3-6 are structured as stand-alone papers, and for this reason there is some repetition of material between the general introduction and the chapter introductions. The research chapters are followed by a concluding chapter (Chapter 7) that shows how the treatments and conditions discussed in chapters 3 to 6 interact to influence the viability and germinability of the seeds of the four study species.

Chapter 2: Study site and species selection

Study site location and history

The Worcester Veld Reserve (33°37'21.68"S, 19°28'7.16"E) lies within the Succulent Karoo Biome. It was established by the South African Government in 1935 as a research farm to investigate ways of alleviating the effect of drought conditions prevailing in the early 20th century (Botha & Marais 2004). A 110 ha plot of land, northeast of the town of Worcester, in the Western Cape Province of South Africa was chosen as the site for the research farm. The condition of Karoo rangelands was thought to be deteriorating (Henrici 1935a) and hence the production of seeds of palatable Karoo plants was started with the intention to provide them to land owners for the improvement of degraded rangelands (Botha & Marais 2004).

The Worcester Veld Reserve has been the site of many research projects on Karoo vegetation, including several studies involved with the seeding of degraded areas to return desirable species and the germination testing of these species (Van Breda 1939, 1958; Barnard 1987; Witbooi & Esler 2004; Botha & Saayman 2014). Similar studies regarding the germination of a variety of Karoo seeds, as well as the chemical composition of fodder plants, were investigated by Henrici (1935b, 1939) at Fauresmith. Despite these studies there seems to be no guaranteed method of successfully establishing seedlings for rangeland improvement.

The seed used for this project was harvested and pre-treated at the Worcester Veld Reserve, as well as being subjected to germination trials in the Worcester Veld Reserve nursery. Further germination trials were carried out at the germination room of the Western Cape Department of Agriculture offices at Elsenburg.

Climate and soils of the Worcester area

Worcester is located in the semi-arid region of the Western Cape province of South Africa. The area experiences a Mediterranean climate, with hot, dry summers and cold, rainy winters. The long-term mean annual precipitation is relatively low, at 270 mm (Climate-Data.org 2018). The region receives predominantly winter rainfall, with the majority of precipitation occurring from May to August (Figure 2.1). The low rainfall of the area is due to the rainshadow effect of the surrounding mountain ranges (Mucina *et al.* 2006a).

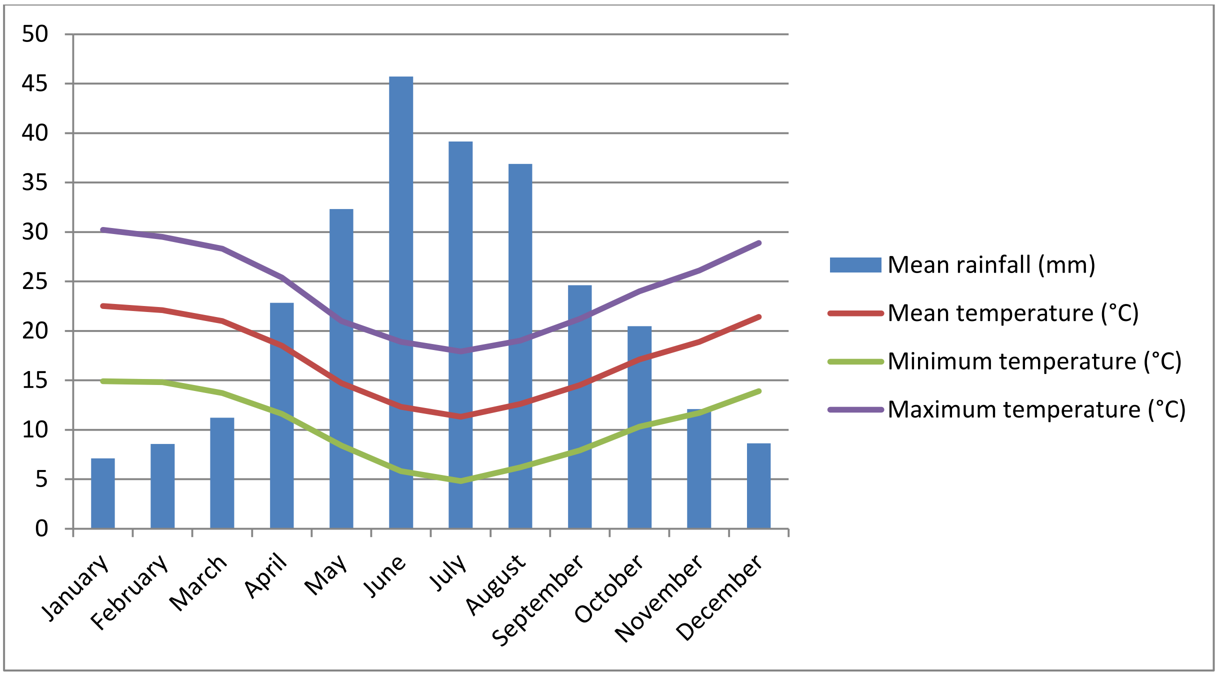

Figure 2.1: Mean monthly rainfall, and mean monthly maxima, minima and average temperatures for Worcester (Climate-Data.org 2018)

Summer temperatures are general high, with temperature in December to February often exceeding 40°C. The area receives light frost in winter with an average of 7 days per year receiving frost. The long term mean temperature of the area is above 17°C (Figure 2.1).

Figure 2.2: Vegetation and soils of the Worcester Veld Reserve (photo: R. Swart).

The geology of the Worcester Veld Reserve area is dominated by shales from the Cape Supergroup, with occasional diamictite and Jurassic Enon conglomerates (Mucina *et al.* 2006a). The soils are generally poorly developed with Mispah and Glenrosa soil forms prevailing (Western Cape Department of Agriculture 2018). Soils on the Worcester Veld Reserve are generally shallow and rocky with high clay content (Figure 2.2) (Western Cape Department of Agriculture 2018).

Study species

The seeds of four Karoo plant species are produced on a large scale at the Worcester Veld Reserve, namely the grass, *Chaetobromus involucratus* subsp. *dregeanus,* and the shrubs, *Eriocephalus africanus* subsp. *africanus, Osteospermum sinuatum* (= *Tripteris sinuata*) and *Gorteria integrifolia* (= *Hirpicium integrifolium*)*,* which are all members of the family Asteraceae. These species have been selected for seed production at the Worcester Veld Reserve since they are palatable to livestock, relatively easy to establish and produce large numbers of seed that can be harvested with relative ease. These four species are generally well-known to farmers and are the best-selling seeds at the Worcester Veld Reserve. Each of the four species is adapted to survive under slightly different soil and climatic conditions (Vlok & Schutte-Vlok 2015). These four species were selected for this study since most have previously been used for seed based restoration (Witbooi & Esler 2004; Visser & Botha 2005; Saayman & Botha 2010; Saayman *et al.* 2017) and since their seeds are produced in large enough quantities to provide enough materiel for this study.

Osteospermum sinuatum DC. (Karoobietou)

Common throughout most of the southern Karoo, *O. sinuatum* is a small shrub, growing up to 50 cm tall in loamy and clayey soils (Figure 2.3) (Herman *et al.* 2006; Vlok & Schutte-Vlok 2015). It has white or grey bark with grey-green leaves which vary in shape between populations (Vlok & Schutte-Vlok 2015; Stuart & Barker 2010). This species is widespread and occurs throughout most of the Karoo, from the Eastern Cape through the Western Cape province into southern Namibia (Figure 2.4) (Herman *et al.* 2006; Vlok & Schutte-Vlok 2015), and shows little genetic variation over its range (Stuart & Barker 2010).

These shrubs react quickly to rainfall after droughts and produce high volumes of very palatable foliage, soon followed by yellow flower heads (Le Roux *et al.* 1994). The winged seeds are wind dispersed and often collect underneath adjacent shrubs. These drought-adapted shrubs can flower and set seed two, or three times per year, responding to favourable rainfall (Milton & Dean 1990). Karoobietou is highly palatable (Van Breda & Barnard 1991) and an abundance of this plant can be used as an indication of well managed rangeland (Vlok & Schutte-Vlok 2015). Seedlings are dependent on good follow-up rain, as 100% mortality has occurred in *O. sinuatum* seedlings after 120 days without watering (Esler & Phillips 1994).

Figure 2.3: *Osteospermum sinuatum* (photo: R. Swart).

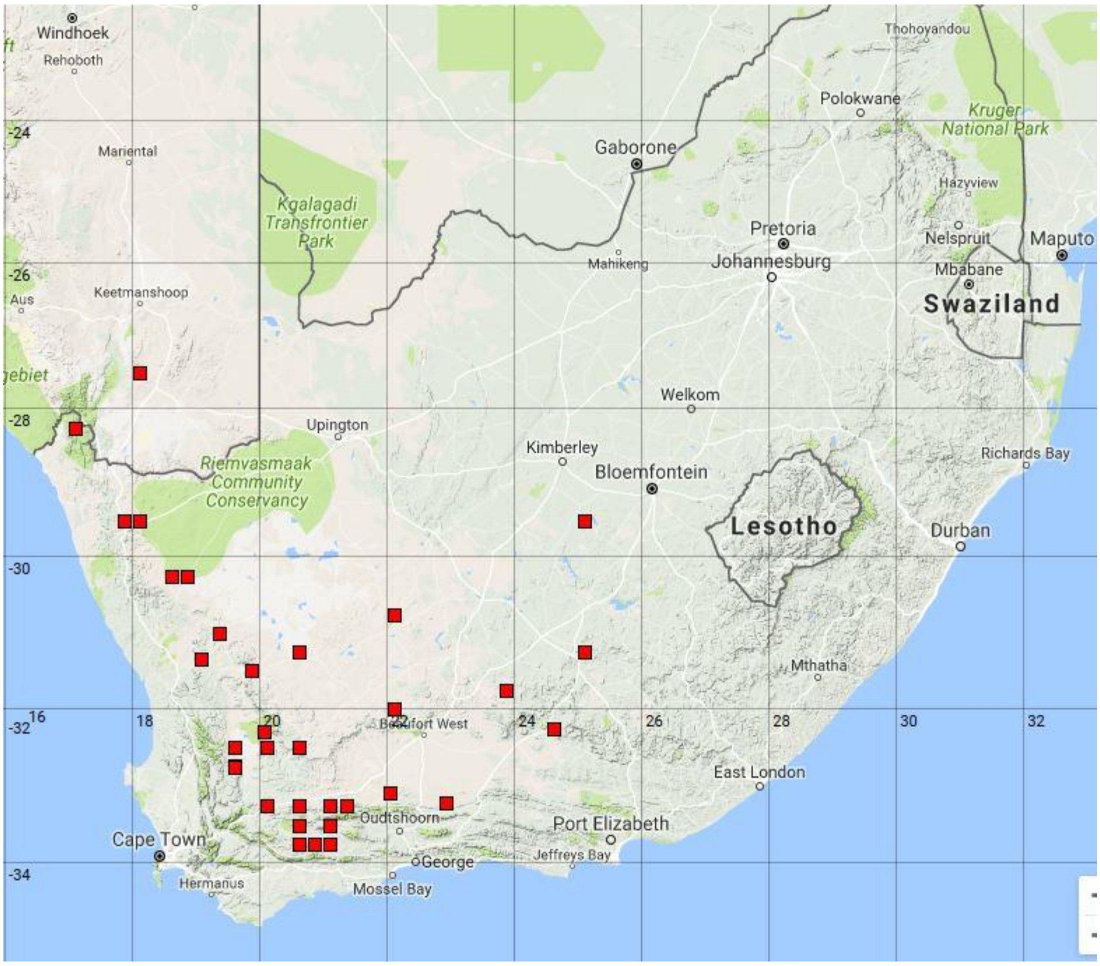

Figure 2.4: Distribution range of *Osteospermum sinuatum* (SANBI 2017).

Gorteria integrifolia Thunb. in Skr. Naturhist.-Selsk. (Haarbossie)

A small, bristly shrub, *G. integrifolia,* previously known as *Hirpicium integrifolium,* (Figure 2.5) rarely grows up to 50 cm tall (Vlok & Schutte-Vlok 2015). The leaves are dark green and bristly on the upper surface and white-woolly on the lower surface (Vlok & Schutte-Vlok 2015). Flowering occurs after rain and plants produce orange flower heads with distinctive long, dark bristles on the bracts (Le Roux *et al.* 1994). Mature flowers form capitula which act as diaspores (Stångberg 2009) and are dispersed by wind.

These plants occur on ridges with clayey and stony soils and sometimes form dense stands (Vlok & Schutte-Vlok 2015). Its distribution is limited to the Little Karoo region of the Western Cape province (Figure 2.6) (Stångberg & Anderberg, 2014). The very similar and closely related species, *Gorteria alienata* is more widely distributed and also occurs in the Northern Cape province, along the west coast and in the south west of the Nama Karoo (Stångberg & Anderberg, 2014). Despite the plant's hard, bristly appearance it is highly palatable and provides valuable fodder to game and livestock (Van Breda & Barnard 1991; Le Roux *et al.* 1994; Vlok & Schutte-Vlok 2015).

Figure 2.5: *Gorteria integrifolia* (photo: R. Swart).

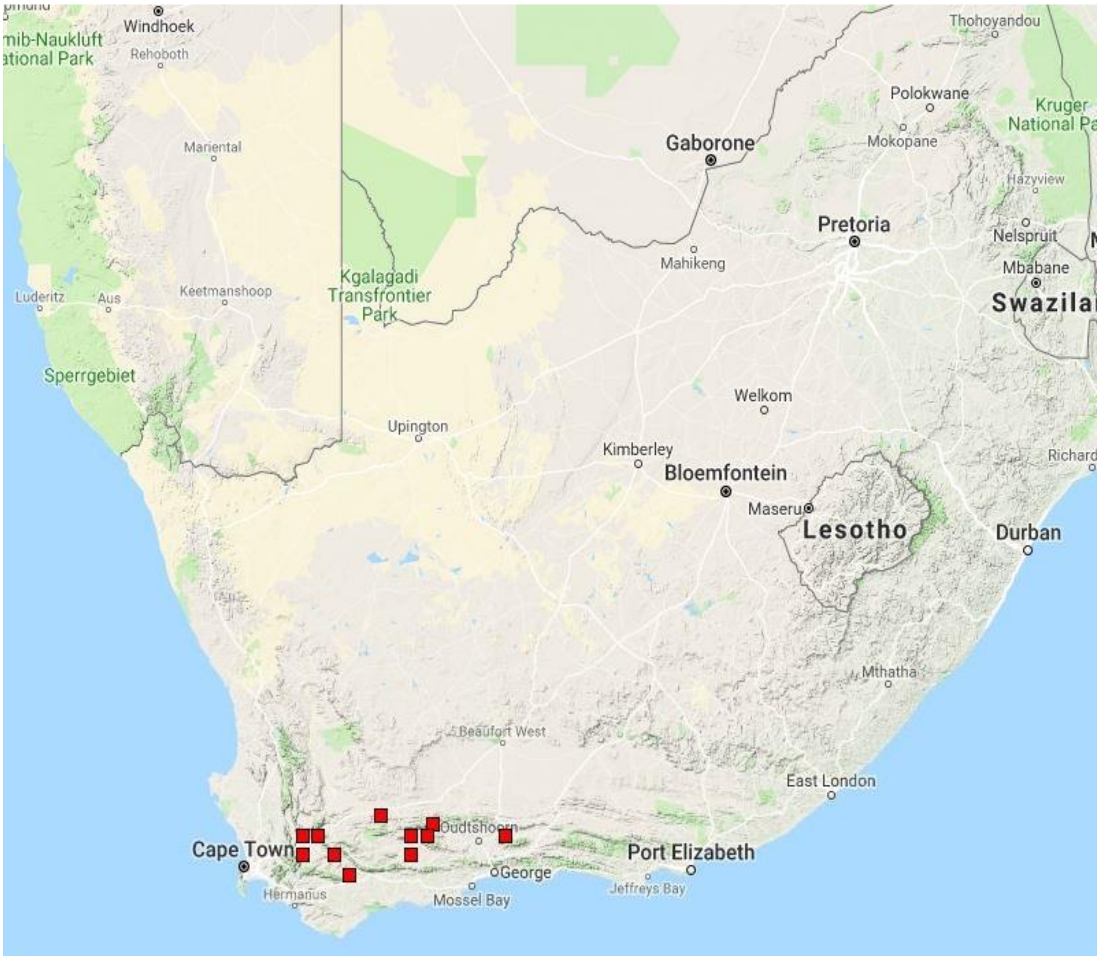

Figure 2.6: Distribution range of *Gorteria integrifolia* (SANBI 2017).

Eriocephalus africanus L. *africanus* (Cass.) M.A.N. Müll., P.P.J Herman & H.H. Kolberg (Kapokbos, Wild rosemary)

This silvery grey shrub grows up to 1 m tall (Vlok & Schutte-Vlok 2015) and has small, woolly leaves that have a strong rosemary scent when crushed (Figure 2.7). These plants occur at altitudes of 100 to 3000 meters above sea level in the Northern-, Western-, and Eastern Cape Provinces (Figure 2.8) (Herman *et al.* 2006; Rebelo *et al.* 2006). The plants flower during spring and produce small flower heads with mauve-purple central florets and white or light pink ray florets (Vlok & Schutte-Vlok 2015). Woolly fruitlets are produced and are dispersed by birds that use the seeds as nesting material. These plants occur widely in sandy or loamy soils, often in moist areas. Wild rosemary is considered palatable (Van Breda & Barnard 1991) and is regularly browsed by game and livestock (Vlok & Schutte-Vlok 2015).

Figure 2.7: *Eriocephalus africanus* (photo: R. Swart).

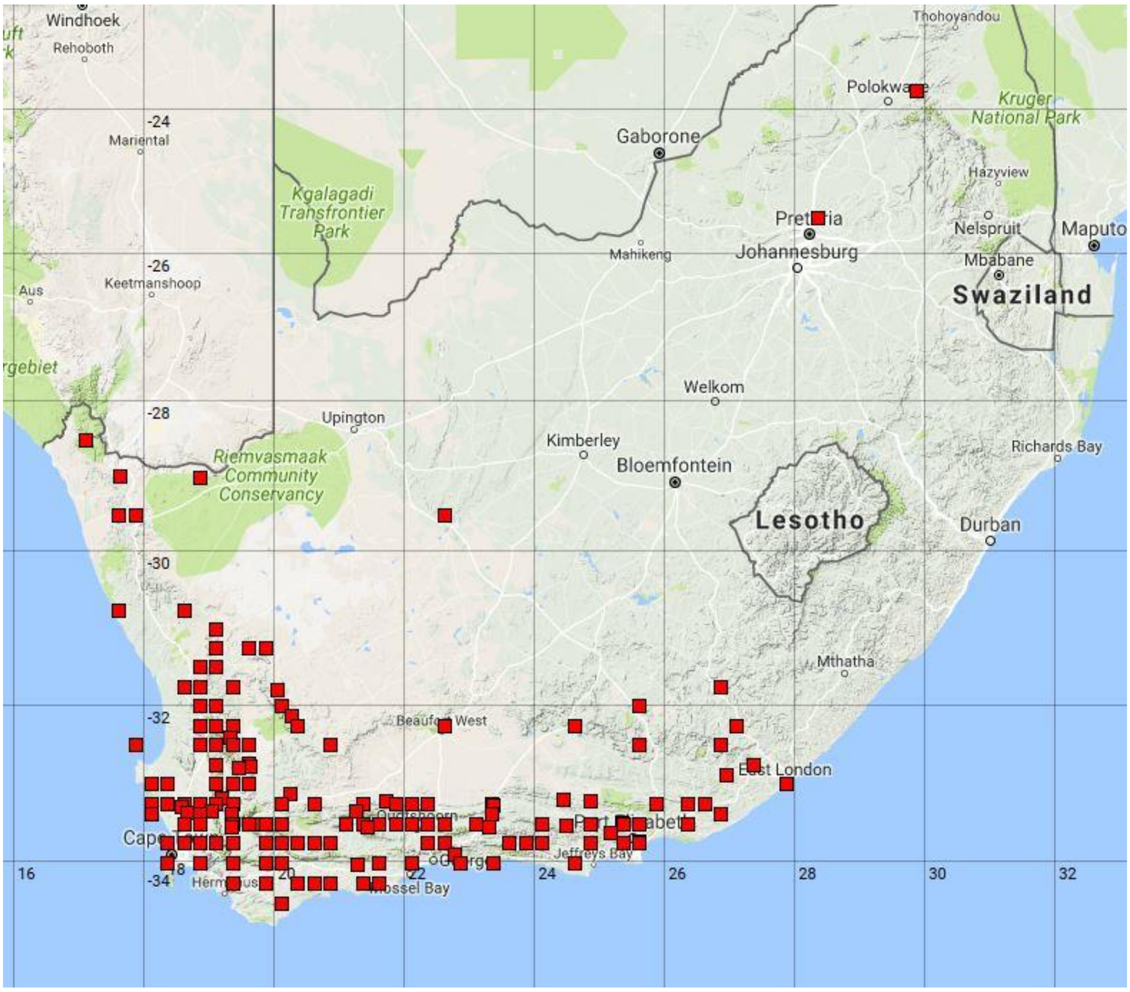

Figure 2.8: Distribution range of *Eriocephalus africanus* subsp. *africanus* (SANBI 2017).

Chaetobromus involucratus (Schrad.) Nees. subsp. *dregeanus* (Nees.) Verboom. (Hartbeesgras, Gha grass).

A perennial, tufted grass that grows up to 50 cm tall when in flower (Figure 2.9) (Fish 2006; Van Oudtshoorn 2012). It is most common in the winter-rainfall region, but is also found scattered further east, especially along major roads (Figure 2.10). It mostly flowers during spring, but responds opportunistically to rainfall and may produce seeds throughout the year (Vlok & Schutte-Vlok 2015). This seed is produced in a panicle with only a few spikelets (Fish *et al.* 2015). The tuft of hair at the base of the spikelet offers an easy means of identification. This grass is considered highly palatable (Van Breda & Barnard 1991) and is found on rocky or sandy soils where it is well utilised by livestock and game (Vlok & Schutte-Vlok 2015; Van Oudtshoorn 2012; Fish *et al.* 2015). This species has been shown to be effective in the rehabilitation of an open-cast phosphate mine on the West Coast of South Africa (Van Eeden 2010).

Figure 2.9: *Chaetobromus involucratus* (photo: R. Swart).

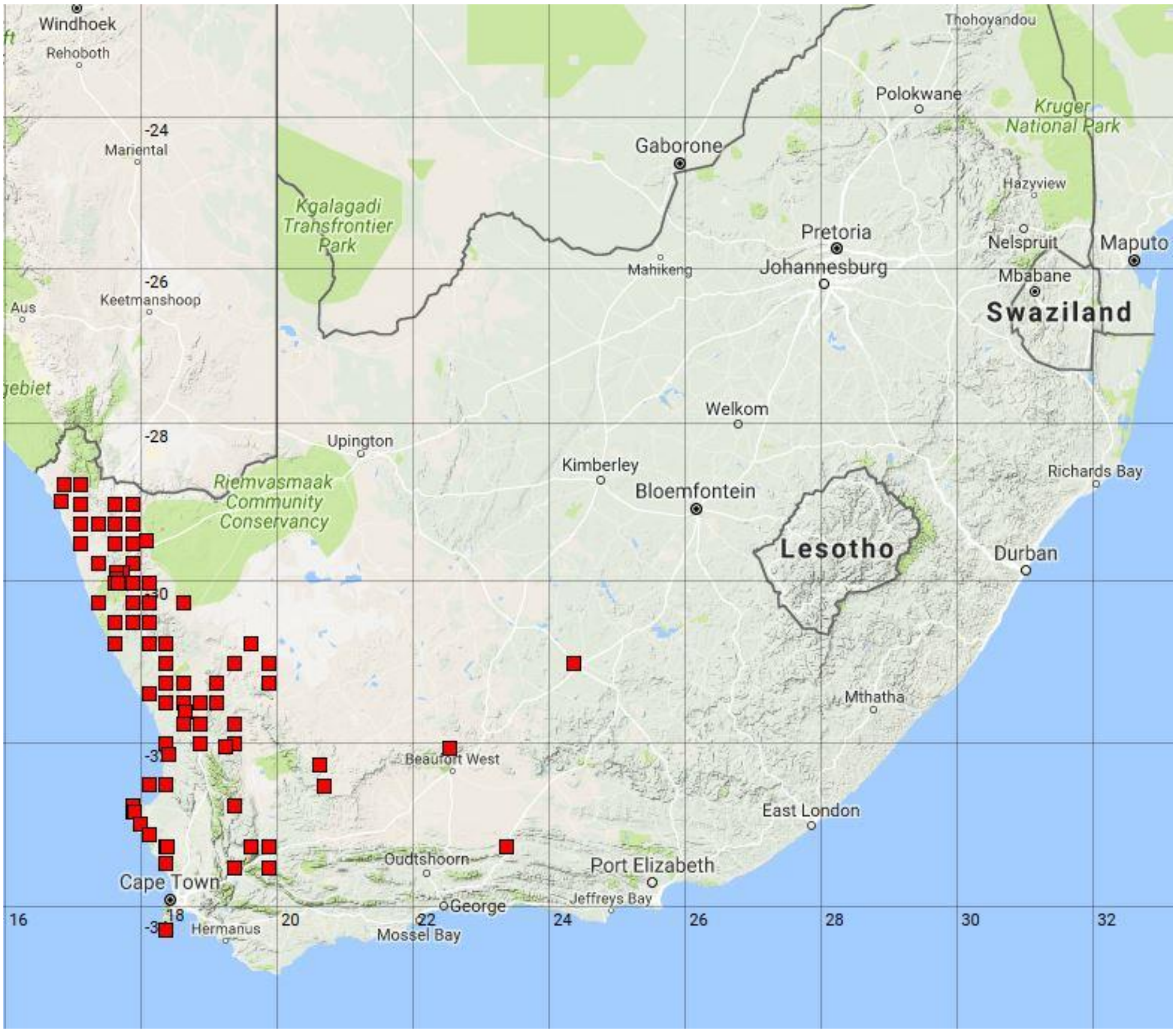

Figure 2.10: Distribution range of *Chaetobromus involucratus* subsp. *dregeanus* (SANBI 2017).

Chapter 3: Seed viability

Introduction

Rangeland degradation is a recurring feature throughout the Karoo (Esler *et al.* 2006). This phenomenon is often caused by poor management of livestock over successive seasons (Esler *et al.* 2006). Continuous, selective herbivory by livestock or game leads to a reduction in the abundance of palatable plant species (Milton 1993). This is often the case where rotational grazing systems are absent or rangelands are stocked with more herbivores than can be supported by the available vegetation. As palatable species are reduced in abundance, the rangeland loses productivity and the ability to sustain herbivores (Esler *et al.* 2006; Kinyua *et al.* 2009). Palatable plants can be slow to colonise degraded rangelands, due to increased competition and high reproduction rates from established unpalatable species (Schantz *et al.* 2015). In order for these degraded rangelands to be restored to a productive state, palatable plants need to be returned by active management intervention (Seabloom *et al.* 2003; Esler *et al.* 2006; Van Eeden 2010; Matthee 2015).

Returning palatable plants to a degraded rangeland can be achieved by the reintroduction of seeds from extirpated palatable species (Pywell *et al.* 2002; Mahood 2003; Seabloom *et al.* 2003; Esler *et al.* 2006; Kinyua *et al.* 2009; Van Eeden 2010). These seeds could be collected near the degraded area, or be purchased from seed production companies. Seeds can then be introduced to the seedbank of the degraded rangeland by broadcasting or planting. Large quantities of seed are often required, since seeds may be damaged or destroyed by pathogens or seed predators before germinating, or may germinate under unfavourable rainfall conditions and thus few survive to become established plants (Saayman *et al.* 2017).

Seed based restoration projects are dependent on good germination performance, and as such the seed used for restoration needs to be of a high quality (Van den Berg & Kellner 2005; Waller *et al.* 2015). Restoration projects are often very expensive (Esler *et al.* 2006) and seed is often difficult to obtain. Seedlings established during restoration projects may fail to survive due to damage by herbivores, inadequate soil moisture and competition from established unpalatable plants and their seedlings (Saayman *et al.* 2017; Matthee 2015). It is therefore important that seed used for restoration has high viability and germinability in order to produce as many seedlings as possible, so that a sufficient number may survive to reach maturity.

Restoration projects often achieve poor results when seeding degraded areas (James *et al.* 2011). Simons and Allsopp (2007) found that after a restoration trial of two years in Namaqualand, 90 seedlings emerged from approximately 20 000 seeds sown. The difficulty in reintroducing palatable plants is partly due to failed seed germination (Commander *et al.* 2009a). The failure of many rehabilitation projects are ascribed to the lack of suitable rainfall events (Milton 1995a), however, this study will focus on the possible mortality of seeds prior to sowing. Karoo seed harvested for restoration purposes at the Worcester Veld Reserve often show visual signs of defects or damage caused by seed predators (personal observation).

Seeds that show obvious malformation or damage can be separated by visual inspection (Mallik & Karim 2008), while germination trials (Travlos *et al.* 2007; Commander *et al.* 2009a) or chemical staining (Association of Official Seed Analysts 2005) provide a more robust measure of seed viability. Chemical staining to detect seed viability is done with 2, 3, 5-triphenyl tetrazolium chloride (Association of Official Seed Analysts 2005). This compound stains tissues that show active cell metabolic activity and as such is used to determine whether individual seeds are viable or not (Association of Official Seed Analysts 2005).

The aim of this study was to compare the seed viability of the most commonly used plants in rangeland restoration at the Worcester Veld Reserve. This will allow one to calculate the amount of seeds required to produce a desired number of seedlings for each species, and to calculate the proportions of seed from each species in seed mixtures. Furthermore, the causes of seed mortality are determined to allow new management measures to be taken to improve seed production.

Methods

The test for seed mortality was done at the Worcester Veld Reserve (33°37'20.75"S, 19°28'8.66"E). Five replicates of 20 seeds were randomly selected from harvested seed stock. It should be noted that "seeds" in this sense refers to single fruiting bodies. The seeds were inspected under a dissecting microscope and the empty, shrivelled and deformed seeds were considered non-viable (Commander *et al.* 2009b). Non-viable seeds were removed, recording the nature of the damage and the number of damaged and deformed seeds. Seeds that showed no superficial signs of damage or deformation were imbibed in tap water (Association of Official Seed Analysts 2005; Mallik & Karim 2008) and placed in 90 mm petri dishes. Imbibing the seeds was done to encourage active cellular respiration within viable seeds (Association of Official Seed Analysts 2005). The petri dishes were then stored at room temperature for 48 hours. The products of the cellular respiration were detected by using the 2, 3, 5-triphenyl tetrazolium chloride stain (Association of Official Seed Analysts 2005).

Seeds were dissected after being imbibed and stored for 48 hours. Seed that showed signs of germination were recorded as viable. The rupturing of the seed coat and emergence of the radicle are considered signs of germination (Travlos *et al.* 2007; Commander *et al.* 2009a). Seeds that showed no clear signs of germination were treated with a 1% solution of 2, 3, 5-triphenyl tetrazolium chloride (Association of Official Seed Analysts 2005). At this stage only seeds that show no signs of germination were tested with the tetrazolium solution, since germinated seeds had already been determined to be viable. The treated seeds were left for 24 hours in order for the living tissues to be stained. The treated seeds were then evaluated for viability according to the guidelines of the Association of Official Seed Analysts (2005).

The tested seeds were categorized as follows: Seeds unviable due to malformation, damage by seed predators, damage by pathogens or unviable after the Tetrazolium test. Seeds were considered viable when showing signs of germination or being confirmed viable after the Tetrazolium test.

The proportions of viable and non-viable seeds were recorded in percentage format for each species. The Shapiro-Wilk test was performed on the standardised residuals to test for normality (Shapiro and Wilk 1965). The percentage data were subjected to analysis of variance (ANOVA) to determine whether there were significant differences between the mean percentages of viable seed between the different species used, using SAS statistical software (SAS Institute 2016). Fisher's least significant difference was calculated at the 5% level to compare species means (Ott & Longnecker 2001). The data were also analysed with a principal component analyses (PCA) with XLSTAT software (Addinsoft 2015). The principal component analysis allowed the determination of the most significant causes of seed mortality for each of the seed species investigated in this study.

Results

Gorteria integrifolia had the highest mean percentage of viable seeds at 48% (Table 3.1). *Eriocephalus africanus* showed significantly lower viability than the other species tested, at only 22% ($F = 21.45$; $p<0.0001$).

Table 3.1: The mean percentage viability of seeds after visual screening and TTC test. Means with different superscripts differ significantly ($p<0.001$). Sample size (N) refers to the number of replicates of 20 seeds.

Species	Mean % Confirmed Viable	Standard deviation	Sample size (N)	Total seeds sown
Osteospermum sinuatum	44^{a}	8.37	5	100
Eriocephalus africanus	22^{b}	13.96	5	100
Chaetobromus involucratus	43^{a}	5.70	5	100
Gorteria integrifolia	48^{a}	8.22	5	100

A principal component analysis (Figure 3.1) shows the leading association between causes of seed mortality and seed species. The majority of un-viable *E. africanus* seeds were discovered after dissection of the fruiting bodies. *Gorteria integrifolia* and *O. sinuatum* showed a high number of seed mortality due to damage by seed predators including rodents and insects. *Chaetobromus involucratus* had the highest mortality due to seed malformation.

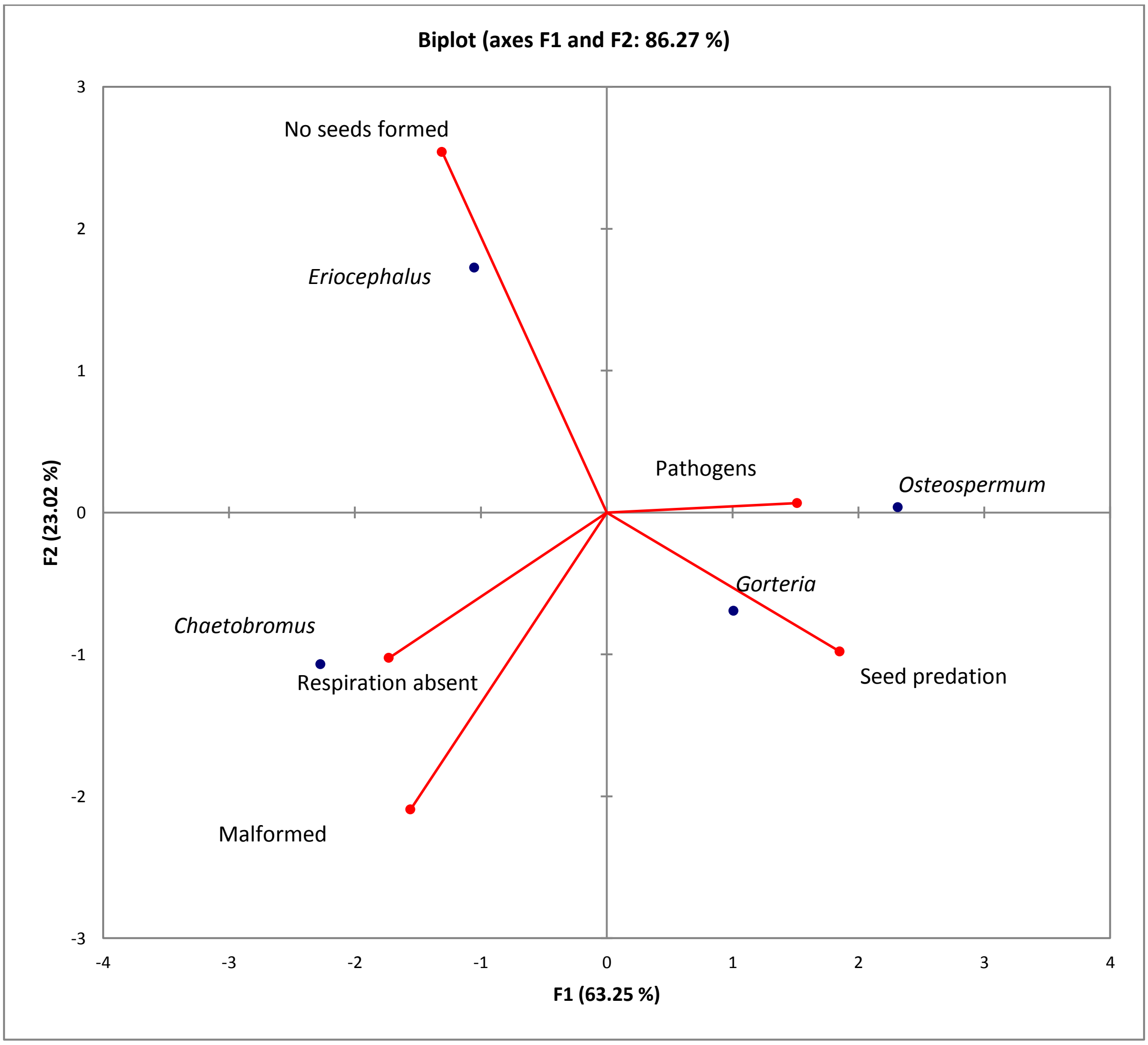

Figure 3.1: Relative frequencies of causes of seed mortality per species tested.

Discussion

The viability of all four species tested was below 50%. The causes of seed mortality varied considerably between species. This may be due to the considerable difference in seed morphology between the investigated species.

The larger seeds of *Osteospermum sinuatum* and *Gorteria integrifolia* were prone to similar causes of seed mortality. Their relatively large and oily seeds seemed to attract rodents and insects (Milton 1995b) and were also prone to infection by pathogens. Rodent damage was particularly common in *O. sinuatum,* while *G. integrifolia* showed more signs of insect damage to seeds. The seeds of both *O. sinuatum* and *G. integrifolia* are preferentially predated on by rodents over a variety of other Karoo seeds (Kerley & Erasmus 1990). The woolly fruiting bodies of *Eriocephalus africanus* often contained no seeds upon dissection, despite seeming intact with only visual inspection. Many of the seeds of *Chaetobromus involucratus* were malformed and soft. Many *C. involucratus* seeds did not indicate any cellular respiration when subjected to the TTC test, despite seeming to be undamaged and plump. It may be that these *C. involucratus* seeds were still affected by a form of chemical dormancy and were thus kept from initiating cellular respiration and subsequent germination or that a proportion was still unripe at the point of harvest, similar to other Southern African grass species, such as *Themeda triandra* (Venter 1962; Baxter 1996).

The damage by seed predators may be reduced by prompt harvesting of seed as soon as it ripens. Many of the seeds harvested at the Worcester Veld Reserve are collected from underneath parent plants. This dense collection of seeds would likely be very attractive to seed predators. Seed predation by rodents may also be reduced by attracting predators of rodents to the seed orchards. The addition of owl boxes may be a viable, environmentally sound way to reduce rodent numbers (Leirs 2003). Harvesting the seeds as soon as they ripen and while they are still on the plants should mitigate this problem. Malformed and damaged seeds were generally lighter than the plumper, healthy seeds. A proportion of the seeds may also have been unripe at the time of harvest, particularly that of *C. involucratus.* A wind winnowing machine may be useful to separate healthy seeds from damaged seed and chaff.

Conclusion

The viability of all four study species were found to be quite low, with all species having less than 50% seed viability. The seed viability of *E. africanus* was significantly lower than the other studied species. The causes of seed mortality differed between species. Some of the causes of seed mortality, such as predation by rodents or fungal decay, could be mitigated by appropriate management intervention. Other causes of dead or missing seed, such as the tendency of a portion of harvested seed to be unripe, will be more difficult to address and may be an unavoidable issue in the collection of seed.

Chapter 4: The effect of drying on seed germination

Introduction

The seeds harvested at the Worcester Veld Reserve are air dried in a drying shed before being packaged for storage. The air drying of the seeds is intended to improve the longevity of the seeds by mild desiccation to prevent rotting or fungal damage and to release innate dormancy that may affect the germination of otherwise undried seed (Gutterman 2000; Commander *et al.* 2009a; Probert *et al.* 2009). The drying is also intended to place the seeds in a state of enforced dormancy, whereby the seeds are not exposed to any germination cues, thus preventing premature germination during storage (Harper 1977).

While the purpose of drying the seeds is to maintain viability, reducing the moisture content of seeds below a certain threshold may cause a reduction in viability (Ellis *et al.* 1988; Saipari *et al.* 1998). This threshold varies considerably between different plant families and species (Ellis *et al.* 1988), yet all the seed species harvested at the Worcester Veld Reserve receive the same drying treatment. The average temperature measured over 8 weeks in the drying shed at the Worcester Veld Reserve was 33.8 °C, with maximum temperature readings over 45 °C. This is similar to the conditions prescribed by Commander and others (2009b) who found that storing seeds between 30 °C and 45 °C for a period of less than 3 months improved seed germination.

The objective of this study is to determine how air drying of the seeds at the Worcester Veld Reserve affects their germinability. An improvement in seed germination after the drying treatment will be attributed to the removal of seed dormancy and the preservation of seed viability.

Methods

The effect of drying on seed germinability was tested at the Elsenburg germination room and laboratory (33°50'41.61"S, 18°50'13.09"E). The germination room at Elsenburg allows seed samples to be exposed to controlled light and temperature conditions. Day-night length and temperature were selected to simulate conditions during late summer/early autumn, the purported ideal time for seed germination in an arid, winter rainfall climate (Milton 1995a; Esler *et al.* 2006; Kulkarni *et al.* 2006; Saayman 2013). It has been found that germination can be strongly influenced by ambient day/night temperatures (Travlos *et al.* 2007; Commander *et al.* 2009a). Conditions in the germination room were based on similar trials by Kulkarni *et al.* (2006).

Seeds were visually inspected, and damaged or deformed seeds were not used for the germination trial. The seeds were placed in 90 mm petri dishes lined with filter paper. Each petri dish was watered with 5 ml sterile, de-ionised tap water and then sealed with parafilm to prevent seed desiccation (Nadjafi *et al.* 2006; Mallik & Karim 2008; Vieira *et al.* 2018). A description of the light and temperature settings for the germination tests is given in Table 4.1.

Table 4.1: Simulated day and night conditions in the germination room.

Condition setting	Lighting	Temperature	Duration
Day	On	20 °C	14h
Night	Off	10 °C	10h

Prior to drying, five replicates of 20 seeds each were randomly selected from harvested seed. These seeds were kept in brown paper bags at room temperature (Van der Walt & Witkowski 2017). These seeds were then tested for germination in the germination room at Elsenburg as described above. The five replicates of each species were arranged in a randomised block design

Seeds were considered to have germinated once the radicle has ruptured the seed coat and extended for 2 mm or more (Travlos *et al.* 2007; Commander *et al.* 2009a; Vieira *et al.* 2018). Germinated seeds were counted and removed weekly. Additional de-ionised tap water was added to the petri dishes if the seeds appeared to be drying out. The seeds were assumed to be non-viable if they did not germinate within four weeks' time, since by this time most seeds would have either germinated or started showing signs of fungal growth and decay.

The remainder of the harvested seed was exposed to drying. Drying the seed was done by spreading the seed on the floor of a drying shed. The seeds were left to air dry (Commander *et al.* 2009a) for four weeks before being removed. Five replicates of 20 seeds each of the dried seed of each of the four species was tested for germination using the same design and germination room as is used for the undried seed described above. Four trials (species) were conducted. The experimental design was a randomised block design with 5 replications and 2 treatments (control and drying treatment). The germination data were expressed as percentages out of a total of 20 seeds to convert binary to continuous data for analysis of variance (ANOVA) (Snedecor and Cochran 1967). Levene's test for homogeneity of variance showed heterogeneous species variances (Levene 1960). The Shapiro-Wilk test confirmed normality of the standardized residuals (Shapiro and Wilk 1965). The data were subjected to weighted ANOVA using General Linear Models Procedure (PROC GLM) of SAS software (Version 9.4; SAS Institute Inc, Cary, USA). The weight was the reciprocal of the species variances (1/MSE). Fisher's least significant difference was calculated at the 5% level to compare treatment means (Ott and Longnecker 2001).

Results

No germination of un-dried seeds occurred, except for a single *Eriocephalus africanus* seed. The only statistically significant improvement in germination after drying was found with *Osteospermum sinuatum* ($F = 9.86$; $p<0.05$), although germination of *Chaetobromus involucratus* and *Gorteria integrifolia* seeds did show germination after drying, and no germination without drying (Table 4.2).

Table 4.2: A comparison of the mean germination of air dried seeds between four species used in Karoo rangeland restoration. Superscripts with different letters in a row indicate significant differences ($p<0.05$). Sample size (N) refers to the number of replicates of 20 seeds each.

Species	Treatment	Germination (%)	Standard Deviation	Sample Size (N)	Total seeds sown
Chaetobromus involucratus	Dried	3^{a}	4.47	5	100
	Control	0^{a}	0	5	100
Eriocephalus africanus	Dried	0^{a}	0	5	100
	Control	1^{a}	2.24	5	100
Gorteria integrifolia	Dried	14^{a}	11.40	5	100
	Control	0^{a}	0	5	100
Osteospermum sinuatum	Dried	47^{b}	33.47	5	100
	Control	0^{a}	0	5	100

The germination percentage of dried *O. sinuatum* was found to be significantly higher ($F = 2.28$; $p < 0.05$) than that of the other tested species.

Discussion

All four of the tested species have seeds that are wind dispersed. It can be presumed that these seeds will be exposed to considerable desiccation once dislodged from the parent plant, since these species tend to set seed just prior to the dry summer season of the winter rainfall region in which they occur (Van Breda & Barnard 1991). Gutterman (2000) suggests that the seeds of annuals in the Negev Desert may have their dormancy reduced by the sudden onset of high soil temperatures during summer. A similar mechanism may explain the improvement in germination of *O. sinuatum* seeds tested in this study. The distribution range of *O. sinuatum* includes both the summer and winter rainfall regions of the Nama Karoo and Little Karoo biomes (SANBI 2017). The survival strategy of *O. sinuatum* seems to be opportunistic in the sense that it responds immediately to rainfall with new growth and flowers (Milton & Dean 1990), regardless of the seasonal timing of the rain. It can be deduced that the seeds of *O. sinuatum* would follow a similar opportunistic strategy, germinating as soon as moisture becomes available, and having a relatively short period of dormancy.

Summer rainfall is sporadic and unreliable in the western region of the Succulent Karoo biome. As such it would be reasonable to presume that seeds of local plants in this region would follow a more cautious strategy by remaining dormant throughout the summer, and germinating during the start of the more predictable winter rains. This could explain the low germination of, *C. involucratus* and *G. integrifolia*, both of which are native to winter-rainfall regions.

The reason for the effective lack of germination of *E. africanus* is unknown, but may be due to a combination of dormancy preventing germination, as well as the morphology of the *E. africanus* fruits. The woolly exterior of the fruits may provide sufficient insulation to reduce moisture loss and thus prevent the lifting of seed dormancy, however, further study may be required to confirm this.

The increased germination of *O. sinuatum* after drying indicates the requirement to dry seeds of this species before sowing. The low or absent germination of non-dried seeds of all tested species was likely caused by a lack of ripening, combined with high seed moisture content, which lead to the growth of mould, killing the embryo before germination was possible. It is also possible that the seeds of *C. involucratus*, *E. africanus* and *G. integrifolia* have a dormancy-enforcing mechanism that is not effectively removed by drying, thus allowing them to remain dormant until the rainfall and favourable germination conditions of the winter months arrive.

Conclusion

The drying procedure has proven to be essential to enable germination of freshly harvested *O. sinuatum* seeds. While the drying did not have a significant effect on the germination of the other species tested, it should still be considered as a way to lower seed moisture content, thus preventing the growth of harmful fungus and improving seed shelf life.

Chapter 5: The effect of storage time on seed germination

Introduction

Seeds harvested for restoration projects often have to be stored for extensive periods of time in order to be available for sowing during favourable conditions for germination and seedling establishment. The longevity of stored seeds can be improved by storing the seeds at low temperature and humidity, as well as by reducing the seed moisture content before storage (Probert *et al.* 2009), causing a state of enforced dormancy (Harper 1977). Large seedbank storage facilities utilize advanced equipment to precisely regulate the temperature and relative humidity of seed storage rooms (Probert *et al.* 2009). These facilities are generally not available to small scale seed harvesting projects undertaken by landowners. For the purpose of this trial, seed was harvested at the Worcester Veld Reserve and stored at ambient (room) temperature and then tested for germination over the timespan of a year. The seeds harvested at the Worcester Veld Reserve are usually stored in a cold room at 4°C and sold or used within a period of one year.

Seeds of Karoo shrubs often have a level of innate dormancy and may need a significant amount of after ripening time before optimum germination can be achieved, or germination could be triggered by seasonal cues (Henrici 1935; Harper 1977; Weiersbye & Witkowski 2002; Commander *et al.* 2009a). It has been suggested that seeds of Succulent Karoo plant species will germinate best during autumn when temperatures are moderate and when the first rains arrive (Milton 1994). Germination cues and the level of dormancy are likely to differ between species (Esler 1993; Esler 1999; De Villiers *et al.* 2003; Nadjafi *et al.* 2006; Probert *et al.* 2009).

Plants that are native to arid areas with a predictable rainy season are likely to evolve mechanisms for their seeds to germinate prior to or during this rainy season in order to improve the chances of successful seed germination and seedling establishment. The mechanism required to allow this delay in germination would likely be a form of seed dormancy which is lifted over time, or by specific environmental cues (Weiersbye & Witkowski 2002; Commander *et al.* 2009a). Conversely, plants that have evolved in arid areas with an unpredictable rainfall regime would likely evolve mechanisms to opportunistically exploit rainfall events. To this end their seeds would have little or no dormancy in order for them to quickly respond to a rainfall event (Esler 1999).

The aim of this study is to determine whether there is a relationship between seed storage time and germination over the timespan of a year, and whether such a relationship is positive or negative.

The germination successes of the four species will also be compared with each other to determine the species with the highest germination potential. This is intended to provide insight into the relative composition of seed mixes for use in restoration projects, rather than simply selecting the single species with the highest germination potential for such projects.

Methods

The effect of storage time on the germination of harvested seed was determined by testing seed germination over time at intervals of three weeks. The first test was done directly after seed harvested at the Veld Reserve had been dried and cleaned for storage. Subsequent tests were done over time at intervals of three weeks for a total of 17 intervals, thus 48 weeks until the start of the last interval. This time period of approximately one year was selected, since seed at the Worcester Veld Reserve are generally sold out within one year after harvesting. Five replicates of 20 seeds were collected randomly per species and tested at every interval. Seeds were screened for damage by visual inspection (Mallik & Karim 2008; Saaed *et al.* 2018), before being used in the trial.

The tests were conducted at the germination room and laboratories of the Western Cape Department of Agriculture at Elsenburg (33°50'41.61"S, 18°50'13.09"E). Seed germination was tested at the germination room at Elsenburg at every interval. Each test consisted of five replicates of 20 seeds each. Each replicate comprised two 90 mm petri dishes containing 10 seeds each.

The petri dishes were lined with filter paper, sown, watered with 5 ml de-ionised water and then sealed with parafilm to prevent seed desiccation (Nadjafi *et al.* 2005; Mallik & Karim 2008). The light and temperature settings, as well as the arrangement of petri dishes were the same as described in Chapter 4 (Table 4.1).

The number of seeds germinating in each replicate were counted and removed weekly. The seeds in each petri dish were checked for desiccation and additional de-ionised tap water was added if necessary. After a three week interval passed the remaining seeds of that interval were assumed to be non-viable and were discarded. Germination data were expressed as percentages out of a total of 20 seeds.

The experimental design was a randomised block design with 5 replications. The Shapiro-Wilk test was performed on the standardized residuals from the model to test for normality (Shapiro and Wilk 1965). The percentage data were subjected to analysis of variance (ANOVA) using General Linear Models Procedure (PROC GLM) of SAS software (Version 9.2; SAS Institute Inc, Cary, USA) to determine if there was a significant difference between the mean % germination of the four species across the entire germination period.

A linear regression using PROC REG of SAS software (Version 9.4; SAS Institute Inc, Cary, USA) was fitted to the data on the mean germination percentages over time intervals for each of the four species. Normality of the standardised residuals of the model was confirmed by Shapiro-Wilk test (Shapiro and Wilk 1965) before the regression and variance analyses (ANOVA). Fisher's least significant difference was calculated at the 5% level to compare treatment means (Ott and Longnecker 2001).

Results

The germination results from the seeds planted in the growth room with controlled light and temperature conditions indicate that *O. sinuatum* had a significantly higher number of seeds germinating than *G. integrifolia* which in turn had a significantly higher germination than both *E. africanus* and *C. involucratus* (Table 5.1).

Table 5.1: The mean germination of four trial species tested under controlled light and temperature conditions over 51 weeks. Means with different superscripts indicate significant differences ($p<0.001$). Sample size (N) refers to the number of replicates of 20 seeds each tested over 17 intervals.

Species	Mean germination	Standard deviation	Sample size (N)	Total seeds sown
Chaetobromus involucratus	18.94^{c}	16.33	85	1700
Eriocephalus africanus	18.12^{c}	12.51	85	1700

Gorteria integrifolia	43.53[b]	22.31	85	1700
Osteospermum sinuatum	52.94[a]	19.91	85	1700

The regression analyses of the germination data from the growth room indicate that there are strong positive relationships between the mean germination and the storage time of both *Chaetobromus involucratus* and *Gorteria integrifolia* seeds (Table 5.2).

Table 5.2: A regression analyses of the mean germination of four Karoo plant species over time and under controlled light and temperature conditions.

Species	R Square	F value	P (significance)
Chaetobromus involucratus	0.704	35.761	< 0.001
Eriocephalus africanus	0.187	3.453	ns
Gorteria integrifolia	0.690	33.463	< 0.001
Osteospermum sinuatum	0.154	2.731	ns

There was a significant increase in the mean percentage germination of *C. involucratus* seeds over time, with less than 20% of seeds germinating in the first 30 weeks and up to 45% germinating after 42 weeks (Table 5.2; Figure 5.1).

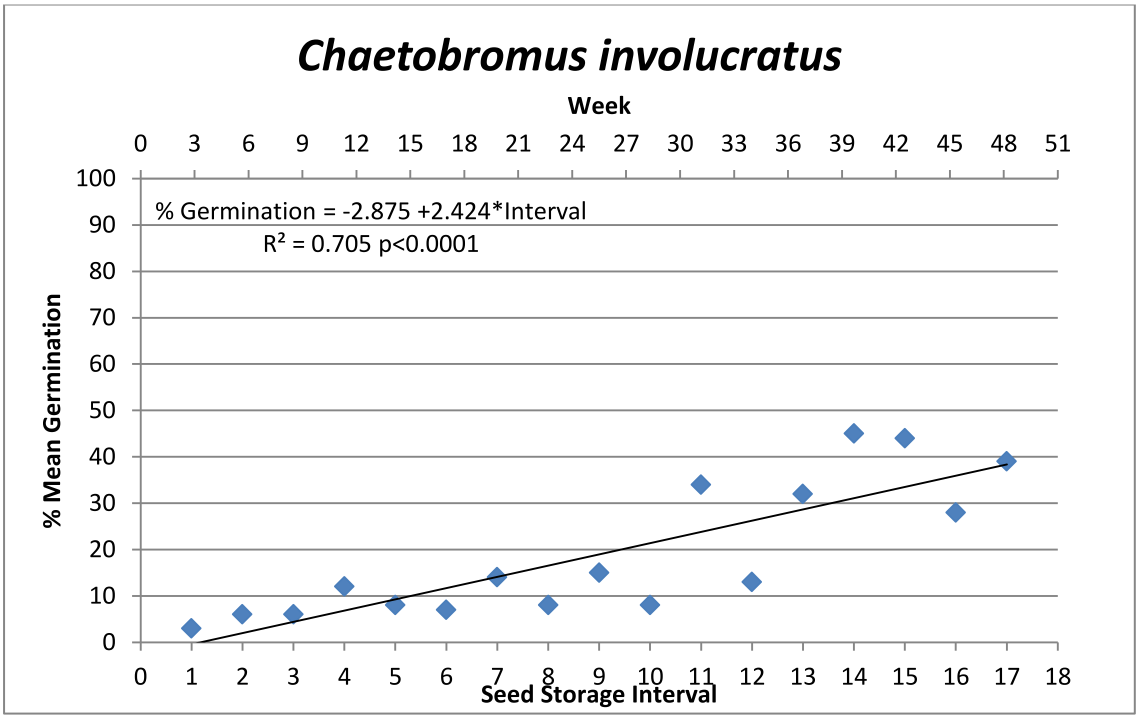

Figure 5.1:Mean percentage germination over storage time of *C. involucratus* seeds in a temperature and light controlled growth room. Each interval represents a three week period during which newly germinated seeds were recorded weekly.

The seeds of *E. africanus* did seem to show a slight increase in germination over time, with no germination in the growth room during the first interval (Figure 5.2). This increase was not statistically significant though (Table 5.2).

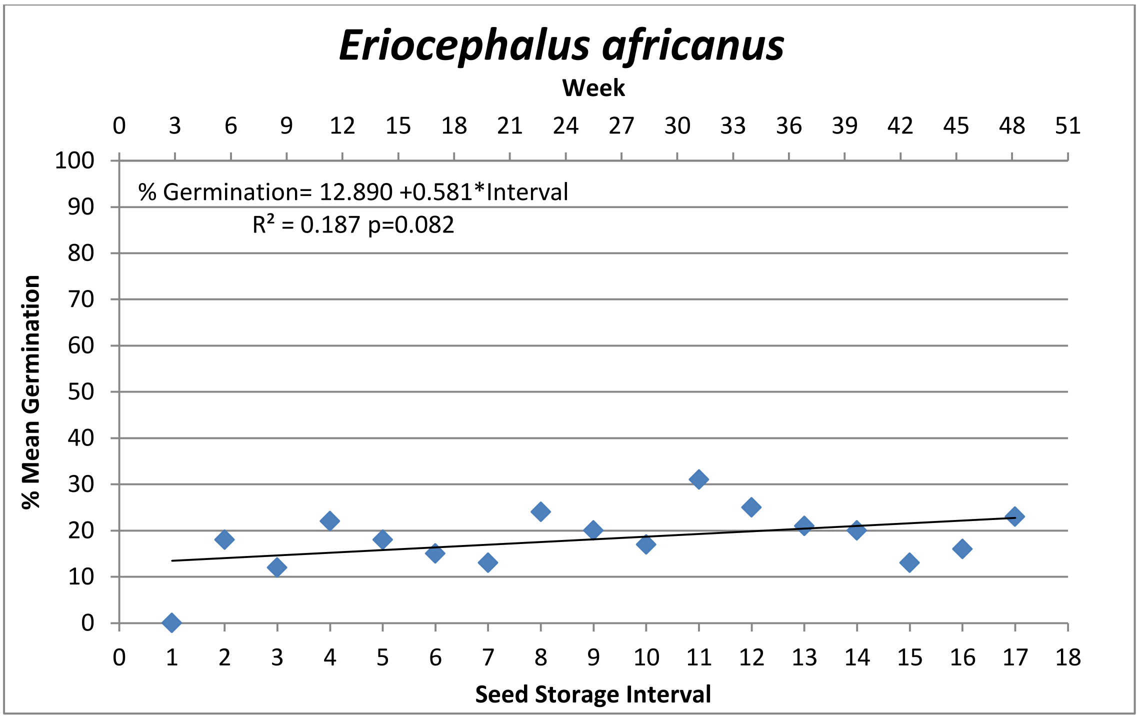

Figure 5.2: Mean percentage germination over storage time of *E. africanus* seeds in a temperature and light controlled growth room. Each interval represents a three week period during which newly germinated seeds were recorded weekly.

The germination of *G. integrifolia* in the controlled conditions of the growth room showed a significant increase over time, with the highest mean germination occurring after 51 weeks (Table 6.2; Figure 6.3).

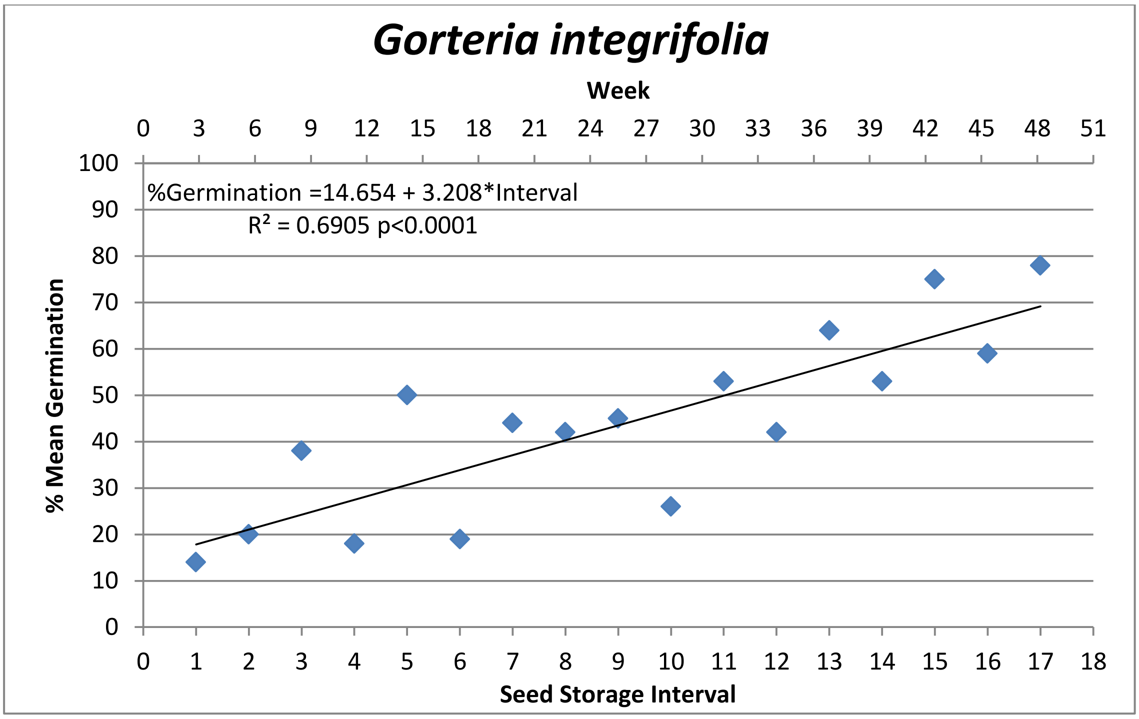

Figure 5.3: Mean percentage germination over storage time of *G. integrifolia* seeds in a temperature and light controlled growth room. Each interval represents a three week period during which newly germinated seeds were recorded weekly.

There is no association between the mean germination of *O. sinuatum* seeds and the storage time of one year (Table 5.2), however, there was a slight, but non-significant increase in germination over time (Figure 5.4).

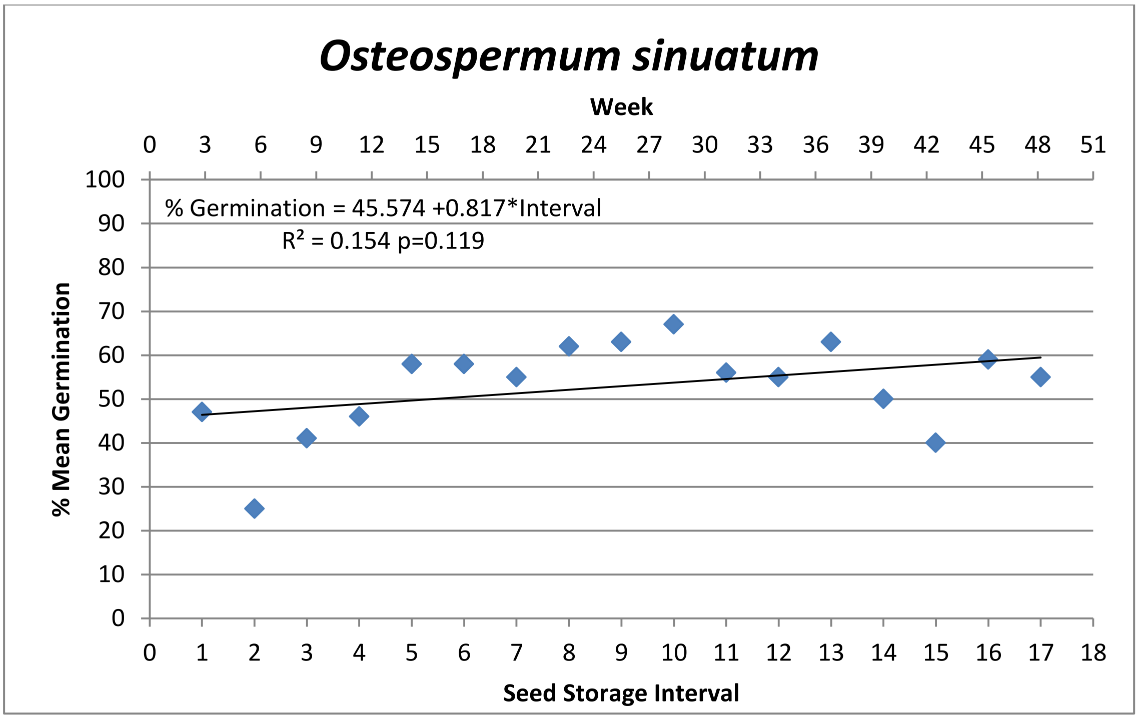

Figure 5.4: Mean percentage germination over storage time of *O. sinuatum* seeds in a temperature and light controlled growth room. Each interval represents a three week period during which newly germinated seeds were recorded weekly.

Discussion

Seeds of *O. sinuatum* have often been used for germination trails or sown with the intent to introduce a palatable species to a degraded rangeland (Barnard 1987; Milton, 1994; Saayman *et al.* 2017). One of the reasons this species is so popular among restoration practitioners is the relative ease with which its seeds will germinate. The results of this study suggest that *O. sinuatum* seeds react in a similarly opportunistic way to established *O. sinuatum* plants. These plants can endure long dry spells and then respond quickly to rain with a flush of new leaves and flowers (Milton 1992; Le Roux *et al.* 1994), rather than having a defined growth and flowering season. Seeds of *O. sinuatum* seem to respond similarly, having little or no dormancy and germinating whenever sufficient moisture becomes available. This strategy makes sense when one considers that the distribution range of *O. sinuatum* includes areas with a variety of rainfall regimes.

The germination strategy of *E. africanus* seems to be similar to that of *O. sinuatum,* with seeds germinating throughout the year and not showing significant signs of seed dormancy. The distribution range of *E. africanus* is also similar to that of *O. sinuatum,* including areas with summer and winter rainfall regimes (Figure 2.2; Figure 2.6).

The distribution ranges of *C. involucratus* and *G. integrifolia* are located in the west coast and the western Little Karoo regions of Southern Africa respectively, regions which have a predominantly winter rainfall regime (Figure 2.4; Figure 2.8). The germination of both these species showed a significant increase over time, which is likely an indication of some form of dormancy, preventing the seeds from germinating soon after ripening. Given that these species, particularly *G. integrifolia,* tend to flower and set seed in spring, after the winter rains (Le Roux *et al.* 1994), it would be advantageous for these seeds to remain dormant throughout the dry summer and to start germinating after the next winter rains. The data from this study suggests that the seeds of these two species continue to germinate more successfully over time, with higher germination occurring after the middle of winter.

Conclusion

Care should be taken to select the best time of year to sow seeds of the four studied species for reseeding purposes. While the seeds of *O. sinuatum* and *E. africanus* are capable of germinating reasonably well throughout the year, the rainfall regime of the area where the reseeding is planned needs to be considered so that the seeds will receive enough moisture to germinate and for seedlings to establish.

The majority of the seeds of both *C. involuctratus* and *G. integrifolia* were found to be dormant during the first six months after harvesting. Sowing the seeds of these species in the field during the autumn or early winter directly after harvest will likely provide poor germination results. The seedlings that do germinate in this scenario will have the advantage of being exposed to the whole rainy season and thus may be well established and able to survive the subsequent dry summer.

Alternatively, these seeds can be kept in storage and sown during the latter part of winter or early spring. In this scenario a higher proportion of the seeds will germinate and the seedlings won’t have to survive the coldest winter months, however, the seedlings won’t have as much time to establish and grow before the onset of the dry summer.

It may also be prudent to store these seeds and sow them after 18 months, however further study may be needed to determine seed longevity before this can be advised.

Chapter 6: The effect of planting depth on seed germination

Introduction

It has been demonstrated that viable seeds in Karoo rangelands occur only in a relatively thin layer of the topsoil, to a depth of approximately 40 mm from the soil surface (Esler 1993; Dreber 2010). Bittencourt and others (2017) also found that the seeds of *Eragrostis plana* show no emergence from planting depths of 40 mm or greater. It would therefore be inadvisable to attempt restoration of degraded areas by ploughing and seeding in deep furrows. Barnard (1987) found that Karoo seeds of various shrubs and grasses germinated better at depths of 10 mm and 20 mm than on the soil surface. The poor germination of surface planted seeds may be due to seeds being dislodged by wind or rainfall, or due to rapid desiccation of seeds after watering (Van Breda 1939; James *et al.* 2011). Attempts to introduce palatable plants to denuded lands through seeding alone have provided poor results compared with seeding in combination with tilling or brush-packing (Saayman & Botha 2010).

Wind-dispersed seeds become trapped underneath shrubs or debris where they are exposed to a suitable micro-climate and are then able to successfully germinate (Milton and Dean 1990; Saayman & Botha 2010). It may thus be necessary to prevent sown seeds from being dislodged to facilitate better germination. This study will test the hypothesis that planting seeds at a depth of 10 mm provides a significant advantage in terms of seedling emergence compared to surface planting where seeds are covered with only enough substrate to prevent them from being dislodged. The results of this study could provide insights that improve the success of restoration projects reliant on reseeding and inform restoration projects on the need for soil cultivation to allow germination.

Methods

The effect of planting depth on the germination of the four species of palatable Karoo plants described in Chapter 2 was tested at the nursery of the Worcester Veld Reserve. The seeds were harvested from the Worcester Veld Reserve and then air dried for four weeks in a drying shed as a treatment to reduce seed moisture content, with the intention to remove seed dormancy and to prolong shelf life by limiting bacterial or fungal growth (Commander *et al.* 2009a; Probert *et al.* 2009). The dried seeds were then stored at room temperature in brown paper bags (Mallik & Karim 2008; Van der Walt & Witkowski 2017).

Seeds were visually screened and only intact, plump seeds were used for this trial (Mallik & Karim 2008; Saaed *et al.* 2018). Five replicates of 20 seeds of each species were sown onto the substrate surface, while a further five replicates of 20 seeds were planted at 10 mm depth. Each replicate consisted of a seed tray filled with sterilized sand and planted with 20 seeds. The sand which was used as a growth medium was sterilized by heat treating for 7 days at 70°C in order to remove any foreign seeds from potentially contaminating the sand prior to seeding with the trial species. Seeds sown on the surface were partially covered with a thin layer of sand less than 1 mm deep. This was intended to address a problem encountered by Barnard (1987) whereby surface sown seeds were inadvertently moved when watered. This thin layer of sand was intended to prevent the seed from being dislodged by wind or by the impact of dripping water. The replicates were arranged in a completely randomized design. Seed trays were placed on racks in a nursery that is covered by 50% shade cloth to exclude birds and contamination from wind-blown seeds, as well as to protect the replicates from desiccation due to excessive wind or exposure to sunlight. The trays were watered with tap water twice daily for 5 minutes by an automated sprinkler system. This approach is based on that followed by Esler (1993) and Milton and Dean (2001).

Emergent seedlings in each replicate were recorded and removed weekly. Germination trials were repeated over time at intervals of three weeks for a total of 17 intervals.

The germination data was expressed as percentages out of a total of 20 seeds to convert binary to continuous data for analysis of variance (ANOVA) (Snedecor and Cochran 1967). Levene's test for homogeneity of variance showed heterogeneous species variances (Levene 1960). Shapiro-Wilk test confirmed normality of the standardized residuals (Shapiro and Wilk 1965). The data were subjected to weighted ANOVA using General Linear Models Procedure (PROC GLM) of SAS software (Version 9.4; SAS Institute Inc, Cary, USA). The weight was the reciprocal of the species variances (1/MSE). Fisher's least significant difference was calculated at the 5% level to compare treatment means (Ott and Longnecker 2001).

Results

Using the data of all the plant species combined, the germination of surface planted seed was significantly higher than the germination of seed planted to a depth of 10 mm (Table 6.1).

Table 6.1: Mean germination percentage of seeds planted on the substrate surface compared to seeds planted at 10 mm depth. Mean values with different superscripts indicate significant differences ($p<0.001$). The sample size (N) refers to the number of replicates of 20 seeds each.

Treatment	Mean germination %	Standard deviation	Sample size (N)	Total seed sown
Surface planting	28.21^{a}	2.32	340	6800
10 mm planting	21.33^{b}	2.13	340	6800

When analyzing the data of all the plant species separately, the germination of surface planted seed was significantly higher than the germination of seed planted at a depth of 10 mm for all species tested (Table 6.2).

Table 6.2: A comparison of the mean germination of trial species planted at the substrate surface and at 10 mm depth. Within a species, mean values with different superscripts indicate significant differences ($p<0.001$). The sample size (N) refers to the number of replicates of 20 seeds each.

Species	Planting depth	Mean germination	Standard deviation	Sample size (N)	Total seeds sown
Chaetobromus involucratus	Surface	28.12^{a}	2.49	85	1700
	10 mm	24.82^{b}	2.35	85	1700
Eriocephalus africanus	Surface	16.59^{a}	1.21	85	1700
	10 mm	10.94^{b}	1.14	85	1700
Gorteria integrifolia	Surface	26.47^{a}	1.74	85	1700
	10 mm	19.18^{b}	1.72	85	1700
Osteospermum sinuatum	Surface	58.65^{a}	1.71	85	1700
	10 mm	43.53^{b}	1.91	85	1700

Discussion

The combined emergence of seedlings planted at 10 mm depth was significantly lower than that of the seedlings planted on the substrate surface. This result is contrary to what was found by Merino-Martín *et al.* (2017), when testing several Australian *Acacia* species, however, these seeds were sown on an exposed mine rehabilitation site, and were likely to be exposed to extreme temperature conditions and desiccation. The results of this study are also contrary to the findings of Barnard (1987), who tested the seeds of a range of palatable karoo species, including *O. sinuatum,* and found that they generally achieved higher germination success when planted at 20 mm depth than at the soil surface. The thin layer of sand used to keep the seeds in place in this study likely played a significant role in keeping the seeds from being dislodged by water, since the seedlings emerged from the same positions from where they were planted. This stabilisation of the seed was likely the reason for the increased germination success compared to the results of Barnard (1987). A stabilisation treatment for seeds in the form of seed coatings or agglomerating the seeds into pellets has been shown to improve emergence (Madsen *et al.* 2012). Pioneering work on coatings for seeds, including *Gorteria integrifolia* and *Eriocephalus africanus*, was carried out at the Worcester Veld reserve (Van Breda 1939). Coatings protected the seeds from being washed or blown away and from predation.

The results of this study would suggest a similar approach to reseeding as is prescribed by Saayman (2013), who suggests that the soil surface should be broken before sowing, but that the seed should not be covered or buried in the soil. It should be noted that suitable cultivation of soils may be required to address soil compaction and to create microsites for successful seedling establishment (Van der Merwe & Kellner 1998; Witbooi & Esler 2004; Matthee 2015).

While the germination of surface sown seeds of all species studied were significantly higher than that of seeds sown at 10 mm depth, the recorded number of emerging *C. involucratus* seeds from 10 mm depth was likely in error, due to the way the seedlings were removed after being recorded. The emerging seedlings were pulled from the substrate by hand. This often caused the seedlings to break, sometimes leaving the roots intact in the substrate. The seedlings of the Asteraceous shrubs would likely not survive this, since their apical meristem would be removed. It is possible though that the seedlings of the grass *C. involucratus* could resprout and thus be counted twice. A future study might devise a way to remove the entire seedling without it breaking and disturbing the adjacent seedlings.

Conclusion

The relatively low emergence of seed planted at 10 mm depth compared to the emergence of lightly-covered, surface planted seeds found in this study is similar to the results of other studies showing the low number of viable seeds and seedling emergence from deeper substrate depths. This suggests that soil preparation for reseeding projects in karoo rangelands should not attempt to bury seeds deeply, but rather to provide just enough of a stabilisation effect to ensure the sown seeds are not dislodged by wind or rainfall.

Chapter 7: Synthesis and Conclusions

Summary of findings

Landowners and managers require access to large quantities of good quality, viable seeds in order to achieve success with the reseeding of degraded rangelands (Merrit & Kingsley 2011). Obtaining such seeds can be difficult and time consuming (Esler *et al.* 2006), therefore seed production ventures can provide a valuable product to enable restoration of degraded lands. Various different methods have been tested to determine how such degraded lands should be prepared and sown in order to achieve the best germination and establishment of desired plant species. While many such restoration projects have provided disappointing results, they have produced valuable lessons and have highlighted the need for further research into this field.

Instead of looking into further restoration methodologies, this study has focused on the seeds themselves in order to develop procedures that will allow a favourable starting point for restoration projects, namely preparing and sowing seeds at the correct age and depth in order to achieve the best germination results. The species tested in this study are shown to differ in terms of seed viability and the germination of seeds over time.

The viability of *E. africanus* seeds is significantly lower than that of *C. involucratus, G. integrifolia* and *O. sinuatum.* Each of these species also had differing causes of seed mortality. The germination of *C. involucratus* and *G. integrifolia* was shown to increase over the storage time of one year, while the germination of *O. sinuatum* and *E. africanus* did not increase significantly over the same time period.

The drying of seeds after harvesting is shown to significantly improve the germination of only one of the study species namely *O. sinuatum.* Nevertheless, the drying of all species has the beneficial effect of placing the seeds in a state of enforced dormancy. The drying treatment also extends the shelf life of stored seeds by preventing decay due to fungal growth. It should thus be recommended that a seed drying treatment be applied to the species examined in this study. Freshly harvested seeds at the Worcester Veld Reserve are dried by spreading the seeds on a drying floor in a storeroom where there is sufficient heat and airflow to allow drying.

The seeds are dried for eight weeks before being cleaned of debris and placed in bags for storage.

When sowing the seeds of the species investigated in this study, care should be taken that the seeds are not buried. A thin layer of soil that partially covers the seeds and prevents them from being dislodged by rainfall or wind is beneficial and sufficient to allow germination, however, seeds sown and subsequently buried will provide poorer germination results.

Limitations of the study

The seeds collected for this study were all sourced from the seed orchards of the Worcester Veld Reserve. The seeds were sourced here since they were readily available in sufficient quantities to run the germination trials described in chapters 4, 5 and 6. The single source of seeds would limit the applicability of the results, since seeds were not sourced from different populations of the study species. The results may thus not be representative of other populations or of the entirety of the study species.

The seeds may also be affected by the weather conditions specific to the year during which they were harvested. The availability and timing of sufficient rainfall and the suitability of conditions for pollination, abundance of seed predators and harvesting of seeds could affect seed viability (Milton 1995b).

Only four species were selected for this study. There is a wide variety of other species that could show potential for reseeding restoration projects and which could be subjected to similar germination and viability testing as was done in this study. Once again the availability of seeds was a limitation of the number of species that could be included.

Drying of the seeds used in this study was done in a shed where the temperature and humidity could not be directly regulated and was determined by ambient conditions. These ambient conditions would vary between years and the drying treatment would thus be difficult to replicate.

The repeated germination tests described in chapter 5 were done over a period of one year, with the expectation that a peak in germination would be reached for each species within that year. This was assumed since the literature reports that perennial, palatable species tend to have seeds with little or low dormancy and that their seeds do not persist in the seedbank for much longer than a year (Esler 1993; Carrick & Krüger 2007). Nevertheless, when exploring the data with polynomial regressions prior to a formal statistical analysis, it became obvious that no peaks in germination occurred. The data were therefore analysed only to see if linear relationships existed between seed storage time and seed germination. It may be possible that the one year study period during which the seeds were tested for germination was in actual fact too short to reach a point of peak germination. This implies that a longer period of testing would be required in order to determine the point at which the seeds start declining in viability due to ageing. This would allow a "shelf life" to be determined for each of the study species.

Possibilities for future research

The limitations of the study present several possible changes that could be applied to a similar future project. The germination of the study species of this project, or other species selections, could be tested over a longer period of time to determine when viability is affected by seed age.

A future study may include a wider range of species, perhaps a range of species that are not selected based on palatability, but rather on ease of seedling establishment and facilitation of further colonisation by other native species.

The data collected in this study suggests that there may be a correlation between ambient temperature and seedling emergence (Appendix 1). A future study may investigate the effects of storage time and soil temperature on the emergence of seedlings intended for rangeland rehabilitation.

The relatively successful germination of seeds partially covered by a thin layer of soil as opposed to being buried or simply sown on the seed surface suggests that seed coating treatments for Karoo reseeding projects may warrant further research.

Finally, the germination trials in this study were done in the relatively controlled conditions of a growth room and a shaded nursery. A future study with a similar design may attempt to test the germination of seedlings in a field setting over time.

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APPENDIX 1: Germination of *C. involucratus* planted in a nursery at the Worcester Veld Reserve over time compared with average monthly temperatures. (Climate-Data.org 2018).