NATIVE LEGUME SPECIES FROM THE NORTHERN CAPE PROVINCE OF SOUTH AFRICA AND THEIR POTENTIAL USE AS FORAGE CROPS.

By

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DECLARATION

I declare that “***NATIVE LEGUME SPECIES FROM THE NORTHERN CAPE PROVINCE OF SOUTH AFRICA AND THEIR POTENTIAL USE AS FORAGE CROPS***” is my own work, and has not been submitted for any degree or examination in any other University and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

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ABSTRACT

The lack of bioclimatically suitable forage species for livestock production in the water-limited agro-ecological areas of South Africa has resulted in significant feed shortages within these areas during the ongoing drought experienced within the country. This, in turn, has resulted in significant livestock mortalities leading to financial difficulties for the farmers and farming communities within these areas. Thus, many of the water-limited agro-ecological areas in South Africa have been declared disaster areas. These cyclic long-term droughts, as well as more common short-term droughts are expected to increase in frequency, duration and intensity under the predicted future bioclimatic conditions. Although there has been significant investment into the development of improved, better-adapted forage crops for these bioclimatically marginal agro-ecosystems, these efforts, to date, have largely been unsuccessful. Therefore, in this study, we propose to identify and evaluate species that are native to the water-limited South African agro-ecosystems and that can potentially be implemented in alternative fodder flow programs within these water-limited agro-ecological areas.

To do this, the study aimed to: 1. Screen for and prioritize various native legume species with fodder potential for use in dryland agricultural systems. 2. Determine the suitability of a selected legume species as a potential new fodder crop under the predicted future bioclimatic conditions. 3. Determine the requirements for dormancy breaking and successful seed germination of the selected native legume species. 4. Determine the ability of seeds of the selected native legume species to germinate and establish under reduced water availability, high temperatures and increased seed burial depths. 5. Determine the effects of reduced water availability on the establishment, growth and physiology of the selected legume species.

Using a combination of ecological niche modelling techniques, plant functional traits, and indigenous knowledge, 18 perennial, herbaceous or stem-woody legume species were prioritized for further evaluation as potential fodder species within water-limited agricultural areas in South Africa. From these species, *Calobota sericea* was selected for further characterization due to its known significant contribution to livestock diets within the semi-arid rangelands of Namaqualand. After selection, the adaptability of *C. sericea* to future bioclimatic conditions was evaluated using ecological niche models under various climate change scenarios. It was found that *C. sericea* will have a limited reduction in its distribution range of less than 2 % of its current distribution. This loss in the already limited adapted range of *C. sericea* will results in the potential loss of approximately 5 % of the existing populations. Shifts in the different adaptation zones under future bioclimatic conditions is predicted to result in further loss of populations, as current populations will have to deal with more marginal future bioclimatic conditions. It is therefore suggested that special attention should be given to the collection of genetic resources from populations that are currently located within the different adaptation zones of the ecological niche of *C. sericea* to conserve as much of the genetic variability within the species. These genetic resources will likely be the key to successfully exploit the potential of C. sericea as a fodder crop under future bioclimatic conditions.

Seeds of *C. sericea* were then collected from native populations from the Namaqualand rangelands, and success of different dormancy breaking treatments were evaluated after determining that seed germination was constrained by dormancy. Mechanical scarification using an abrasive sandpaper was found to be the most effective method to break dormancy. It was found that once the dormancy was removed, germination commenced rapidly and uniformly. However, it was highlighted that further research is needed to determine more efficient means to scarifying larger quantities of seeds for commercial applications. Once dormancy could successfully be removed, the germination requirements of *C. sericea* at different temperatures and water availabilities were determined. Seeds of *C. sericea* were found to germinate best at temperatures ranging between 10 and 20 °C, but still had a germination percentage greater than 80 % at 5 °C. The seeds were also found to require a water potential of not lower than – 0.3 MPa to reach a germination percentage of at least 60 %, below which germination was severely reduced. Thereafter, the optimum planting depth for optimum seedling emergence was investigated by planting seeds of *C. sericea* at depths of up to 5 cm at 1 cm increments. Seedling emergence was found to be highest at burial depths of 2 cm to 4 cm. A decrease in shoot height and a concomitant increase in seedling mass was observed with increasing seed burial depth. From these studies, it was concluded that

C. sericea seeds should be planted between 2 and 3 cm deep, early in winter, when temperatures are lower, and rainfall more prevalent and less erratic.

After determining the requirements for seed germination and seedling establishment, we examined the growth and recovery characteristics of *C. sericea* under controlled water-limitation and subsequent re-watering, imposed on the plants at different ages. Results indicate that *C. sericea* plants have a wide range of morphological and physiological measures that enable them to cope with water-limited conditions. Resource allocation to the roots of water-limited plants, as well as reduced stomatal conductance and transpiration rate were early responses to water-limitation, irrespective of the age at which water-limitation was imposed, or the duration of water-limitation. Increased production of protective pigments such as carotenoids and anthocyanins was also observed. After re-watering, it was found that generally, all negative impacts of water-limitation on morphology and physiology recovered and some even returned to well-watered levels, suggesting that *C. sericea* plants are well adapted to areas with rainfall variability.

The promising preliminary results obtained in this study verify the potential of *C. sericea* as a species that can survive significant degrees of water-limitation and can recover rapidly once the drought stress has been relieved. The potential of this species is also attributed to its wide range of adaptive responses to drought and its potential for expanding its agronomic use into novel agro-ecosystems. This makes it a good candidate for inclusion into breeding programs for the development of a fodder crop for semi-arid and arid water-limited areas.

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F.L. Müller, M. Trytsman, L.M. Raitt, S.B.M. Chimphango., M.I. Samuels, C.F. Cupido, J.S. Boatwright. 2019. The impacts of climate change on the agronomic potential of *Calobota sericea.* 54th Annual Congress of the Grassland Society of Southern Africa. 1 – 4 July 2019. Upington, Northern Cape, South Africa. https://grassland.org.za/gssac54-programme-20190626-final.pdf

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CHAPTER 1

General Introduction

1.1. Rationale and Motivation

With the global human population estimated to reach nine billion by the year 2050, it is estimated that approximately 70 – 80 % more food will be required in 2050 than in 2000 (Bruinsma 2009; Herrero *et al.* 2009; 2015; Godfray *et al.* 2010; Nardone *et al.* 2010; Wright *et al.* 2011; Alexandratos and Bruinsma 2012; Rao *et al.* 2015). With this increase in the demand for food products, it is expected that the demand for livestock products (meat, milk and eggs) will double by 2050, with the largest increases in demand occurring in the developing countries (Delgado *et al.* 2001; Herrero *et al.* 2009; Rao *et al.* 2015). This rapid and global increase in the demand for livestock products has been termed 'the livestock revolution' (Delgado *et al.* 1999; Wright *et al.* 2011).

Livestock production is the largest land use system on earth. It occupies approximately 45 % of the global surface area, primarily in the form of pastoral systems (both intensive and extensive) (de Fraiture *et al.* 2007; Reid *et al.* 2008; Herrero *et al.* 2009; Rao *et al.* 2015). Taking into account the rapid increase in the human population, and the subsequent increase in the demand for livestock products, it has become important to find ways to increase livestock production. This, however, needs to be done in a manner that does not put unsustainable pressure on the already diminishing natural resources such as fossil fuels, mineral nutrients (mined for chemical fertilizers), and indigenous forests and rangelands (Wright *et al.* 2011; Herrero *et al.* 2015). Considering that the livestock industry is only one of many sectors that will need to grow substantially in the near future, significant trade-offs in the sustainable use of these natural resources can be expected. These trade-offs, along with the expected global climatic changes, will not only have global consequences, but also local impacts on livelihoods and the environment (Morton 2007; Herrero *et al.* 2009; 2015; Thornton *et al.* 2009; Silvestri *et al.* 2012).

Through its effects on livestock and livestock production systems, climate change can significantly influence the health, food security and livelihoods of various vulnerable populations. This is primarily due to the projected declines in agricultural production in these countries that will affect both food availability and access (IPCC 2007; Rufino *et al.*

2013). This is said to occur primarily as a result of changes in biodiversity, water availability and quality, forage availability and quality, and the subsequent decrease in animal health (Luseno *et al.* 2003; McPeak 2006; Thornton *et al.* 2007; 2009; Silvestri *et al.* 2012, Thornton and Herrero 2014). Countries in Sub-Saharan Africa are particularly vulnerable to climate change impacts as they are affected by widespread poverty which further limits their adaptive capacity to climate change. However, these countries have the largest potential to increase their livestock production systems in a sustainable manner due to their abundance of natural resources (Silvestri *et al.* 2012).

Just like in other developing countries (Thornton and Herrero 2014), livestock farming in South Africa is an important contributor to food security, poverty alleviation and socio-economic upliftment (Meissner *et al.* 2013a). Approximately 38 500 commercial farmers and an estimated 2 million emerging and communal farmers contribute to livestock production in South Africa (Meissner *et al.* 2013a). The gross value of livestock products in South Africa has increased from 42 % to 47 %, primarily due to the South African middle-class population increasing in the last 10 years resulting in an increased demand for livestock products (Meissner *et al.* 2013a, b). However, with the South African human population rapidly increasing, the demand for livestock products are becoming more than what can be sustainably produced under current livestock production systems. This is believed to further deteriorate with the projected climatic changes, which predict hotter and drier conditions with more erratic and unpredictable rainfall events (Mukheibir 2008).

According to the United Nations Council on Combatting Desertification (UNCCD), approximately 80 % of South Africa's land surface is classified as semi-arid to arid (Palmer and Ainslie 2006). Approximately 82 % of these areas are used for agricultural activities of which only 14 % receive sufficient rainfall for arable crop production. The remainder of the agricultural land is used for extensive livestock production, forestry and wildlife/nature conservation (Palmer and Ainslie 2006; Jordaan *et al.* 2013). Under these semi-arid and arid conditions, the most extensive agricultural activities are livestock (sheep, goats, cattle and ostriches) farming under rangeland conditions where livestock make use of the natural veld (Jordaan *et al.* 2013). However, along with low annual precipitation, these semi-arid and arid rangelands are, in many instances, also subjected to recurrent droughts, cyclic long-term droughts, extreme temperatures and marginal edaphic conditions (Jordaan *et al.* 2013). During these dry periods, livestock production in these rangelands is often severely reduced due to the lack of adequate good quality, nutritious forage available to the livestock (Palmer and Ainslie 2006; Jordaan *et al.* 2013, Müller *et al.* 2019). In certain areas where rainfall is less erratic, dryland fodder in the form of low input cereal crops are grown on cleared patches within the rangelands, where crop residues make an important contribution to livestock diets on both commercial and communal farming areas, especially during dry periods (Palmer and Ainslie 2006). In general, however, the nutritional quality of these crop residues is poor, mainly due to its low digestibility, protein and available carbohydrate content (Brand *et al.* 2000; Brundyn *et al.* 2005). In many instances, in these dry communal rangeland areas, these croplands are left uncultivated and unmanaged due to the costs involved in maintaining them, as well as a lack of forage species suitable for these marginal agro-ecological conditions. An example of this can be found in the Leliefontein communal rangelands of Namaqualand in the Northern Cape province of South Africa.

Within the agro-pastoral areas of the Leliefontein communal area, approximately 12 % of the communal rangelands has been demarcated for cultivating crops and forages. This equates to approximately 23 049.8 ha of land that has been divided into 559 sowing plots (Samuels 2013). Through the years, these sowing plots have been left uncultivated, unmanaged and fallow, primarily due to cereal crops regularly failing as a result of the increased variability in rainfall and uncertainty regarding the onset of the rainy season within these areas. This in turn, has resulted in only 3741.7 ha (or 16.2%) of available croplands being used per annum (Samuels 2013). When these sowing plots are left uncultivated and unmanaged for prolonged periods, it has been shown that various non-palatable plant species such as *Galenia africana* L. (kraalbos) and *Dicerothamnus rhinocerotis* (L.f.) Koekemoer (renosterbos) occupy these spaces. This has resulted in serious consequences for livestock production within these communal areas as many animals succumb to hunger, particularly during dry periods. This, in turn, has had negative economic impacts on these rural communities, which are often found to be food insecure, and live well below the poverty line (Ntombela 2017).

One of the ways to try and improve livestock production within these water-limited areas is to implement better fodder flow programs. This can be done on these uncultivated and unmanaged old croplands using fodder crops that are nutritionally superior to the low input cereal crops, as well as species that are naturally better adapted to the climatic and edaphic conditions experienced within these areas. Currently, there are only a few commercially available fodder species such as *Opuntia ficus-indica* (spineless cactus) or *Atriplex numularia* (old man saltbush) that is suitable for these dry conditions (Dickinson *et al.* 2010). Also, these commercially available forage species are usually exotic germplasm, reducing their potential in certain areas due to the risk of becoming weedy and/or invasive e.g. cactus pear fodders. Furthermore, past attempts to introduce various exotic forage species into these water-limited agro-ecosystems in South Africa have failed, often to the detriment of the farmers who have participated in these trials, where failure resulted in reduced production and increased livestock deaths due to a lack of forage during marginal times (per communication with the South African red meat industry (2017) and local farmers). An alternative to the use of exotic germplasm is to use indigenous species, specifically leguminous species, which are naturally adapted to these climatic and edaphic conditions.

South Africa houses a large diversity of indigenous legume species with diverse growth forms, including creepers, climbers, herbs, dwarf shrubs, shrubs and trees, and are found in a wide range of environments, ranging from fertile soils, to poor soils, and areas receiving large amounts of rainfall, to areas that are regularly drought stricken (Trytsman *et al.* 2011, 2016, 2019). However, even with this large diversity, very little information exists regarding the forage potential of native leguminous plant species from South Africa (Trytsman *et al.* 2011, 2016, 2019). This, in turn, has resulted in very little efforts to domesticate and develop native South African legume species, resulting in the South African National Forage Genebank housing less than 5 % of native legume germplasm that can be evaluated for their forage potential (Trytsman 2013, Trytsman *et al.* 2019).

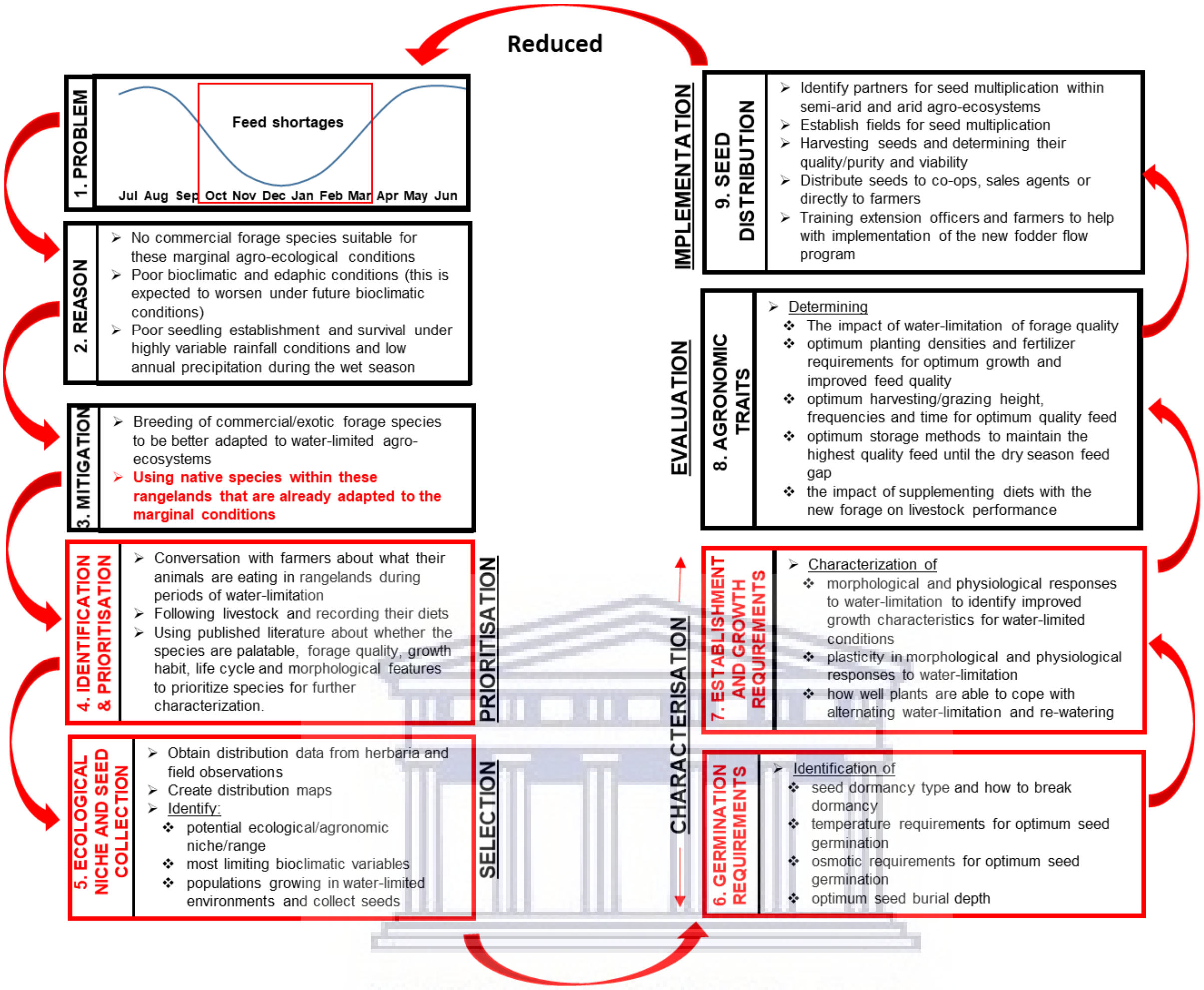
The lack of germplasm accessibility, and a general lack of basic information regarding the characteristics of native leguminous species that would merit their inclusion in forage breeding and improvement programs, has resulted in the decline in the domestication and development of native legume species in South Africa. As a result, in South Africa, there has been a significant investment into the development of introduced forage species that can withstand the marginal bioclimatic conditions in the country. These introduced species however, have put a limit on the production potential of various drylands due to the fact that many (if not all) of these introduced species are not well adapted to the dry agro-ecological conditions in most parts of South Africa (Truter *et al.* 2015, Müller *et al.* 2017, Trytsman *et al.* 2019). Although the development of improved cultivars for these marginal agro-ecological areas is a continuous effort, the time taken to develop an adapted cultivar of exotic germplasm through conventional breeding practices (selective breeding and cross hybridization) is extremely long and can take several years to complete. These constraints, along with others, have resulted in significant feed shortages in most arid and semi-arid livestock production areas throughout South Africa, with numerous dry agro-ecological livestock production areas in South Africa recently being declared as disaster areas due to the current (2015-2020) drought being experienced within the country.

In order to effectively improve livestock production, and subsequently the socio-economic standing of communities, which lie within these drought-stricken areas, it is imperative to develop better fodder flow programs for these marginal agro-ecological areas. This can be done by identifying specific native legume species, already well-adapted to these areas, which can be characterized further and evaluated for their production potential within these areas, and thereafter be included into fodder flow programs. Native species that are naturally occurring within these marginal agro-ecological areas would be a more sensible alternative to the development of exotic germplasm for these areas because they are naturally better adapted to the climatic and edaphic conditions of these areas than the exotic germplasm.

1.2. Research Aims and Objectives

A theoretical representation of the steps that are needed to identify, characterize, evaluate and implement indigenous species into water-limited agro-ecosystems, and the desired impacts it will have for fodder flow in these water-limited areas is given below (Fig. 1.1), with the specific steps done in this thesis highlighted in red. Instead of focusing on the entire forage development cycle this study only focused on certain sections of this process, with the overall aims of this study being to identify native South African legume species adapted to water-limited conditions. Thereafter, the aim was to characterize specific legume species that were selected for their ability to deal with water-limited conditions. To do this, the following specific objectives were pursued:

1. To screen for and select various legume species with fodder potential for use in dryland agricultural systems in South Africa using plant functional traits and ecological niche modelling techniques.
2. To determine the suitability of the selected legume species as potential new fodder crops under the predicted future bioclimatic conditions of South Africa.
3. To determine the requirements for dormancy breaking and successful seed germination of the selected native legume species.
4. To determine the ability of seeds of the selected native legume species to germinate and establish under reduced water availability, high temperatures and increased seed burial depths.
5. To determine the effects of reduced water availability on the establishment, growth and physiology of the selected legume species.



2. REASON

- No commercial forage species suitable for these marginal agro-ecological conditions
- Poor bioclimatic and edaphic conditions (this is expected to worsen under future bioclimatic conditions)
- Poor seedling establishment and survival under highly variable rainfall conditions and low annual precipitation during the wet season

3. MITIGATION

- Breeding of commercial/exotic forage species to be better adapted to water-limited agro-ecosystems
- **Using native species within these rangelands that are already adapted to the marginal conditions**

4. IDENTIFICATION & PRIORITISATION

- Conversation with farmers about what their animals are eating in rangelands during periods of water-limitation
- Following livestock and recording their diets
- Using published literature about whether the species are palatable, forage quality, growth habit, life cycle and morphological features to prioritize species for further characterization.

5. ECOLOGICAL NICHE AND SEED COLLECTION

- Obtain distribution data from herbaria and field observations
- Create distribution maps
- Identify:
  - potential ecological/agronomic niche/range
  - most limiting bioclimatic variables
  - populations growing in water-limited environments and collect seeds

6. GERMINATION REQUIREMENTS

- Identification of
  - seed dormancy type and how to break dormancy
  - temperature requirements for optimum seed germination
  - osmotic requirements for optimum seed germination
  - optimum seed burial depth

7. ESTABLISHMENT AND GROWTH REQUIREMENTS

- Characterization of
  - morphological and physiological responses to water-limitation to identify improved growth characteristics for water-limited conditions
  - plasticity in morphological and physiological responses to water-limitation
  - how well plants are able to cope with alternating water-limitation and re-watering

8. AGRONOMIC TRAITS

- Determining
  - The impact of water-limitation of forage quality
  - optimum planting densities and fertilizer requirements for optimum growth and improved feed quality
  - optimum harvesting/grazing height, frequencies and time for optimum quality feed
  - optimum storage methods to maintain the highest quality feed until the dry season feed gap
  - the impact of supplementing diets with the new forage on livestock performance

9. SEED DISTRIBUTION

- Identify partners for seed multiplication within semi-arid and arid agro-ecosystems
- Establish fields for seed multiplication
- Harvesting seeds and determining their quality/purity and viability
- Distribute seeds to co-ops, sales agents or directly to farmers
- Training extension officers and farmers to help with implementation of the new fodder flow program

Figure 1.1. A theoretical representation of the steps needed to identify, characterize, evaluate and implement indigenous species into water-limited agro-ecosystems, and the desired impacts it will have for fodder flow in these areas.

1.3. Research Questions

1. What are the priority legume species that should be evaluated for their pasture and/or fodder potential in water-limited agro-ecosystems in South Africa?
2. How will climate change influence the distribution and agronomic potential of the selected legume species prioritized for further evaluation as potential alternative fodder crops?

3. Do the seeds of the selected legume species with fodder potential display any form of dormancy and if so, what are the patterns of seed dormancy breakdown displayed by these legume species?
4. What are the germination and early seedling growth requirements of the selected legume species under water-limited conditions, after seed dormancy has been broken?
5. What mechanisms of drought tolerance do the selected legumes display?
6. Can the indigenous legume species evaluated in this work be proposed as possible alternative pasture and/or fodder crops for further evaluation for their use under marginal climatic conditions, and what possibilities exist for them in South Africa?

1.4. Thesis layout

This thesis starts (**Chapter 1**) with the rationale and motivation as to the importance of the current study where the research problem is described in a global and national light. Special focus is then given to South African livestock systems in semi-arid and arid areas, and what could be done to improve livestock production within these areas. At the end of the chapter, specific research objectives and questions are provided that will be answered in the later chapters.

Chapter 2 describes the selection and prioritization of native legume species from South Africa that could potentially be used as fodder crops in water-limited agro-ecosystems. The initial selection of legume species was performed based on a list of legume species from southern Africa produced by Trytsman (2013) from the Agricultural Research Council, for further evaluation for their forage potential along with other selection criteria such as the occurrence of the legume species in the Northern Cape province of South Africa. The list was further characterized by evaluating various published sources pertaining to the functional traits of the selected plant species. The new handbook for standardized measurements of plant functional traits worldwide, published by Perez-Harguindeguy *et al.* (2013), was used as a guideline as to what the best traits are to consider. Thereafter, the initially selected legume species were evaluated for their climate and soil adaptation using ecological niche modelling techniques.

Chapter 3 introduces *Calobota sericea*, the native perennial leguminous species selected for further evaluation for its fodder potential in the semi-arid rangelands of Namaqualand in South Africa. The chapter deals with how *C. sericea* will cope with the predicted future bioclimatic conditions in South Africa. In this chapter, MaxEnt software was used to produce suitability maps across South Africa, highlighting areas of suitability for potentially implementing *C. sericea* as a fodder crop. In this chapter, it was also determined whether the distribution of this species will expand or shrink under the predicted future bioclimatic conditions. The results obtained from this chapter informs future collection and breeding priorities which would help with effectively exploiting this species as an alternative fodder resource under the predicted future bioclimatic conditions.

Chapter 4 deals with the requirements for seed dormancy breaking of *C. sericea*. In this chapter, methods to break seed coat dormancy are evaluated. Here, it is reported how dormancy in *C. sericea* can be artificially broken, aiding with rapid seed germination and establishment. This is regarded as one of the most important components of evaluating the suitability of a new forage species to dry agro-ecological areas. Rapid break down of dormancy, and subsequent seed germination and seedling establishment is extremely important for areas which only receive limited amounts of rainfall, and especially in areas where the rainfall season is short.

Chapter 5 deals with the impacts of temperature, osmotic stress, and seed burial depth on the germination and establishment of *C. sericea*. For germination to commence, seeds must imbibe water from the surrounding soil, and there is a base minimum water potential needed for germination. In arid and semi-arid ecosystems, the adverse effects of water constraints on seed germination may be further aggravated by the exposure of seeds to temperatures that are either below or above the optimum germination temperatures, with temperature being the major determinant of germination rate. In this chapter, the base water potential required for seed germination, and the optimum germination temperature ranges for *C. sericea* seed germination were identified.

When all other requirements for seed germination are met, the depth at which seeds are buried becomes one of the most important factors that influence successful seed germination, seedling emergence and the rate of seedling emergence. *Calobota sericea* has very small seeds, and therefore, deeper seed burial depths may be detrimental for seedling establishment. However, under water-limited conditions, and areas where rainfall is highly erratic, deeper seed burial is preferred due to extended periods of moisture and nutrient availability associated with deeper seed burial. In this chapter the optimum seed burial depths of *C. sericea* was identified by looking at the success of seedling establishment from different seed burial depths, as well as the seedling characteristics after establishment from the different burial depths.

Chapter 6 deals with the growth, development and persistence of *C. sericea* under different levels of water-limitation, at different ages. To evaluate the plant tolerance to water-limitation, plant functional traits including root, stem and shoot growth, resource allocation, plant water status, and dry matter production was evaluated. Apart from morphological characterization, in this chapter, the plant's photosynthetic responses to water-limitation were also quantified. Here it was evident that *C. sericea* displays phenotypic plasticity under water-limitation. It was found that the plants could adjust growth and photosynthetic responses to deal with the stress conditions and have the ability to rapidly recover once the stress condition subsides. This is done through a combination of resource allocation to improve root production, and strict stomatal control, which results in improved water-use efficiency under water-limited conditions.

Chapter 7 concludes this thesis with a general discussion, conclusion and recommendations for further evaluation. It summarizes the findings of the previous chapters into a broader perspective, but also highlights the shortcomings of this work and provides recommendations for future research activities.

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CHAPTER 2

Prioritisation of native legume species for further evaluation as potential forage crops in water-limited agricultural systems in South Africa

2.1. Abstract

In the face of climate change, identification of forage species suitable for dryland farming under low rainfall conditions in South Africa is needed. Currently, there are only a limited number of forage species suitable for dryland farming under such conditions. The objective of this study was to identify and prioritize native legume species that could potentially be used in water-limited agro-ecosystems in South Africa. Using a combination of ecological niche modelling techniques, plant functional traits, and indigenous knowledge, 18 perennial, herbaceous and stem-woody legume species were prioritized for further evaluation as potential fodder species within water-limited and marginal edaphic agricultural areas. These species should be evaluated further for their forage quality, and their ability to survive and produce sufficient biomass under water-limitation and poor edaphic conditions.

Key Words: arid environments; Fabaceae; ecological niche models; perennial forage species; South African native legumes

2.2. Introduction

With the rapidly growing human population, and the severity of the projected future bioclimatic scenarios, significant trade-offs in the sustainable use of natural resources can be expected to meet the future demands for livestock products. These trade-offs could have significant negative impacts on the health, food security and livelihoods of various vulnerable populations (Luseno *et al.* 2003; McPeak 2006; Morton 2007; Thornton *et al.* 2007; 2009; Herrero *et al.* 2009; 2015; Silvestri *et al.* 2012; Thornton and Herrero 2014). Generally, in South Africa, the projected climate change scenarios indicate a trend of becoming hotter and drier (Mukheibir 2008; Meissner *et al.* 2013a; b), and this, in turn, is expected to result in declines in agricultural production that will affect both food availability and access (IPCC 2007; Rufino *et al.* 2013). In South Africa, approximately 20 % of the country is already receiving less than 200 mm of rainfall per annum, and a further 47 % receives less than 400 mm of rainfall per annum (DEA 2013). Furthermore, future climate change projections for South Africa are generally indicating that the current unpredictability and high variability in the amount and distribution of rainfall, will likely increase with climate change, and this, in turn, will be further aggravated with the predicted increases in temperatures under future bioclimatic conditions (DEA 2013).

Current commercial livestock systems are mostly running at full production capacity and this implies that to meet the future demand for livestock products in South Africa, livestock production will have to increase in areas that are generally not regarded as highly productive. These areas include the more marginal livestock production areas under rangeland conditions, and those livestock production systems that are characterized by water-limitation, poor and restrictive edaphic and extreme bioclimatic conditions. Currently there are few commercial perennial forage options, primarily old man saltbush (*Atriplex numularia* Lindl.) and spineless cactus (*Opuntia ficus-indica* (L.) Mill.), adapted to these marginal agro-ecological areas (Palmer and Ainslie 2006; Dickinson *et al.* 2010; Truter *et al.* 2015). The use of the already limited forage options for these areas is, however, further limited in areas that fall within, or near, protected areas or, areas with high levels of plant endemism, due to their risk of becoming weedy or invasive e.g. cactus pear forages. In addition, these species are non-leguminous, and therefore, farmers do not have the added benefits of the symbiotic nitrogen fixation that legumes offer.

Therefore, for the more arid Mediterranean regions of South Africa, as well as others around the world, there is an urgent need to develop fodder plants that are suitable for use under these marginal conditions and allow for the added benefits such as nitrogen fixation. These new fodder plants in turn, should allow for improved livestock production in areas that are currently underutilized or unproductive (Edwards *et al.* 2019). The use of native leguminous species, that are naturally adapted to these marginal areas, would therefore, be a more sensible alternative than the non-leguminous, exotic germplasm, and will allow farmers to expand their agricultural systems by diversifying their fodder flow programs within these water-limited agro-ecosystems.

In 2013, Trytsman produced a list of legume species from southern Africa for further characterization and evaluation as potential forage crops. The selection of these species was based on six factors which included the distribution, height and life cycle of the plants, the presence of any anti-nutritional and toxic factors, adaptation to low soil phosphate conditions, and lastly, whether or not the plants were grazed/browsed and/or cultivated (Trytsman 2013). From this, Trytsman (2013) produced an extensive list of species with varying potential to be evaluated as possible forage crops. Unfortunately, further efforts at evaluating these species have been minimal.

Several reasons exist for the lack of interest in evaluating and developing native legume species for forage production in South Africa itself. The most commonly reported reasons are the easily accessible exotic forage germplasm in South Africa (Palmer and Ainslie 2006; Trytsman 2013), large and well-known South African legume genera that are not generally recognized as livestock feed in South Africa itself due to their perceived toxic qualities, the general lack of knowledge about their agronomic potential (Trytsman 2013), the time required to domesticate new forage species, and the costs associated with producing marketable amounts of seeds (Loi *et al.* 2008; Nichols *et al.* 2007; 2010; Muir *et al.* 2014; Mitchell *et al.* 2015). This, in turn, resulted in forage breeding programs in South Africa focusing primarily on producing exotic legume cultivars (e.g. *Medicago sativa* cv SA Standaard and SA Select) that are adapted to specific agro-ecological conditions (Palmer and Ainslie 2006; Trytsman 2013; Truter *et al.* 2015). As a result, the National Forage Genebank of South Africa holds less than 5 % of the native South African legume species that could be evaluated as possible forage crops, as opposed to nearly all native grass species (Trytsman 2013, Trytsman *et al.* 2019, 2020).

The identification of native legume species that are already well adapted to the water-limited and marginal edaphic and bioclimatic conditions would however, provide a more sustainable means to meet the future demand for livestock products in South Africa. Also, with the general climate change trend in South Africa indicating hotter and drier conditions, new forage species that can withstand these conditions throughout South Africa are needed in order to prepare for future bioclimatic scenarios. The objective of this study was, therefore, to identify and prioritize native legume species that could potentially be used in dryland farming systems in South Africa.

2.3. Materials and Methods

2.3.1. Species identification and selection

Distribution records of native legume species occurring in South Africa were obtained from the Global Biodiversity Facility. The distribution records were cleaned by removing all incomplete and replicated data records. Thereafter, legume species occurring within the borders of the Northern Cape Province of South Africa were selected. The Northern Cape is characterized by large arid and semi-arid plains with a mean annual precipitation of 200 mm but ranges from 20 mm in the far west and up to 540 mm in the east. Temperatures range from as low as -10 °C in winter to temperatures often exceeding 40 °C in the summer months (Jordaan *et al.* 2013). However, even under these extreme bioclimatic conditions, the Northern Cape houses a large diversity (ca.402 species) of indigenous legume species. This makes the Northern Cape ideal to study native legume species for water-limited agro-ecosystems.

The legume species occurring within the province were thereafter compared to a list of priority legume species from southern Africa produced by Trytsman (2013). Legume species from the Northern Cape that occurred on the list by Trytsman (2013) were divided into different priority classes as described by Trytsman (2013). From this list, only species that occurred in the classes characterized by not having any known (from literature surveys) toxic or anti-nutritional (containing substances that can cause illness to livestock or reduce feed intake) qualities were selected (Trytsman 2013). Furthermore, all trees, climbers and creepers were removed from this list. This was due to climbers needing a supporting plant or structure in order to be effective. Creepers were removed because the species identified need to be used as cut and carry crops, and trees were removed because of the time that it takes for a tree to grow and produce sufficient quantities of edible biomass under water-limited conditions. A further 13 legume species were identified as important livestock forages through informal interactions with botanists and pastoralists from the Northern Cape, as well as data gathered from following livestock to determine livestock diets in the communal rangelands of Leliefontein (Samuels *et al.* 2016). This meant that 13 species were added to the final list without being screened for containing anti-nutritional qualities. Thereafter, plant functional traits (Pérez-Harguindeguy *et al.* 2013) were used to further characterize the remaining legume species. Based on the availability of information, a total of 11 plant functional traits (Pérez-Harguindeguy *et al.* 2013) were used to describe the selected legume species with regards to their agronomic potential.

2.3.2. Species distributions, climate and soil adaptation

The distribution records for the final selection of legumes occurring in the Northern Cape were plotted across their potential distribution range in South Africa using DivaGIS version 7.5 (Hijmans *et al.* 2001; 2005). The DOMAIN model (Carpenter *et al.* 1993) was used to estimate the climatic adaptation of the legume species using the 19 bioclimatic variables of the WorldClim climate database version 1.4 (Hijmans *et al.* 2005) at the resolution of 2.5 ARC minutes. The output Gower distance statistics generated by the DOMAIN model were categorized into four adaptation zones namely, “Possible adaptation trend” (Gower scores of 50 – 70), “adaptive trend” (Gower scores of 71 – 90), “adapted range” (Gower scores of 91 – 95) and “highly adapted range” (Gower scores of 96 – 100). The suitability of these adaptation zones was assessed by dividing the distribution records of *Lessertia frutescens* subsp*. frutescens* and *Indigofera alternans* into proportions of 25 % (training set) and 75 % (testing set). The DOMAIN model was used to define these adaptation zones using the training sets while the testing set was used to calculate the proportion of the records for each of these species that fall within these adaptation zones. *Lessertia frutescens* subsp. *frutescens* and *Indigofera alternans* were used for this assessment as they contained the largest number of distribution records. Thereafter, maps of South Africa with “adapted” and “highly adapted” ranges were generated in DivaGIS. The grid files generated were imported into IDRISI (Terrset) after which the vector files were converted to raster images and adaptation ranges for each legume species quantified. The bioclimatic variables most limiting the adaptation of the native legume species were then identified using the DOMAIN Most Limiting Factor analysis in DivaGIS.

The prevalence of the different soil classes in South Africa (BGIS.org) was determined by calculating the area of each soil class using IDRISI. The distribution records of the selected legume species were thereafter plotted onto the different soil classes using ArcView version 3.3. The occurrence of each species within each soil class was calculated as a percentage of the total number of occurrence records for each legume species. The adaptation zones of each of the legume species were thereafter overlaid onto the different soil classes on which the legume species occur, and clipped. The remaining areas were then calculated as the ‘new’ adaptation zones and were given as a percentage of the total surface area of South Africa.

2.4. Results

2.4.1. Species identification and selection

A total of 402 legume species in 67 genera occur within the borders of the Northern Cape. Approximately 54 % of the legume species found within the Northern Cape occur in only four genera namely, *Aspalathus* (18 %), *Indigofera* (16 %), *Lotononis* (12 %) and *Lessertia* (8 %) while 36 genera (11 %) contain only one or two species. Of the 402 legume species, a total of 129 species were found on the list of priority species for further evaluation by Trytsman (2013), and after the removal of all species not occurring in Trytsmans’ categories of higher (A1), medium (B1) and lower (C1) forage potential, grazed/browsed, and/or cultivated as well as all trees, creepers and climbers, a total of

24 legume species remained. A further 13 species identified as important livestock forages by the botanists and pastoralists from the Northern Cape were added to the list resulting in 37 initially selected legume species (Table 2.1).

Of the 11 plant functional traits initially selected, only seven had sufficient available information to use in the characterisation of the selected legume species. Table 2.2 provides information regarding the plant functional traits in the categories whole plant traits and reproductive traits for the initially selected legume species. The majority of the species on the list were found to have a perennial life cycle while only four species had an annual life cycle. A total of 27 species were found to have a herbaceous growth form and all species had terminally placed seed pods. Only perennial, herbaceous, stem woody, spineless, and species not already being developed as forage crops elsewhere (i.e. *Lebeckia ambigua* (Howieson *et al.* 2013, de Meyer *et al.* 2014)) were selected for further screening. Therefore, only 18 legume species remained and were considered for the remainder of the work that focused on the climate and soil adaptability of the species.

Table 2.1: Selected native legume species from the Northern Cape Province of South Africa using Trytsman's (2013) prioritised categories and those species identified as important livestock forages in the Northern Cape. High priority (A1), Moderate priority (B1), Grazed/browsed (*), cultivated (+), Identified as important by farmers and botanists (⌂⌂)

	Name	**USE**	
1	*Crotalaria effusa* E.Mey.	*	⌂⌂
2	*Crotalaria excisa* (Thunb.) Baker f. subsp. *namaquensis* Polhill	*	⌂⌂
3	*Crotalaria laburnifolia* L. subsp. *laburnifolia*	+	⌂⌂
4	*Crotalaria pearsonii* Baker f.		B1
5	*Cullen tomentosum* (Thunb.) J.W.Grimes	+	B1
6	*Indigastrum argyroides* (E.Mey.) Schrire	*	
7	*Indigofera alternans* DC. var. *alternans*	*	A1
8	*Indigofera heterotricha* DC.		B1
9	*Indigofera meyeriana* Eckl. & Zeyh	*	
10	*Indigofera nigromontana* Eckl. & Zeyh.	+	⌂⌂
11	*Indigofera pungens* E.Mey	*	
12	*Lebeckia ambigua* E.Mey.	*	
13	*Calobota sericea* (Thunb.) Boatwr. & B-E.van Wyk (*Lebeckia sericea* Thunb.)	*	⌂⌂
14	*Calobota spinescens* (Harv.) Boatwr. & B-E.vanWyk (*Lebeckia spinescens* Harv.)	*	
15	*Lessertia brachypus* Harv	*	⌂⌂
16	*Lessertia depressa* Harv	*	A1
17	*Lessertia diffusa* R.Br	*	
18	*Lessertia excisa* DC.	*	
19	*Lessertia frutescens* (L.) Goldblatt & J.C. Manning subsp. *frutescens*	*	B1
20	*Lessertia frutescens* (L.) Goldblatt & J.C. Manning subsp. *microphylla* (Burch ex DC.) J.C.Manning & Boatwr.	+	A1
21	*Lessertia incana* Schinz	*	
22	*Lessertia inflata* Harv	*	
23	*Lessertia pauciflora* Harv. var. *pauciflora*	*	A1
24	*Lessertia spinescens* E.Mey	*	
25	*Lotononis falcata* (E.Mey.) Benth	*	⌂⌂
26	*Lotononis leptoloba* Bolus	*	⌂⌂
27	*Melolobium adenodes* Eckl. & Zeyh.	*	⌂⌂
28	*Melolobium humile* Eckl. & Zeyh.	*	⌂⌂
29	*Melolobium microphyllum* (L.f.) Eckl. & Zeyh		B1
30	*Melolobium obcordatum* Harv.		A1
31	*Psoralea glaucescens* Eckl. & Zeyh.	*	⌂⌂
32	*Rhynchosia adenodes* Eckl. & Zeyh.		A1
33	*Rhynchosia emarginata* Germish	*	
34	*Rhynchosia schlechteri* Baker f	*	⌂⌂
35	*Senna italica* Mill. subsp. *arachoides* (Burch.) Lock	+	B1
36	*Wiborgia fusca* Thunb. subsp. *fusca*	*	⌂⌂
37	*Wiborgia monoptera* E.Mey	*	

Table 2.2: Plant functional traits used to characterize native legume species for their forage potential.

Name	Life Cycle	Height [up to (m)]	Growth Form	Growth Habit	Spines	Flower - Fruit Time	Fruit Placement	Fruit size (mm long)	Fruit attributes	Seed Size (mm long)	Seeds per pod
Calobota sericea[1,2]	P	1.8	SW	Erect	No	May–Oct	Terminal	-	Dehiscent	-	-
Calobota spinescens[1,2]	P	1	W	Erect	Yes	Sept-May	Terminal	-	Dehiscent	-	-
Crotalaria effusa[4]	A	0.7	H	-	-	-	Terminal	-	-	-	-
Crotalaria excisa[1,2]	P	1.2	H	Sprawling	No	Aug.–Oct	Terminal	20 – 25	-	-	-
Crotalaria laburnifolia subsp. *laburnifolia*[4]	P	2	H	-	-	-	Terminal	-	-	-	-
Crotalaria pearsonii[2]	P	0.5	H	Erect	No	Aug-Nov	Terminal	18 - 35	Dehiscent	2 - 4	-
Cullen tomentosum[4]	P	1.2	H	Prostrate	No	July-Jan	Terminal	-	-	-	-
Indigastrum argyroides[2]	A	0.5	H	Prostrate	No	Sept-May	Terminal	-	-	-	-
Indigofera alternans subsp. *alternans*[4]	P	2	H	Prostrate	No	Sept - Dec	Terminal	< 10	-	-	6 – 8
Indigofera heterotricha[4]	P	1.5	H	Erect	No	-	Terminal	-	-	-	8
Indigofera meyeriana[1,2]	P	1	H	Prostrate	No	Jun - Nov	Terminal	-	-	-	-
Indigofera nigromontana[1]	P	1.5	H	-	No	-	Terminal	-	-	-	-
Indigofera pungens[2]	P	1.2	SW	Erect	Yes	May-Oct	Terminal	-	-	-	-
Lebeckia ambigua[1,2]	P	0.8	H	Erect	-	Aug.–Dec	Terminal	-	Dehiscent	-	-
Lessertia brachypus[2,3]	P	1	H	Erect	No	July–Aug	Terminal	-	-	-	-
Lessertia depressa[1,3]	P	0.6	H	Prostrate	No	Jun - Dec	Terminal	10 - 15	-	-	-
Lessertia diffusa[1-3]	P	0.5	H	Sprawling	No	Aug.–Sept	Terminal	10 - 15	-	-	6 – 8
Lessertia excisa[1,3]	P	0.4	H	Procumbent	No	Aug-Oct	Terminal	-	-	-	-
Lessertia incana[1-3]	P	0.6	H	-	No	Sept. - Nov.	Terminal	-	-	-	-
Lessertia inflata[2,3]	P	0.2	H	Decumbent	No	Aug - Dec	Terminal	10 - 15	-	-	-
Lessertia pauciflora var. *pauciflora*[3]	P	1.2	H	Prostrate	No	-	Terminal	30 – 43	-	-	10 – 12
Lessertia spinescens[1-3]	P	0.8	H	Erect	Yes	Aug-Sept	Terminal	-	-	-	1 – 2
Lessertia frutescens subsp. *frutescens*[1-3]	P	1.3	H	Erect	No	-	Terminal	-	-	-	-
Lessertia frutescens subsp. *microphylla*[1-3]	P	1.3	H	Erect	No	-	Terminal	-	-	-	-
Lotononis falcata[1,2]	A	0.3	H	Prostrate	-	May–Sept.	Terminal	-	-	-	-
Lotononis leptoloba[1,2]	A	0.8	H	Sprawling	-	Sept.–Oct.	Terminal	-	-	-	-
Melolobium adenodes[1,2,4]	P	0.3	H	-	Yes	Sept.–Oct.	Terminal	-	-	-	-
Melolobium humile[1,2]	P	0.5	H	-	Yes	Sept.–Oct	Terminal	-	-	-	-
Melolobium microphyllum[4]	P	1	W	Branched	Yes	-	Terminal	12 - 18	Dehiscent	2 - 3	2 - 4
Melolobium obcordatum[4]	P	0.5	W	Decumbent	Yes	-	Terminal	12 - 16	Dehiscent	2 - 2.3	2 - 4
Psoralea. Glaucescens[2]	P	3	H	Branched	-	Nov.–Apr.	-	-	-	-	-
Rhynchosia adenodes	P	0.6	W	Prostrate	No	?	Terminal	-	-	-	-
Rhynchosia emarginata[2]	P	0.8	W	Erect	No	Aug.–Sept.	Terminal	-	-	-	-
Rhynchosia schlechteri[2]	P	0.4	W	?	?	Jul - Sept	Terminal	-	-	-	-
Senna italica subsp. *arachoides*[4]	P	1	H	Erect	No	?	Terminal	-	-	-	5 - 7
Wiborgia fusca[1,2]	P	1.6	W	Erect	Yes	Aug.–Oct.	Terminal	-	-	-	-
Wiborgia monoptera[2]	P	1	W	Erect	Yes	Jul-Sept	Terminal	-	Indehiscent	-	-

1=Schutte 2012, 2=Campbell-Young 2013, 3=Nkonki 2013, 4=Nkonki *et al.* 2003, A = Annual; P = Perennial; SW = Stem Woody; W = Woody; H = Herbaceous

2.4.2. Distribution and climate adaptation

Potential distribution ranges, determined from the output Gower statistics for each of the legume species are shown in Figure 2.1. Nine of the legume species (*Crotalaria pearsonii, Crotalaria excisa* subsp. *namaquensis*, *Calobota sericea, Indigofera meyeriana, Lesseria diffusa, Lessertia excisa, Lessertia incana, Lessertia inflata,* and *Psolarea glaucescens*) were only found within the boundaries of two or three provinces i.e. Northern Cape, Western Cape and Eastern Cape or North West Province. The remaining nine legume species (*Crotalaria laburnifolia* subsp. *laburnifolia, Cullen tomentosum, Indigofera alternans* var. *alternans, Indigofera heterotricha, Indigofera nigromontana, Lessertia depressa, Lessertia pauciflora* var. *pauciflora, Lessertia frutescens* subsp. *frutescens, and Senna italica*) had distribution records that span across the boundaries of five to seven of the South African provinces. A total of seven species (*Cullen tomentosum, Indigofera alternans, Indigofera heterotricha, Lessertia depressa, Lessertia pauciflora* var. *pauciflora, Senna italica,* and *Lessertia frutescens* subsp. *frutescens*) were found to have a combined 'adapted' and 'highly adapted' range covering over 40 % of the South Africa's land surface (Figure 2.1).

The results of the analysis of the most limiting bioclimatic factors influencing the distributions of the legume species in South Africa is shown in Table 2.3. Based on the two major bioclimatic themes (temperature and precipitation) of the WorldClim climate database, the 18 native legume species can be divided into three broad categories based on the bioclimatic variables limiting their distributions. The first category consists of a total of seven legume species (*Crotalaria laburnifolia, Crotalaria pearsonii, Indigofera alternans* var. *alternans, Indigofera nigromontana, Lessertia pauciflora* var. *pauciflora*, *Lessertia frutescens* subsp. *frutescens and Psolarea glaucescens*) that have distributions limited primarily by temperature variables. The second category consisted of six legume species (*Calobota sericea, Crotalaria excisa, Indigofera meyeriana, Lessertia diffusa, Lessertia excisa*, and *Lessertia incana*) that have distributions limited primarily by precipitation variables. The third category consisted of six legume species (*Cullen tomentosum, Indigofera heterotricha, Lessertia depressa, Lessertia inflata and Senna italica*) that have distributions limited by a combination of temperature and precipitation variables.

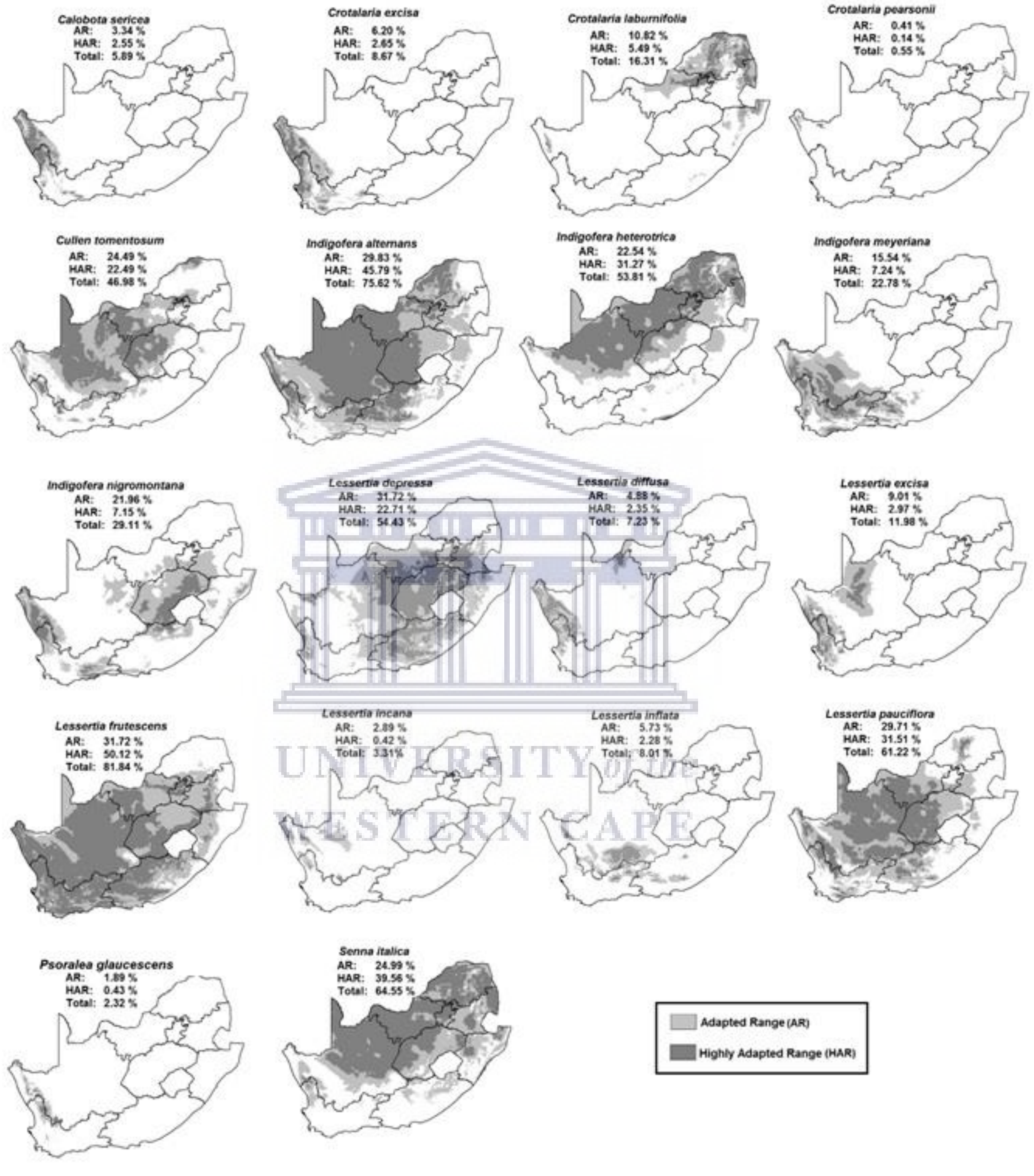

Figure 2.1: Adapted and highly adapted ranges of 18 native legume species from South Africa.

Table 2.3: The percentage of South Africa's total land surface (rounded to the nearest integer) where variables of the WorldClim climate database is most limiting the adaptation of each of the native legume species.

	BIO 1	BIO 2	BIO 3	BIO 4	BIO 5	BIO 6	BIO 7	BIO 8	BIO 9	BIO 10	BIO 11	BIO 12	BIO 13	BIO 14	BIO 15	BIO 16	BIO 17	BIO 18	BIO 19
exc.	2	1	1	0	0	0	0	21	3	1	2	0	0	1	2	0	1	65	0
lab.	1	5	6	43	1	5	0	0	12	1	0	1	0	5	7	1	5	1	6
pea.	0	0	1	0	0	5	0	0	51	0	0	0	0	0	19	0	5	0	19
tom.	1	5	17	6	5	6	0	2	3	4	1	1	6	18	2	2	6	12	3
alt.	5	11	12	7	2	11	2	3	4	6	2	1	2	11	7	0	3	10	1
het.	1	4	23	2	1	10	1	8	9	1	0	5	3	12	8	1	5	2	4
mey.	0	1	7	2	0	1	0	2	2	1	5	0	46	0	6	18	0	9	0
nig.	2	17	19	7	1	1	1	6	1	2	2	2	20	8	3	1	1	2	4
dep.	7	5	11	3	1	2	1	3	4	2	7	2	15	2	11	10	4	5	5
dif.	1	1	4	0	0	1	0	6	5	1	5	10	12	3	1	2	5	42	1
exci.	2	5	3	0	1	1	0	8	4	0	3	0	4	1	5	0	0	63	0
inc.	1	1	3	0	0	1	0	2	2	0	1	1	27	1	3	24	5	25	3
inf.	3	29	3	2	1	1	0	1	1	8	0	1	23	1	4	10	6	5	1
pau.	6	5	12	6	1	10	6	2	4	2	7	2	4	2	3	5	3	16	4
ser.	0	0	2	1	0	1	0	7	23	1	0	1	7	0	0	1	0	55	1
gla.	6	5	12	6	1	10	7	2	4	2	7	2	4	2	3	5	3	15	4
ita.	2	2	20	4	5	3	1	2	2	6	3	3	1	9	21	3	4	2	7
fru.	5	6	17	8	1	5	1	2	2	1	12	3	8	2	21	2	1	2	1

Species names are abbreviated as: *exc.: C. excisa, lab: C. laburnifolia, pea: C. pearsonii, alt: I. alternans, het: I. heterotricha, mey: I. meyeriana, nig: I. nigromontana, dep: L. depressa, dif: L. diffusa, exci: L. excisa, inc: L. incana, inf: L. inflata, pau: L. pauciflora, gla: P. glaucescens, ita: S. italica, tom: C. tomentosum, ser: C. sericea, fru: L frutescens.* BIO1 = Annual Mean Temperature; BIO2 = Mean Diurnal Range (Mean of monthly (max temp - min temp)); BIO3 = Isothermality (BIO2/BIO7) (* 100); BIO4 = Temperature Seasonality (standard deviation *100); BIO5 = Max Temperature of Warmest Month; BIO6 = Min Temperature of Coldest Month; BIO7 = Temperature Annual Range (BIO5-BIO6); BIO8 = Mean Temperature of Wettest Quarter; BIO9 = Mean Temperature of Driest Quarter; BIO10 = Mean Temperature of Warmest Quarter; BIO11 = Mean Temperature of Coldest Quarter; BIO12 = Annual Precipitation; BIO13 = Precipitation of Wettest Month; BIO14 = Precipitation of Driest Month; BIO15 = Precipitation Seasonality (Coefficient of Variation); BIO16 = Precipitation of Wettest Quarter; BIO17 = Precipitation of Driest Quarter; BIO18 = Precipitation of Warmest Quarter; BIO19 = Precipitation of Coldest Quarter

2.4.3. Soil adaptations and new adaptation zones

Table 2.4 shows the percentage that the 19 general soil classes contribute to the total land surface of South Africa as well as the occurrence (given as a percentage of the total distribution records for each species) of each of the native legume species within each of the general soil classes. Three soil classes namely Arenosols 2 (AR2), Leptosols 2 (LP2) and Regosols (R) were found to be the soil classes on which the largest percentage of occurrence records for most of the native legume species were recorded (Table 2.4). Arenosols 2 are red and yellow, well-drained sandy soils with high base status. Leptosols 2 are soils with minimal development. They are usually shallow, on hard or weathering rock, with or without intermittent diverse soils and lime is generally presents in part or most of the landscape. Regosols are characterized as rocky areas with limited soil (FAO 2005). After overlaying the 'adapted' and 'highly adapted' ranges of the legume species on the different soil classes, and clipping only those soil classes with occurrence records, a significant reduction in the overall 'adapted' and 'highly adapted' ranges were observed. Table 2.5 provides the new 'adapted' and 'highly adapted' range of the native legume species as a percentage of the total land surface of South Africa. From this table those seven species (*Cullen tomentosum, Indigofera alternans* subsp. *alternans, Indigofera heterotricha, Lessertia depressa, Lessertia pauciflora* var. *pauciflora, Senna italica* and *Lessertia frutescens* subsp. *frutescens*) that had a combined 'adapted' and 'highly adapted' range covering over 50 % of the total South African land surface, remained the species with the largest 'adapted' and 'highly adapted' ranges (Table 2.5).

Table 2.4: Percentage of each soil class in South Africa, the percentage of the total species distributions on individual soil classes and their associated soil pH ranges.

Soil Class	***Soil as a % of SA***	**Occurrence (%) on each soil class**																	
		exc.	**lab.**	**pea.**	**tom.**	**alt.**	**het.**	**mey.**	**nig.**	**dep.**	**dif.**	**exci.**	**inc.**	**inf.**	**pau.**	**gla.**	**ita.**	**ser.**	**fru.**
AC	*4.5*	5	2	-	-	1	1	-	-	1	-	3	-	-	-	-	-	-	2
AR1	*4.1*	-	-	-	5	8	2	-	3	-	-	-	-	-	4	-	5	-	-
AR2	*9.1*	11	5	25	17	9	14	1	7	11	30	11	25	-	8	8	19	21	6
AR3	*1.3*	11	-	-	-	-	-	-	-	-	5	7	-	-	-	-	-	-	1
CM	*8.1*	8	12	-	25	9	20	7	4	9	3	-	-	-	9	-	22	5	3
FL	*1.2*	-	2	-	-	2	2	-	-	-	-	-	-	-	-	-	2	-	1
FR	*0.1*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LP1	*7.8*	-	14	-	-	-	10	-	4	7	-	-	-	-	-	-	6	-	2
LP2	*28.4*	37	20	25	25	33	16	50	7	19	27	18	75	50	47	43	15	45	40
NT	*0.6*	-	5	-	-	1	3	-	7	-	-	-	-	-	-	-	2	-	-
PH	*1.4*	-	5	-	3	1	1	-	-	6	-	-	-	-	1	-	-	-	-
PL1	*4.5*	3	0	-	3	17	-	7	-	13	-	7	-	25	9	-	1	-	6
PL2	*8*	3	5	-	8	8	1	5	30	11	5	11	-	5	7	8	5	-	6
PT1	*4.4*	-	-	-	-	1	6	-	-	4	-	-	-	-	1	-	3	-	1
PT2	*4.5*	-	12	-	2	2	10	-	15	4	3	4	-	-	1	8	11	-	-
PZ	*0.2*	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	1
R	*8*	22	16	25	7	5	11	30	19	11	27	39	-	20	9	33	9	29	28
SC	*1.6*	-	-	25	5	2	-	-	-	-	-	-	-	-	3	-	-	-	3
VR	*2.1*	-	2	0	-	1	3	-	-	4	-	-	-	-	1	-	-	-	-
W	*0.1*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Soil pH		5.5-7.4	5.5-7.4	5.5-7.5	6.5-8.4	6.5-7.4	5.5-7.4	6.5-8.4	6.4-7.4	6.5-8.4	6.5-8.4	5.5-8.4	6.5-8.4	6.5-8.4	6.5-8.4	6.5-8.4	5.5-7.4	6.5-8.4	6.4-7.5

A dash (-) represents the absence of distribution records on a soil. Species names are abbreviated as: *exc.: C. excisa*, *lab: C. laburnifolia*, *pea: C. pearsonii, tom: C. tomentosum, alt: I. alternans, het: I. heterotricha, mey: I. meyeriana, nig: I. nigromontana, dep: L. depressa, dif: L. diffusa, exci: L. excisa, inc: L. incana, inf: L. inflata, pau: L. pauciflora, gla: P. glaucescens, ita: S. italica, ser: C. sericea, fru: L frutescens, Acrisols (AC), Arenosols1 (AR1), Arenosols2 (AR2), Arenosols3 (AR3), Cambisols (CM), Fluvisols (FL), Ferralsols (FR), Leptosols1 (LP1), Leptosols2 (LP2), Nitisols (NT), Phaezems (PH), Luvisols1 (PL1), Luvisols2 (PL2), Plinthosols1 (PT1), Plinthosols2 (PT2), Podzols (PZ), Regosols (R), Solonchaks (SC), Vertisols (VR), Water (W)*

Table 2.5: Percentage of the 'new' 'adapted' and 'highly adapted' ranges on different soil classes in South Africa.

		AC	AR1	AR2	AR3	CM	FL	LP1	LP2	NT	PH	PL1	PL2	PT1	PT2	PZ	R	SC	VR	Total (%)
exc.	**A**	0.06	-	0.86	0.18	0.02	-	-	1.08	-	-	-	-	-	-	-	1.15	-	-	**4.97**
	HA	0.03	-	0.45	0.24	0.06	-	-	0.42	-	-	-	-	-	-	-	0.44	-	-	
lab.	**A**	0.90	-	0.96	-	1.62	0.15	1.17	1.96	0.21	0.21	-	0.42	-	0.94	-	0.85	-	0.48	**15.08**
	HA	0.17	-	0.27	-	1.11	0.12	0.51	1.48	0.18	0.12	-	0.20	-	0.54	-	0.27	-	0.25	
pea.	**A**	-	-	0.07	-	-	-	-	0.11	-	-	-	-	-	-	-	0.07	0.02	-	**0.36**
	HA	-	-	0.02	-	-	-	-	0.04	-	-	-	-	-	-	-	0.02	0.02	-	
tom.	**A**	-	4.46	3.22	-	2.24	-	-	6.98	-	0.03	2.39	2.14	-	1.74	-	1.53	0.36	-	**51.01**
	HA	-	6.75	4.04	-	3.55	-	-	6.45	-	0.19	0.42	1.71	-	1.11	-	0.70	1.02	-	
alt.	**A**	1.25	0.18	1.50	0.00	1.50	0.39	-	8.63	0.14	0.23	-	-	3.13	2.10	-	3.35	0.36	1.44	**62.30**
	HA	0.25	3.87	7.23	0.00	5.74	0.56	-	13.78	0.08	0.21	-	-	0.43	2.10	-	2.32	1.06	0.49	
het.	**A**	2.77	2.01	2.14	-	2.10	0.22	0.95	4.20	0.21	0.51	-	2.87	1.76	0.94	-	1.07	-	1.02	**55.39**
	HA	2.56	1.97	5.29	-	5.19	0.29	1.31	7.66	0.20	0.08	-	1.14	1.59	3.01	-	1.56	-	0.77	
mey.	**A**	-	-	1.41	-	0.08	-	-	8.45	-	-	0.71	3.23	-	-	-	2.53	-	-	**24.79**
	HA	-	-	0.38	-	0.08	-	-	4.63	-	-	0.26	1.90	-	-	-	1.13	-	-	
nig.	**A**	-	0.04	1.69	-	1.82	-	1.52	4.33	0.06	-	-	2.91	-	1.24	0.04	1.96	-	-	**21.45**
	HA	-	0.03	0.41	-	0.33	-	0.09	1.52	0.04	-	-	2.37	-	0.45	0.01	0.60	-	-	
dep.	**A**	2.06	-	4.51	-	2.01	-	2.29	8.76	-	0.31	1.73	2.44	1.85	1.27	-	2.21	-	0.33	**51.98**
	HA	0.52	-	1.68	-	2.58	-	0.93	4.30	-	0.45	2.14	3.93	1.91	1.86	-	0.88	-	1.05	
dif.	**A**	-	-	1.64	0.20	0.07	-	-	1.72	-	-	-	0.18	-	0.00	-	0.58	-	-	**6.62**
	HA	-	-	0.91	0.09	0.02	-	-	0.80	-	-	-	0.03	-	0.01	-	0.37	-	-	
exci.	**A**	0.06	1.95	1.06	0.17	0.28	0.12	0.18	3.42	-	-	0.07	0.31	0.01	0.004	0.003	0.84	0.55	-	**11.89**
	HA	0.03	0.48	0.52	0.12	0.07	0.03	0.07	1.12	-	-	0.06	0.11	-	0.01	-	0.26	0.09	-	
inc.	**A**	-	0.04	0.52	0.03	0.03	0.02	-	1.80	-	-	0.001	0.004	-	-	-	0.12	0.33	-	**3.30**
	HA	-	0.02	0.14	-	0.001	-	-	0.20	-	-	0.01	0.01	-	-	-	0.03	0.01	-	
inf.	**A**		0.003	0.24	0.003	0.20	0.20	0.04	3.56	-	-	0.72	0.15	-	0.01	-	0.49	0.12	-	**7.99**
	HA		-	0.12	0.01	0.01	0.03	0.02	1.28	-	-	0.49	0.08	-	0.003	-	0.22	0.01	-	
pau.	**A**	-	0.57	2.67	-	1.54	-	-	10.19	-	0.07	1.50	2.64	1.90	1.87	-	2.74	0.44	1.34	**58.46**
	HA	-	3.34	4.67	-	3.71	-	-	9.80	-	0.18	2.73	2.51	0.20	1.50	-	1.20	1.05	0.09	
ser.	**A**	-	-	0.58	-	0.01	-	-	1.45	-	-	-	-	-	-	-	0.59	-	-	**5.00**
	HA	-	-	0.65	-	0.05	-	-	1.17	-	-	-	-	-	-	-	0.50	-	-	
gla.	**A**	-	-	0.20	-	-	-	-	1.03	-	-	-	0.02	-	0.55	-	0.43	-	-	**3.27**
	HA	-	-	0.02	-	-	-	-	0.21	-	-	-	0.02	-	0.69	-	0.10	-	-	
ita.	**A**	-	0.13	1.00	-	1.03	0.30	2.39	5.27	0.22	-	1.60	3.50	2.18	0.88	-	1.74	-	-	**57.07**
	HA	-	3.70	6.77	-	6.50	0.33	1.36	8.31	0.31	-	0.25	2.19	2.08	3.51	-	1.52	-	-	
fru.	**A**	2.25	1.79	2.06	0.30	1.68	0.20	2.96	5.53	0.15	-	1.19	3.59	2.76	2.23	0.11	2.55	0.18	-	**78.97**
	HA	0.58	2.25	6.02	0.30	4.23	0.66	1.19	19.12	0.06	-	3.11	3.56	1.18	1.24	0.07	4.44	1.41	-	

A = Adapted Range; HA = Highly Adapted Range; C = Current Climate; F = Future Climate; A dash (-) represents the absence of distribution records on a soil. Species names are abbreviated as: exc.: *C. excisa*, lab: *C. laburnifolia*, pea: *C. pearsonii*, alt: *I. alternans*, het: *I. heterotricha*, mey: *I. meyeriana*, nig: *I. nigromontana*, dep: *L. depressa*, dif: *L. depressa*, exci: *L. excisa*, inc: *L. incana*, inf: *L. inflata*, pau: *L. pauciflora*, gla: *P. glaucescens*, ita: *S. italica*, tom: *C. tomentosum*, ser: *C. sericea* , fru: *L. frutescens*, Acrisols (AC), Arenosols1 (AR1), Arenosols2 (AR2), Arenosols3 (AR3), Cambisols (CM), Fluvisols (FL), Leptosols1 (LP1), Leptosols2 (LP2), Nitisols (NT), Phaezems (PH), Luvisols1 (PL1), Luvisols2 (PL2), Plinthosols1 (PT1), Plinthosols2 (PT2), Podzols (PZ), Regosols (R), Solonchaks (SC), Vertisols (VR)

2.5. Discussion

2.5.1. Prioritization of native legume species

The initial prioritisation of the native legume species was done primarily on the basis of their life cycle, growth form and degree of spinescence. This led to the selection of only perennial, herbaceous or stem-woody, and spineless species from the 37 species identified. The selection of species with herbaceous or stem-woody growth habits was based on the idea that these species would have a higher relative growth rate compared to woody species (Hunt and Cornelissen 1997; Houghton *et al.* 2013). Therefore, due to the short, wet seasons usually experienced within water-limited agro-ecosystems these species could provide higher edible biomass yields that could be collected and stored as livestock feed. The species selected are, therefore, also intended to be used as 'cut and carry' crops to be stored for when other forages in the veld become reduced or depleted, or as a standing fodder bank that can be utilized when veld conditions deteriorate. This is also the reason why spineless species were prioritised over spinescent species as this would simplify the cut and carry process, as well as the storage of these fodders.

In water-limited environments, perennial species have an advantage over annual species due to a range of physiological adaptations. Perennial species have the ability to grow vegetatively for at least three growing seasons, unlike annual species that senesce after just one growing season after they have set seed (Pérez-Harguindeguy *et al.* 2013). Therefore, the perennial species can survive from one growing season to the next as mature plants, while the annual species would have to establish from seeds each year. Re-establishment from seed in water-limited agro-ecosystems is usually problematic, especially with legumes. Many legume species have been shown to display seed dormancy, primarily imposed on the seeds by the seed coat or testa (Werker *et al.* 1979; Bewley and Black 1994; Nowack *et al.* 2010; Bewley *et al.* 2013; Smýkal *et al.* 2014). In order for these seeds to establish and ensure feed for the following growing season, it would be imperative to first break the dormancy of the seeds before sowing to allow uniform, early germination, and rapid seedling establishment, at the onset of the rainy season.

Unlike annual plants that escape drought or water-limited conditions as seeds, perennial plants have a greater diversity of physiological mechanisms by which they can tolerate and survive these dry conditions (Whalley and Davidson 1969; Harradine and Whalley 1978; Ludlow 1980; Hale and Orcutt 1987; Scott 2000; Pérez-Harguindeguy *et al.* 2013). In perennial plants, the mechanisms of adaptation to drought or water-limited conditions can be divided into four categories. The first category is drought tolerance where plants can withstand near air dryness but rapidly grow after being re-watered (Hale and Orcutt 1987; Scott 2000). The second category is drought tolerance with low plant water potential, a state where plants can endure low tissue water status without desiccation (Sinclair and Ludlow 1985; Hale and Orcutt 1987). The third category is drought tolerance with high plant water potential. Here plants can endure long periods without water while maintaining a high plant water status by reducing the loss of water through transpiration (Sinclair and Ludlow 1985; Hale and Orcutt 1987). The fourth category is drought dormancy, where the shoots of the plant senesce when conditions are unfavorable but growth recommences when conditions become more favorable (Whalley and Davidson 1969; Harradine and Whalley 1978; Hale and Orcutt 1987).

2.5.2. Climate and soil adaptation

The 18 prioritized legume species were found to be limited by a range of different bioclimatic variables, and not all species were equally affected by the same variables. Approximately 39 % of the species prioritized were found to be limited by temperature variables, 33 % of the species were limited primarily by precipitation variables and 28 % of the species were limited equally by temperature and precipitation variables. Each of these categories of species should be evaluated differently to determine the extent to which these variables could limit their use in water-limited agro-ecosystems. Apart from the bioclimatic variables limiting the species distributions, soils are also a major factor influencing species distributions (Nichols *et al.* 2007, Trytsman *et al.* 2016). In a broad evaluation of the potential of these legume species, identifying whether or not species naturally occur on a specific soil type provides a good indication of the extent of a species adaptation to those soils and whether those species can be used in agricultural systems with those soils (Nichols *et al.* 2007). Trytsman *et al.* (2016) studied the diversity and biogeographical patterns of legumes indigenous to southern Africa and found that soil pH as well as mean annual minimum temperatures were the main drivers for distinguishing among legume assemblages. These bioclimatic and edaphic limitations to the distributions of these legume species therefore could reduce the agronomic potential of many of these species, as this reduces the areas in which these species could be used. The 18 species that were prioritized in this study however, can tolerate a wide range of soil pH conditions ranging from as low as 5.5 to a maximum of 8.4 (Table 2.4) and total soil phosphate concentrations ranging from 5 to 35 mg/kg (Trytsman 2013, Trytsman *et al.* 2016). Even so, the modelled ecological niches for the 18 native legume species were found to be much wider than their current distributions. The wider modelled ecological niches compared to the current distributions of the 18 legume species raises the question as to why these species are not filling their entire soil and climate niches. This may simply be because there are many factors, other than climate and soil conditions, that influence the natural distribution of plant species. These factors include competition for limiting resources, herbivory, the mode of seed dispersal, and the biological requirements for seed germination and seedling establishment (Bewley and Black 1994). If it were possible to include all of these variables into the model, it would likely result in an actual niche model with much smaller adaptation ranges. However, from an agricultural point of view, the actual ecological niche, i.e. variables other than climate and soil, may not be entirely relevant. This is due to many aspects of the agro-ecosystem being managed to suit the plant. For example, the mode of seed dispersal and biological requirements for seed germination and seedling establishment is controlled by the routine establishment and/or re-establishment of the species. Inter- and intra-specific competition can be controlled and managed to reduce the competition for limiting resources, and herbivores and insect predation can be managed by fencing off planted areas and using chemical pesticides to control insect predation (Bennet *et al.* 2011).

Some of the genera prioritized in this study are known to be good forages. For example, for some of the *Lessertia* species prioritized (*L. diffusa, L. excisa* and *L. incana*), evidence from Australian trials have indicated that these species are highly palatable and have shown some degree of grazing tolerance. They were also reported to become prostrate under high grazing pressure in trials done in Australia, which allows them to withstand continual grazing pressure (Cocks 2001; Howieson *et al.* 2008; Gerding *et al.* 2013a; b). However, the high numbers of resident *Rhizobium* bacterial strains in the Australian soils that rapidly nodulate these *Lessertia* species but are non-nitrogen fixing, led to the discarding of *Lessertia* as an agricultural legume in Western Australia (Gerding *et al.* 2013a, 2014). Similarly, many *Indigofera* species that have been found to contain high protein concentrations, are able to respond well to small rainfall events and are drought, flood and saline tolerant (Skerman 1982; Hassen *et al.* 2004; 2006a; b; 2007; 2008, Snowball *et al.* 2013). Therefore, the *Indigofera* species prioritized in the present study (*I. meyeriana* and *I. nigromontana*) might have the same potential. Species of *Cullen* have also been evaluated and prioritized as potential forage crops in low-rainfall environments in Western Australia, suggesting that the *Cullen* species identified in the present study (*C. tomentosum*) also merits further evaluation (Bennett *et al.* 2011; 2012). Species in the genus *Crotalaria* have also been evaluated as forage crops elsewhere (Arias *et al.* 2003, Snowball *et al.* 2013, Naim *et al.* 2015). Therefore, *C. excisa*, *C. laburnifolia* and *C. pearsonii* identified and prioritized in this study should also be evaluated for their fodder potential. Furthermore, evidence has shown the importance of *Calobota sericea* (formerly known as *Lebeckia sericea*) as a potentially important dry season fodder species in the semi-arid rangelands of Namaqualand, South Africa (Samuels *et al.* 2016). In Namaqualand, *C. sericea* plays a very important role as a late dry season forage when other more palatable forage species have already been selectively removed from the rangelands. During the late dry season, dry leaves and pods of this plant provide forage that helps to fill the mid to late summer forage gap in these rangelands. Unfortunately, no information regarding the quality of the forage provided by *C. sericea* is available at this stage.

2.6. Conclusion

In conclusion, further research on the forage potential of these 18 prioritized legume species is needed. However, it is evident that native legume species from South Africa could play an important role in improving livestock production in currently water-limited environments in South Africa. Also, these species could become important fodder crops under the predicted future hotter and drier bioclimatic conditions in South Africa. One of the species that stood out in the Namaqualand rangelands that should be evaluated further is *C. sericea*. This species already forms an important component of livestock diets during the dry season in the Leliefontein communal rangelands (Samuels *et al.* 2016) and therefore has the potential to be developed further into a fodder crop. To do so, however, information regarding the germination potential, early seedling vigor, plant growth rate, and responses to water limitation, forage production and the quality of the forage produced need to be determined.

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CHAPTER 3

Modelling the impacts of climate change on the potential distribution of *Calobota sericea*.

3.1. Abstract

Calobota sericea is a perennial legume species from South Africa that has the potential to be utilized as a fodder crop in water-limited agro-ecosystems. Little is currently known about its bioclimatic niche. However, due to its current narrow distribution range, future climate change may influence its adapted niche and therefore, also its agronomic potential. In this study, we aimed to elucidate the bioclimatic niche of *C. sericea* using existing herbarium records to characterize its adaptability to future (2050) bioclimatic conditions by means of ecological niche modelling. This was done in order to prioritize seed collection initiatives for conservation of these plant genetic resources for future forage breeding initiatives. Results from the niche models show that *C. sericea* will have a limited reduction in its distribution range of less than 2 % of its current distribution. This loss in the already limited adapted range results in the potential loss of approximately 5 % of the existing populations. Shifts in the different adaptation zones under future bioclimatic conditions is predicted to result in further loss of populations, as current populations will have to cope with more marginal bioclimatic conditions. Therefore, special attention should be given to the collection of genetic resources from populations that are currently located within the different adaptation zones of the ecological niche of *C. sericea* to conserve as much of the genetic variability within the species as possible. These genetic resources will likely be the key to successfully exploit the potential of *C. sericea* as a fodder crop under future bioclimatic conditions.

Key Words: Bioclimatic niche, water-limitation, drought fodder, Namaqualand

3.2. Introduction

Climate change predictions for South Africa generally indicate that the country will get hotter and drier (Kruger and Shongwe 2004; Benhin 2008; Meissner *et al.* 2013), with further increases in unpredictability and variability in bioclimatic conditions with future climate change (DEA 2013). However, these changes are expected to vary considerably between geographical locations, and in time (Kruger and Shongwe 2004; Benhin 2008; DEA 2013). While temperatures are likely to increase with climate change, projections in rainfall vary greatly (DEA 2013). A reoccurring feature, however, is the slight wetting trend of varying intensity and distribution in the east, and the drying trend that is evident in the west and parts of the north of the country (DEA 2013). Due to the drying trends in most parts of South Africa, coupled with the increases in temperature, and the resulting increases in evapotranspiration, an increase in the average annual irrigation demand of between 4 and 6 % under intermediate warming conditions is expected (Treasury and National Planning Commission 2013). Under more extreme warming conditions however, the irrigation demand may increase to between 15 and 30 % (Treasury and National Planning Commission 2013).

In South Africa, more than 1.2 million hectares of agricultural land is used under irrigation (Van der Stoep and Tylcoat, 2014). Pastures for grazing and hay production accounts for approximately 25 % of the irrigated areas in South Africa (Van der Stoep and Tylcoat, 2014). Therefore, given the reduction in annual precipitation, coupled with the increase in temperature, and evapotranspiration, it is likely that forage production will be done under more arid conditions in the future (Schulze 2016). This, in turn, has led to significant investment into the identification, evaluation and development of grass and legume forages that can withstand the projected future bioclimatic conditions and can be used within the marginal agro-ecological areas of South Africa (Truter *et al.* 2015). These efforts have focused almost exclusively on identifying and developing improved cultivars of introduced forage species (Truter *et al.* 2015). However, native South African grass and legume species have significant potential as fodder crops under the water-limited agro-ecological conditions of South Africa (Müller *et al.* 2017; Trytsman *et al.* 2019; 2020), especially where commercially available exotic fodders are not suitable. Already, in the more water-limited agro-ecological areas of the country, most of the current commercially available forage crops are not suitable for use (Dickinson *et al.* 2010). These unsuitable areas are likely to increase due to the projected changes in climate (DEA 2013). The need to identify native species, that are already well adapted to marginal bioclimatic and edaphic conditions that can fill these gaps, is therefore, becoming increasingly important (Müller *et al.* 2017; Trytsman *et al.* 2019; 2020). This is especially true due to the fact that these native species would probably not need selection and breeding initiatives to improve their adaptability to their native ranges, but rather selection and breeding should focus on improving forage characteristics for these areas.

The newly recognized potential of native South African grass and legume species for use in marginal agro-ecological areas has prompted a number of studies focusing on the identification and prioritization of native species for further evaluation as forage crops (Müller *et al.* 2017; Trytsman *et al.* 2019; 2020; Chimphango *et al.* 2020). From these studies, Müller *et al.* (2017) highlighted the potential of *C. sericea* (Thunb.) Boatwr. & B.-E. van Wyk as a fodder crop for the arid and semi-arid agro-ecological areas of the Namaqualand rangelands of the Northern Cape Province of South Africa. Within these agro-ecological areas, farmers often experience low livestock productivity, partially due to their over dependence on poor quality and inadequate feed supply from natural pastures (Müller *et al.* 2019). However, within these rangelands, wild stands of *C. sericea* fodders contributed up to 16 % of herded sheep and goat diets during the dry summer months (Samuels *et al.* 2016). Therefore, *C. sericea* has the potential to contribute significantly to the filling of the dry summer feed shortages experienced within these semi-arid, water-limited agro-ecosystems, and potentially also other areas experiencing similar bioclimatic conditions.

Within these rangelands, *C. sericea* is highly adapted to the low rainfall conditions of the Namaqualand rangelands, where annual precipitation ranges between 20 mm and 371 mm, with a 36 year (1980 – 2016) mean annual precipitation of 141 mm (Weather SA). Within these rangelands, *C. sericea* has a shrubby growth form, grows up to 1.8 m in height (Schutte 2012, Campbell-Young 2013), and is primarily found on sandy soils with a pH ranging from 6.5 to 8.4, and a total soil phosphate content of 5 to 35 mg/kg

(Trytsman *et al.* 2016, Müller *et al.* 2017). Furthermore, work done on the nodulation of *C. sericea* indicates that the species is nitrogen fixing and is nodulated by the α-proteobacteria that include *Bradyrhizobium* and *Mesorhizobium* species (Phalane 2008). The current narrow distribution pattern of *C. sericea* (Boatwright *et al.* 2018) however, indicates specific ecological niche requirements, and therefore, its agronomic potential could significantly be influenced by climate change. By using ecological niche modelling techniques, this study aims to characterize the bioclimatic niche of *C. sericea* and to determine the adaptability of the species to future bioclimatic conditions under different representative concentration pathways (RCPs). We hypothesize that due to the narrow distribution range of *C. sericea*, and therefore potentially also narrow adequate niche requirements, climate change would significantly influence the ecological niche of *C. sericea* by reducing its adapted range under future bioclimatic conditions.

3.3. Materials and Methods

3.3.1. Species occurrence data

Occurrence data for *C. sericea* was obtained from the global biodiversity information facility (GBIF.org) as well as from carefully curated herbarium records cited in the taxonomic revision of *Calobota* (Boatwright *et al.* 2018). The distribution range from Boatwright et al (2018), which is based on specimens from various national and international herbaria, was used as a means to validate the GBIF occurrence records and all records that were found to fall outside of the distribution range published by Boatwright *et al.* (2018) were removed as they likely represent misidentifications. The GBIF records were further cleaned by removing all incomplete (data points with missing information) and replicated data records.

3.3.2. Bioclimatic data

The 19 bioclimatic variables of the WorldClim climate database were downloaded at a resolution of 2.5 arc minutes (Hijmans *et al.* 2005). The data were downloaded for the ‘current period’, defined as 1950 – 2000, as well as for the future scenario (2050) defined as the average of 2041 – 2060. The future climate predictions are the IPPC5 climate projections from the global climate models (GCMs) for three representative concentration pathways (RCPs). The GCM outputs for this data were downscaled and calibrated (bias corrected) using the WorldClim 1.4 data as a baseline “current” climate. Data for predicting the species distributions under future bioclimatic conditions were downloaded for RCP 2.6, 4.5, and 8.5. These are named after a possible range of radioactive forcing values (difference between sunlight absorbed by the earth and energy radiated back) in the year 2100 relative to pre-industrial values (+2.6, + 4.5, and +8.5 W/m^2, respectively).

3.3.3. Selection of bioclimatic variables

A first model run, under ‘current’ bioclimatic conditions, was performed using the Maximum Entropy (MaxEnt) model (version 3.4.1) using all of the 19 bioclimatic variables. For the initial model we selected 75 % of the data for model training and 25 % for model testing and allowed for a maximum of 5000 iterations to allow for model convergence, and a convergence threshold of 0.0001. This model was replicated 10 times and the average of the 10 replicates were used as the final output for selection of bioclimatic variables. From the output of the initial model, those bioclimatic variables that did not contribute (had a value of zero) to the permutation importance of the model were removed. After removal of these variables the remaining bioclimatic variables were tested for co-linearity using ArcMap v. 10.2. Once the highly correlated variables ($r \geq 0.9$ Pearson’s correlation coefficient) were identified the importance of each variable to model permutation was used to remove the less important variable from each pair of highly correlated variables.

3.3.4. Species distribution modelling

The MaxEnt model used in this study has been shown to perform well with small sample sizes, relative to other modelling methods (Pearson *et al.* 2007, Elith and Leathwick 2009, Kumar and Stohlgren 2009, Qin *et al.* 2017). MaxEnt (Phillips *et al.* 2006) uses presence-only data to predict the distributions of species based on the theory of maximum entropy.

The program attempts to estimate a probability distribution of species occurrence that is closest to uniform, while still subject to environmental constraints (Elith *et al.* 2011). In the model used for this study, we selected 75 % of the data for model training and 25 % for model testing (Phillips 2008). We also allowed for a maximum of 5000 iterations to allow for model convergence, and a convergence threshold of 0.0001. This model was replicated 10 times. A Jackknife test was performed to determine the relative importance of each environmental variable and to determine which variables reduce the model reliability when omitted. MaxEnt measures this by ‘gain’, which represents how much better the distribution fitted the sample points than the uniform distribution (uniform distribution gain = 0). The Area Under the Receiving Operator Curve (AUC) was then used to evaluate the model performance. The value of AUC ranges from 0 to 1, and the closer the AUC value is to 1, the better the model performed. The final probability map (average of the 10 replicates) was then imported into ArcMap for visualization. Within ARCMap, Jenks natural breaks was used to cluster the data. Jenks classification is a data clustering method designed to determine the best arrangement of values into different classes (Jenks 1967) and for this study, four classes i.e. not suitable, adaptive trend, adapted range and highly adapted range were used.

3.4. Results

3.4.1. Selection of bioclimatic variables

Out of the 19 bioclimatic variables assessed (Table 3.1), three variables did not contribute to the permutation of the model and were immediately removed from any further analyses. From the remaining variables, eight pairs of bioclimatic variables were found to be highly ($r \geq 0.9$) correlated (Table 3.2). Using the permutation importance of each pair of highly correlated variables, the least important variable was removed from each pair, resulting in a total of 10 bioclimatic variables (Table 3.1) used to run the subsequent MaxEnt models under current and future bioclimatic scenarios. The relative contribution (%) of each of the selected bioclimatic variables ranged from 0.3 % (BIO 19) to 67.2 % (BIO 18), with the two dominant bioclimatic variables describing the distributions of *C. sericea* under current bioclimatic conditions being BIO 18 (the amount of precipitation in the warmest quarter) and BIO 9 (mean temperature of the driest quarter), contributing 67.2 % and 10.6 %, respectively to the current bioclimatic niche of the species (Table 3.1).

Table 3.1: Relative permutation importance (%) of each bioclimatic variable used to run the initial MaxEnt model. Bold and underlined variables were removed from further analyses either as a result of having a permutation importance of zero or due to being highly correlated with another bioclimatic variable.

Code	Environmental Variables	Permutation importance
BIO 1	Annual Mean Temperature	0.4
BIO 2	**Annual Mean Diurnal Range**	**2.1**
BIO 3	Isothermality	0.6
BIO 4	**Temperature Seasonality**	**0.3**
BIO 5	**Maximum Temperature of the Warmest Month**	**0**
BIO 6	Minimum Temperature of the Coldest Month	0.5
BIO 7	Annual Temperature Range	6.1
BIO 8	Mean Temperature of the Wettest Quarter	2.3
BIO 9	Mean Temperature of the Driest Quarter	10.6
BIO 10	**Mean Temperature of the Warmest Quarter**	**0**
BIO 11	**Mean Temperature of the Coldest Quarter**	**0.2**
BIO 12	**Annual Precipitation**	**1.7**
BIO 13	**Precipitation of the Wettest Month**	**1**
BIO 14	**Precipitation of the Driest Month**	**0.4**
BIO 15	Precipitation Seasonality	0.8
BIO 16	**Precipitation of the Wettest Quarter**	**0**
BIO 17	Precipitation of the Driest Quarter	5.5
BIO 18	Precipitation of the Warmest Quarter	67.2
BIO 19	Precipitation of the Coldest Quarter	0.3

Table 3.2: Collinearity among the remaining 16 bioclimatic variables of the WorldClim Climate database after the removal of variables that did not contribute to the permutation of the initial model. Bold and underlined correlation coefficients indicate highly correlated bioclimatic variables.

	BIO1	**BIO2**	**BIO3**	**BIO4**	**BIO6**	**BIO7**	**BIO8**	**BIO9**	**BIO11**	**BIO12**	**BIO13**	**BIO14**	**BIO15**	**BIO17**	**BIO18**	**BIO19**
BIO1	-	0.1	0.4	-0.2	0.7	-0.1	0.8	0.6	**0.9**	-0.1	0.0	-0.3	0.5	-0.3	0.1	-0.3
BIO2		-	-0.4	**0.9**	-0.6	**1.0**	0.3	-0.3	-0.3	-0.7	-0.5	-0.7	0.4	-0.7	-0.5	-0.6
BIO3			-	-0.8	0.7	-0.7	0.1	0.6	0.7	0.3	0.4	0.1	0.1	0.2	0.4	0.1
BIO4				-	-0.7	**1.0**	0.2	-0.5	-0.5	-0.6	-0.6	-0.5	0.2	-0.5	-0.6	-0.5
BIO6					-	-0.7	0.3	0.8	**0.9**	0.2	0.3	0.3	0.0	0.3	0.3	0.3
BIO7						-	0.3	-0.4	-0.4	-0.7	-0.6	-0.6	0.3	-0.6	-0.5	-0.6
BIO8							-	0.1	0.6	-0.1	0.1	-0.4	0.6	-0.4	0.2	-0.6
BIO9								-	0.7	-0.1	0.0	0.0	0.0	0.0	-0.1	0.3
BIO11									-	0.1	0.2	0.0	0.3	0.0	0.3	0.0
BIO12										-	**0.9**	0.6	-0.2	0.6	**0.9**	0.4
BIO13											-	0.3	0.1	0.4	**1.0**	0.2
BIO14												-	-0.7	**1.0**	0.3	0.8
BIO15													-	-0.7	0.1	-0.6
BIO17														-	0.3	0.8
BIO18															-	0.1
BIO19																-

3.4.2. Model performance using the selected bioclimatic variables

Model performance, as indicated by the area under the receiver operating characteristic curve (ROC) value, for the 10 replicated runs, ranged from 0.968 to 0.989, with a mean of 0.977 (± 0.006 SD). This suggests that the environmental variables selected are good descriptors in predicting the bioclimatic niche of *C. sericea* (Fig. 3.1). The Jackknife test for *C. sericea* highlighted that BIO 18 (precipitation in the warmest quarter) is the environmental variable that gives the highest gain when used in isolation. This is followed by BIO 9 (mean temperature in the driest quarter) and BIO 8 (mean temperature in the wettest quarter). BIO 7 (annual temperature range) however, was found to be the environmental variable that decreases the gain the most when omitted and therefore have the most information that is not present in the other variables (Fig. 3.2).

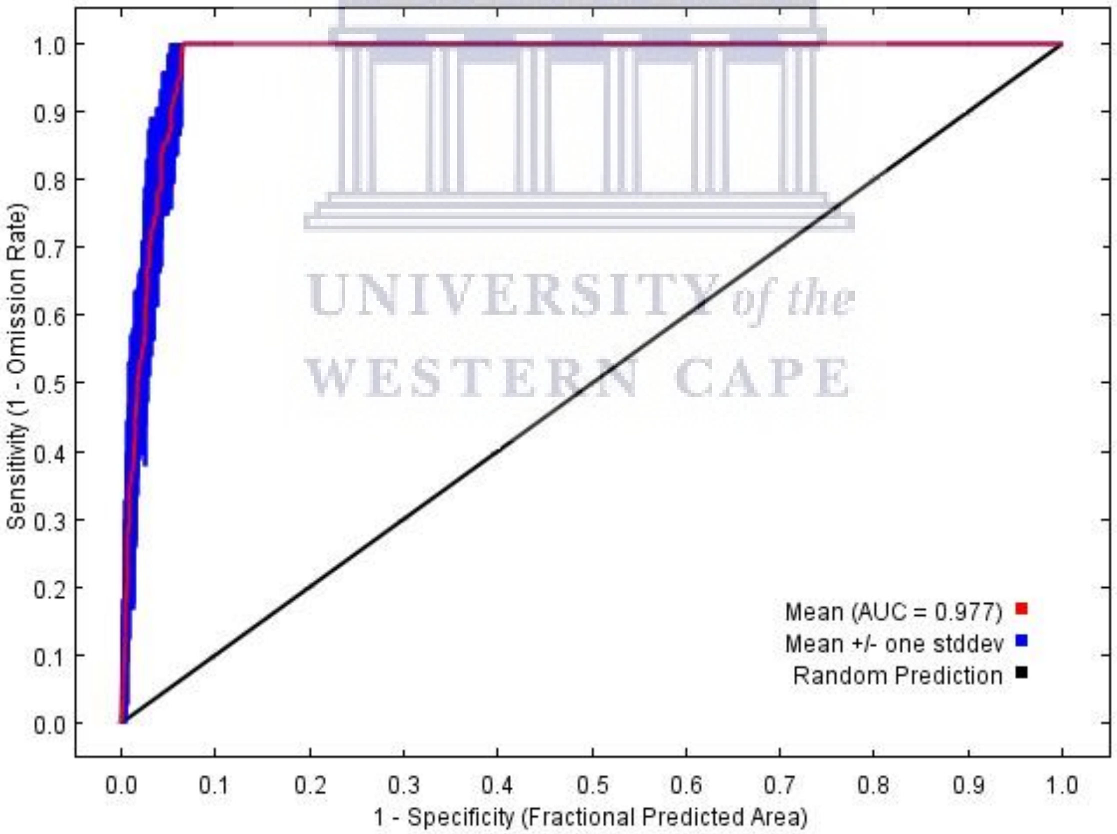


Figure 3.1: Receiver operating characteristic (ROC) showing the average AUC for 10 replicated runs for *Calobota sericea*.

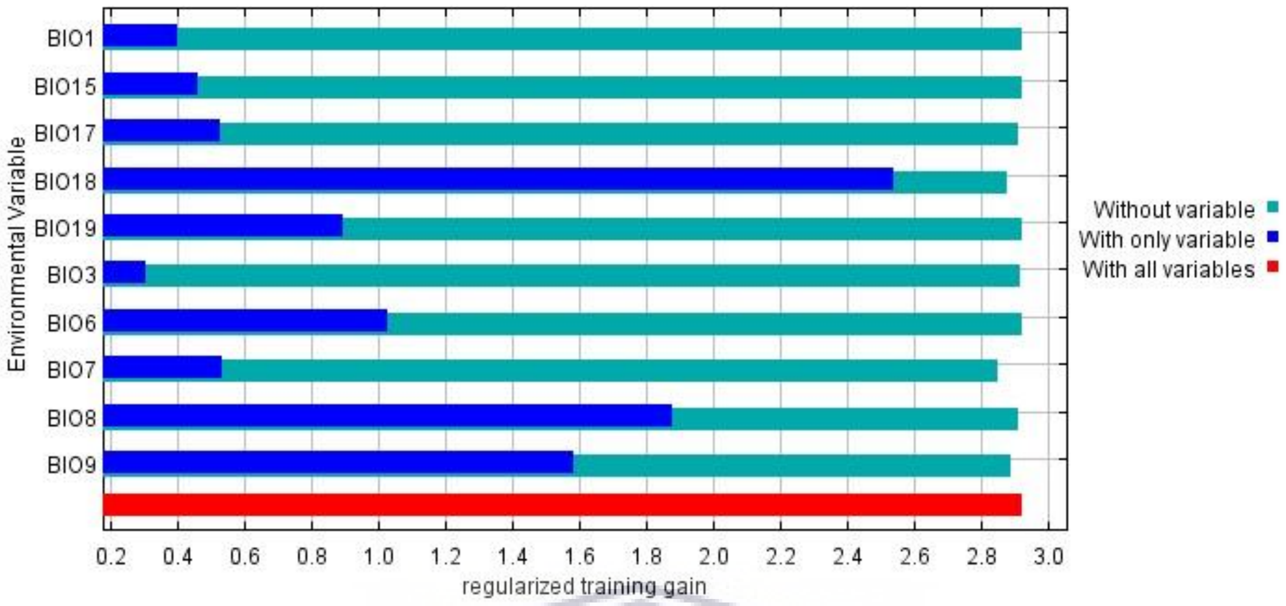
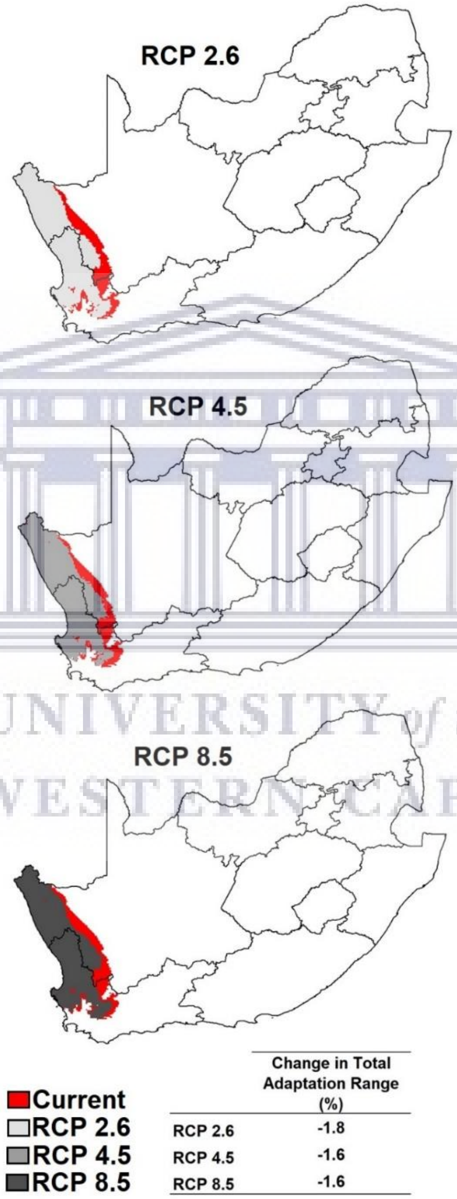


Figure 3.2: Jackknife test results indicating the bioclimatic variables which results in the highest gain when used in isolation, and the bioclimatic variable which decreases the gain the most when omitted for *Calobota sericea*.

3.4.3. Changes in potential distribution ranges from current to future bioclimatic conditions

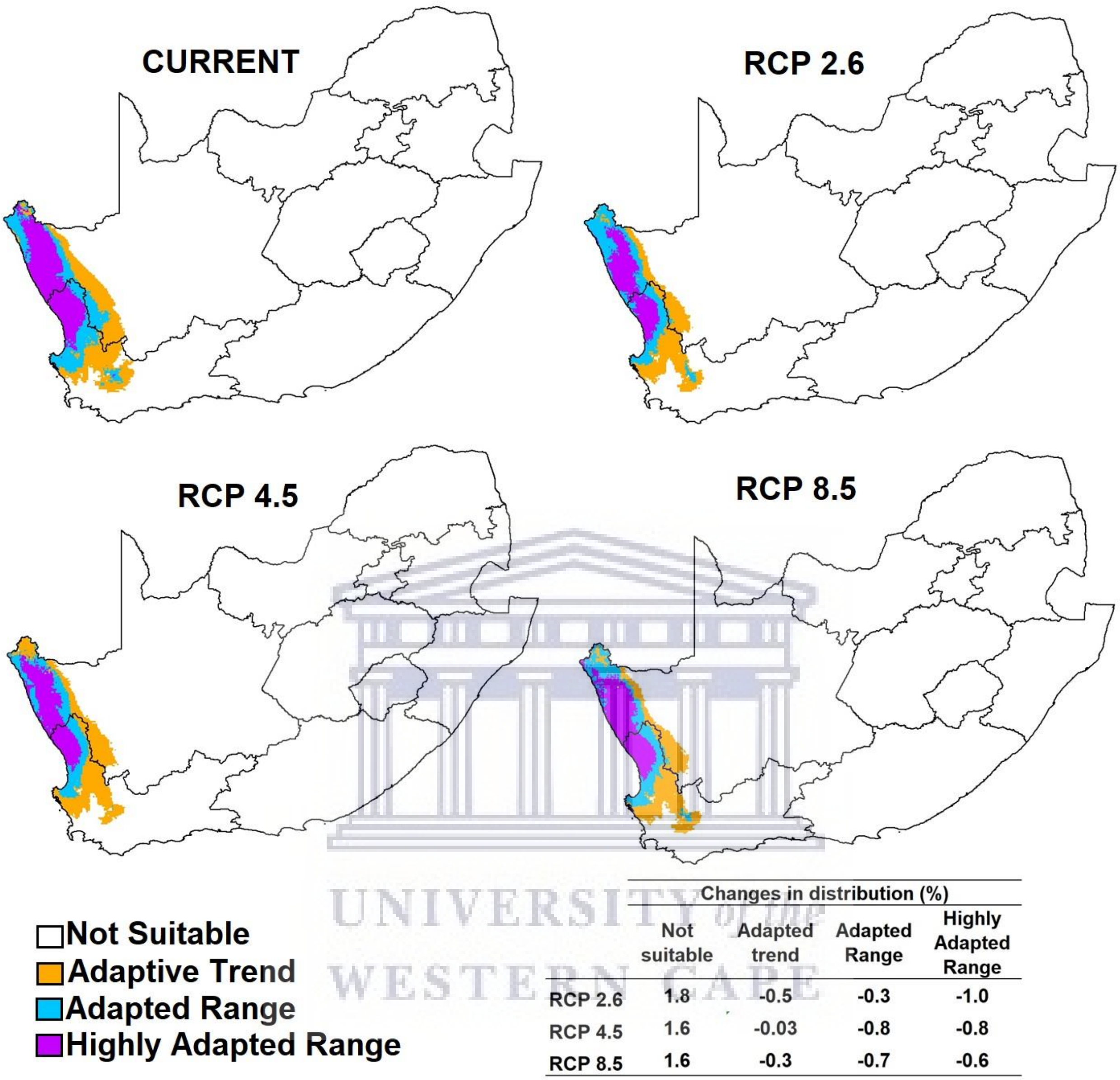
Generally, the predicted total adaptation ranges of *C. sericea* decreased with between 1.6 % and 1.8 % from current to future bioclimatic conditions, with the largest decrease in the adapted range found under RCP 2.6 conditions (Fig. 3.3). The adaptive trend range decreased between 0.03 and 0.5 %, the adapted range decreased between 0.3 and 0.8 % and highly adapted range decreased between 0.6 and 1 % from current to future bioclimatic conditions (Fig. 3.4). When considering specific adaptation ranges based on the occurrence of the current populations 15 %, 27 % and 58 % of the *C. sericea* populations fell within the adaptive trend, adapted and highly adapted ranges, respectively. From this, it is clear that the adapted and highly adapted ranges in this study are good indications of the potential distribution ranges of *C. sericea*. Under future bioclimatic scenarios, RCP 2.6, RCP 4.5 and RCP 8.5, however, 3 %, 5 % and 5 % of *C. sericea* populations respectively, will fall in areas that are outside of the adapted range of *C. sericea*. The portion of *C. sericea* populations in the adaptive trend range, increased from 15 % under current bioclimatic conditions, to 17 %, 20 % and 22 % under the

RCP 2.6, RCP 4.5 and RCP 8.5 scenarios, respectively. The *C. sericea* populations in the adapted range, increased from 27 % under current bioclimatic conditions, to 32 % in each of the future climate change scenarios. The *C. sericea* populations in the highly adapted range however, decreased from 58 % under current bioclimatic conditions, to 48 %, 47 % and 42 % under the RCP 2.6, RCP 4.5 and RCP 8.5 scenarios, respectively.



	Change in Total Adaptation Range (%)
RCP 2.6	-1.8
RCP 4.5	-1.6
RCP 8.5	-1.6

Figure 3.3: Change (%) in total adaptation range of *C. sericea* from current to future bioclimatic conditions (RCP 2.6, RCP 4.5 and RCP 8.5).



	Changes in distribution (%)			
	Not suitable	Adapted trend	Adapted Range	Highly Adapted Range
RCP 2.6	1.8	-0.5	-0.3	-1.0
RCP 4.5	1.6	-0.03	-0.8	-0.8
RCP 8.5	1.6	-0.3	-0.7	-0.6

Figure 3.4: Adaptation ranges of *Calobota sericea* under current and future bioclimatic conditions, and changes in the potential adaptation ranges from current to future bioclimatic conditions.

3.5. Discussion

In this study, we hypothesized that the adapted range of *C. sericea* will be significantly reduced under future climate change scenarios and used ecological niche modelling techniques to test this hypothesis. Species distribution models that correlate occurrence records with bioclimatic variables are a trusted method to predict the potential of species to expand their distributions outside of their native ranges or experience a reduction in their distribution ranges under future bioclimatic conditions (Pulliam 2000; Guisan and Thuiller 2005; Hirzel *et al.* 2006; Barbosa *et al.* 2012). Climatic niche modelling techniques estimate the climate thresholds within which a species can survive. This allows for the screening of the species' potential adaptation to the predicted future bioclimatic conditions. Identifying potential changes in the species' bioclimatic niche early using these ecological niche models could help with decision making regarding collection priorities from specific populations to effectively conserve a wide genetic resources of the species. This, in turn, can help with future breeding initiatives to improve the species agronomic potential.

Results from the current study concur with our hypothesis that future bioclimatic conditions will result in the reduction of the adaptation range of *C. sericea*. However, our results indicate that under future bioclimatic conditions, the adaptation range of *C. sericea* is predicted to decrease with less than 2 % from its current adaptation range. This modelled reduction in the total adaptation range of *C. sericea* under future bioclimatic conditions however, predicts that up to 5 % of the mapped populations could be lost. Taking into consideration the narrow adapted range of *C. sericea* (Boatwright et al. 2018), a reduction of 5 % in its current population numbers could result in a significant loss in genetic variability within the overall *C. sericea* population. The modelled reduction in the total adaptation range of *C. sericea* will primarily be due to a loss in areas that are defined as the adaptive trend zone under current bioclimatic conditions. This adaptive trend zone, within the parameters of this study, is an optimistic representation of the adaptation range of *C. sericea*, with only 15 % of the mapped populations occurring within this distribution zone under current bioclimatic conditions. This optimistic adaptation range however, is important as it can be regarded as the outer most limit of bioclimatic conditions under which natural populations of the species can survive. These populations therefore, are perceived to contain morphological, physiological and/or genetic traits not present in other populations. These traits are potentially allowing these populations to currently persist under the outer most limits of the adapted range of *C. sericea*. Collection of seeds from these populations are therefore, important in order to conserve the genetics of these populations, which, in turn, could help with stabilizing other populations which will also be affected under future bioclimatic conditions.

The models produced in this study also indicate that under future bioclimatic conditions, more of the current *C. sericea* populations will have to cope under bioclimatic conditions that are less suitable. The models also imply that there will be a shift in the different adaptation ranges, which suggest that populations currently persisting within specific adaptation ranges i.e. adaptive trend, adapted range and highly adapted range, under the current bioclimatic conditions, would have to adapt to persist in the future less suitable bioclimatic conditions. For example, under future bioclimatic conditions under RCP 8.5, which represents the scenario that is 'business as usual', with unabated emissions, 12 % of the populations that currently fall within the highly adapted range will under future bioclimatic conditions fall within the adapted range (5 %) and adaptive trend range (7 %). These populations that initially persisted under the modelled most suitable bioclimatic conditions, will under future bioclimatic conditions have to persist under the less desirable conditions, which, in turn, could result in further losses in the current populations.

It is therefore suggested that attention should be given to the collection of plant genetic resources within all of the modelled adaptation ranges. The collection of genetic materials from only the best adapted populations within these different adaptation ranges will most likely be the key to develop cultivars from ecotypes adapted to these specific bioclimatic conditions, which, in turn, will be key to the successful exploitation of *C. sericea* as a fodder crop under future bioclimatic conditions.

3.6. Conclusion

In conclusion, this study has shown that *C. sericea* has a restricted bioclimatic niche, and that under future bioclimatic conditions its adaptation range will decrease by less than 2 % of its current distribution range. This range reduction could lead to the loss approximately 5 % of current *C. sericea* populations, and possibly more, as the different adaptation ranges in this study are modelled to shift. These results compel the collection of *C. sericea* seeds from different populations within the different adaptation ranges to conserve as much genetic variability within the species as possible. It is therefore suggested that the ecological niche of *C. sericea* be further quantified using existing agronomic, climatic and edaphic parameters. These, along with the current bioclimatic niche models, will allow for the prioritization for collection of seeds from ecotypes currently persisting under unique agro-ecological conditions. These ecotypes, in turn, can be used in future breeding initiatives to develop cultivars for specific agro-ecological conditions and to fill agro-ecological niches that may become available due to climate change.

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CHAPTER 4

Dormancy-breaking treatments for *Calobota sericea*, a potential leguminous forage crop from the semi-arid rangelands of South Africa

4.1. Abstract

Native forages have been proposed as a plausible alternative to the use of exotic forage germplasm due to their adaptation to the surrounding bioclimatic and edaphic conditions, and the reduced risk of becoming weedy or invasive. *Calobota sericea* is a perennial legume species from the semi-arid rangelands of Namaqualand and is currently under investigation as a fodder crop for use within these agro-ecosystems. This species displays physical seed dormancy and therefore, we investigated methods to break its dormancy to ensure fast and uniform seed germination and establishment. Three dormancy breaking treatments, namely mechanical scarification, boiling the seeds for five minutes, and placing the seeds in boiled water and leaving them until the water has cooled to room temperature were applied to seeds of *C. sericea* collected from naturally occurring populations within the Namaqualand rangelands. After germinating the seeds in petri-dishes, mechanical scarification was found to be the most effective method to break dormancy, and once the dormancy was removed, germination commenced rapidly. However, further research is needed to determine more efficient means to scarifying larger quantities of seeds.

Key Words: forage legumes; livestock feed; Namaqualand; water-limited agro-ecosystems

4.2. Introduction

Calobota sericea (Thunb.) Boatwr. & B.-E. van Wyk is a perennial legume species (Boatwright *et al.* 2009; Nkonki 2013) that occurs naturally within the semi-arid, water-limited rangelands of South Africa, and therefore, is well adapted to the marginal bioclimatic and edaphic conditions experienced within these areas. *Calobota sericea* was observed to play an important role in livestock diets during the dry summer months in the Namaqualand region of South Africa (Samuels *et al.* 2016, Müller *et al.* 2019), and it was proposed that *C. sericea,* if developed properly, had the potential to fill the summer feed gaps within the semi-arid rangelands in Namaqualand, South Africa, and other areas experiencing similar bioclimatic conditions. The seeds of *C. sericea*, like many other legume species (Smýkal *et al.* 2014), display seed dormancy. This may limit its use within these marginal environments due to seeds not establishing early during the wet season to ensure enough biomass is produced for livestock feeds while the limited water resources are available.

Seed germination and seedling establishment are the most critical stages in the life cycle of plants, and therefore, germination timing is crucial for the survival of newly formed seedlings (Bewley and Black 1994, Foley 2001, Gresta *et al.* 2011, Walck *et al.* 2011, Baskin and Baskin 2014, Ludewig *et al.* 2014, Hu *et al.* 2015). To optimise the time of germination, and therefore, increase the survivorship of seedlings, plants have evolved a range of mechanisms to increase their chance of survival (Foley 2001). One of these mechanisms is seed dormancy (Fenner 2000, Bewley and Black 1994, Foley 2001, Do Canto *et al.* 2013).

Seed dormancy serves at least two ecologically significant roles in the survival of plants. First, it ensures the survival of plants in the absence of seed production through the development of a soil seed bank (Do Canto *et al.* 2013). Second, it enables seeds to avoid germination during periods where conditions for seedling growth are only viable for short periods of time. Therefore, by having seeds with various degrees of dormancy, plants can distribute their offspring across time, as a mechanism to overcome unpredictable and/or variable environments (Venable 2007, Poisot *et al.* 2011, Do Canto *et al.* 2013, Wills *et al.* 2014). Dormancy, therefore, is under strong environmental control, resulting in a wide range of dormancy types, each suitable for specific bioclimatic, ecological and edaphic conditions in which plants occur (Hilhorst 1995, Vleeshouwers *et al.* 1995, Bewley 1997, Li and Foley 1997, Baskin and Baskin 2004, Fenner and Thompson 2005, Finch-Savage and Leubner-Metzger 2006, Donohue *et al.* 2010, Huang *et al.* 2010, Wills *et al.* 2014).

Nikolaeva (1977) developed a classification system for different dormancy types. This classification system reflects the fact that dormancy is determined by both morphological and phenological properties of the seed. Based on this, Baskin and Baskin (1998, 2004) proposed a complete classification system of five dormancy classes. These dormancy classes include morphological dormancy (MD), where seeds have an underdeveloped embryo at the time of dispersal. Physiological dormancy (PD), where seed germination is inhibited by plant hormones. Physical dormancy (PY), where seeds have evolved water impermeable layer(s) in the seed coat or testa. Morphophysical dormancy (MD + PY), where germination is inhibited by a combination of an underdeveloped embryo and physiologically-dormant embryo, and combinational dormancy (PD + PY), where germination is inhibited by a combination of a water-impermeable seed coat and physiologically-dormant embryo (Nikolaeva 1977, Baskin and Baskin 1998, 2004).

In the Fabaceae, the primary form of dormancy is physical dormancy imposed on the seeds by the seed coat or testa (Werker *et al.* 1979, Bewley and Black 1994, Nowack *et al.* 2010, Bewley *et al.* 2013, Smýkal *et al.* 2014). Functionally, there are several constraints imposed on the seed by the seed coat. These include the obstruction of water entering the seed, interference with gaseous exchange, the prevention of germination inhibitor leakage out of the seed, the supply of germination inhibitors to the embryo, the mechanical restraint of a hard seed coat that inhibits radicle emergence and the inhibition of light penetration in species in which light plays a role in germination (Bewley and Black 1994, Wang and Grusak 2005, Nowack *et al.* 2010, Gresta *et al.* 2011, Bewley *et al.* 2013, Smýkal *et al.* 2014). Smýkal *et al.* (2014) provides a recent, in depth, review of the role of the testa on the development and establishment of dormancy in legume seeds. Generally, however, in the Fabaceae, once the constraint on germination, imposed on the seed by the seed coat is removed, germination can commence (Castello *et al.* 2013).

The breakdown of seed coat-imposed dormancy implies the rupturing of the impermeable seed coat, and the subsequent absorption of water and gasses by the seed (Argel and Paton 1999). It has been proposed, that under natural conditions, coat imposed seed dormancy could be overcome after mechanical abrasion by soil particles, decomposition of the seed coat by microbial action, ingestion and passage through the digestive tract of animals, or by cracks in the seed coat caused by crushing or partial consumption (Baskin and Baskin 2000, de Sousa and Marcos-Filho 2001, Fenner and Thompson 2005, Smýkal *et al.* 2014, Tjelele *et al.* 2012, 2015a, b).

Several artificial techniques have also been used to break dormancy imposed by the seed coat. These artificial techniques include alternating and/or constant temperature treatments (Quinlivan 1961, 1966, 1968, Quinlivan and Mellington 1962, Barrett-Lennard and Gladstones 1964, Hagon 1971, McComb and Andrews 1974, Taylor 1981, 2005, Lodge *et al.* 1990), hot-water treatments (Akinola *et al.* 1991), chemical, and/or mechanical scarification (Forbes and Watson 1992, Vilela and Ravetta 2001, Koornneef *et al.* 2002, Baskin 2003, Finch-Savage and Leubner-Metzger 2006, Chen *et al.* 2007, Castello *et al.* 2012). These techniques are used widely in agronomy to ensure fast and uniform germination and establishment of grain and forage legumes (Bewley and Black 1994; Argel and Paton 1999; de Souza and Marcos-Filho 2001, Materechera and Materechera 2001; Ali *et al.* 2011; Asci *et al.* 2011; Khaef *et al.* 2011; Kimura and Islam 2012; Avci *et al.* 2013).

To effectively reveal the agronomic potential of *C. sericea* within these areas, efficient and effective ways to break dormancy, in a manner that can also be implemented by resource poor farming communities, where the species is intended to be used, are needed. Therefore, the present study was aimed at determining the most effective methods of breaking physical seed dormancy of *C. sericea*. The pre-germinating dormancy breaking treatments that were selected in this study however, included only those that use resources that are available to the resource poor communities where these species are intended to be used.

4.3. Materials and Methods

4.3.1. Experimental procedure

Physiologically mature seeds of *C. sericea* were collected in November 2016 from naturally occurring populations within the Leliefontein communal area in the Northern Cape Province of South Africa. The communal area consists of 10 villages, and the seeds were collected from a minimum of 75 plants per sampling location from the rangelands surrounding the Leliefontein, Tweerivier, Spoegrivier and Kharkhams villages (Fig. 4.1).

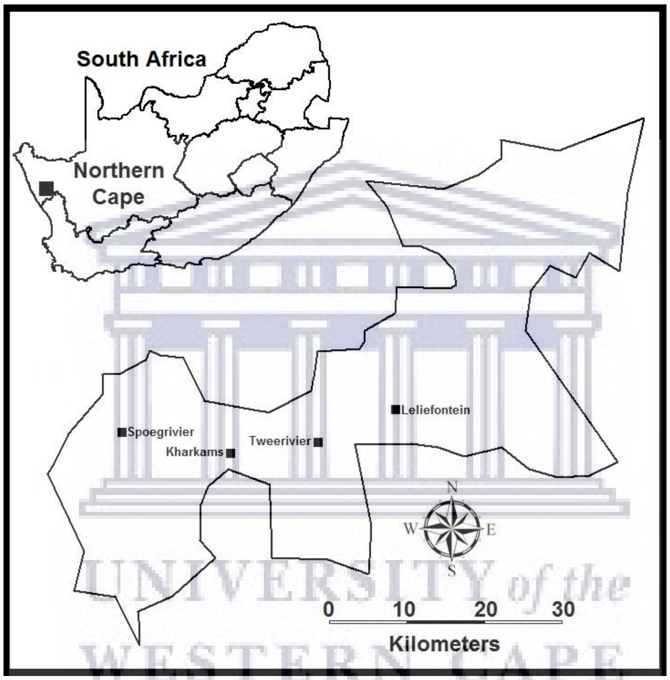

Figure 4.1: Leliefontein Communal Area with four villages from where seeds were collected.

Seeds were removed from seed pods by hand to reduce mechanical scarification of the seeds. A total of 1000 seeds (five replicates of 200 seeds) were thereafter subjected to each of three dormancy-breaking treatments: mechanical scarification with an abrasive sandpaper, boiled for five minutes or placed in boiled water (100 ml of water was used in both wet heat scarification methods) until cooled to room temperature. After applying the treatments, 50 seeds from each replicate, as well as five replicates of 50 seeds that did not undergo any of the dormancy breaking treatments (control), were immersed in 10 ml of dH_2O for 24 hours at room temperature. After 24 hours, the electrolyte leakage through the seed coats was determined by measuring the conductivity of the aqueous solution using a YSI benchtop conductivity meter (United Scientific (Pty) Ltd). A further 50 seeds from each replicate of each treatment and a control group, for each species were placed in glass petri-dishes on a layer of moist soil. The petri-dishes were placed in a growth chamber set to a 12/12 hour day/night and 25/18 °C temperature cycle. The seeds were watered as needed with dH_2O and germination was recorded every day for the duration of the experiment. Seeds with a radicle of 5 mm or longer were regarded as germinated and were removed from the petri-dishes. At the end of the trial, the percentage germinated, imbibed (seeds that were visibly swollen), dead and dormant seeds were determined. Those seeds that were dormant at the end of the trial were tested for their viability using a tetrazolium chloride test (Machlis and Torrey 1956). From these seeds, those that were viable were regarded as dormant and non-viable seeds were considered dead. From this, the germination rate, calculated as the time taken to reach 50 % of the final germination percentage (T_{50}), and germination uniformity, calculated as the time taken from 10% to 90% of the final germination percentage (T_{10} – T_{90}) were calculated following the equation of Farooq *et al.* (2004) only for treatments where more than 50 % germination was achieved.

4.3.2. Statistical analyses

Data were statistically analysed using IBM SPSS Statistics for Windows Version 22.0 (IBM Corporation, Armonk, NY, USA). Electrolyte leakage data were log transformed while the percentage germinated, dormant, imbibed and dead seeds were ARCSINE transformed. Thereafter, a one-way analysis of variance (ANOVA) with a Tukey Post-Hoc test was used to determine whether the different pre-germination treatments influenced seed coat permeability and resulted in differences in the percentage of germinated, dormant, imbibed, and dead seeds. Transformed means were back-transformed for final illustrations.

4.4. Results

The mechanical scarification and boiling treatments resulted in higher electrolyte leakage (Fig. 4.2) from *C. sericea* seeds ($F_{(3,15)} = 3.648$, $p = 0.045$).

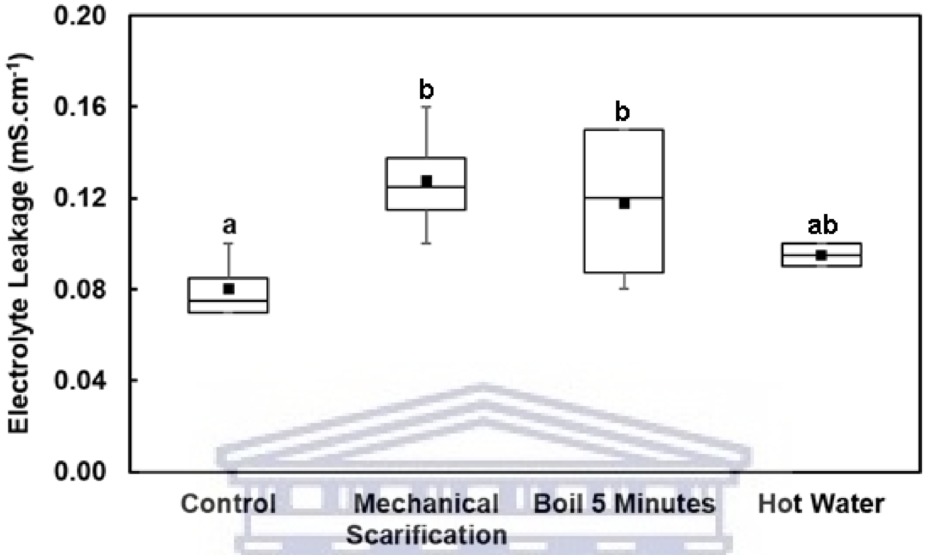

Figure 4.2: Electrolyte leakage from *Calobota sericea* seeds under different dormancy breaking treatments. Significant differences ($p \geq 0.05$) in electrolyte leakage between different treatments are indicated by different letters.

Seeds of *C. sericea* in the control group had a germination percentage of 13 %, and approximately 68 % of the seeds displayed dormancy (Table 4.1). The mechanical scarification of *C. sericea* and seeds resulted in an increase in germination from 13 % to 85 %. The boiling treatment however, resulted in 98 % seed mortality, while the hot water treatment resulted in 99 % mortality (Table 4.1).

Table 4.1: The effects of different dormancy breaking treatments on the seed germination of *Calobota sericea*. Means that were found to be statistically significantly different ($p < 0.05$) between the different pre-germinating treatments within each variable are indicated by different superscript letters.

	Germinated (%)	Imbibed (%)	Dormant (%)	Dead (%)
Control	13.2 ± 2.2^{b}	4.8 ± 1.5^{b}	68.0 ± 2.0^{b}	14.0 ± 1.9^{a}
Mechanical scarification	85.3 ± 2.3^{c}	5.6 ± 4.2^{ab}	2.7 ± 0.8^{a}	6.4 ± 1.7^{a}
Boil 5 minutes	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	2.0 ± 1.1^{a}	98.0 ± 0.0^{b}
Hot water	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	1.0 ± 0.5^{a}	99.0 ± 0.5^{b}

Germination rate (T_{50}) was only calculated for the mechanically scarified seeds, as this was the only treatment resulting in over 50 % seed germination. *Calobota sericea* was found to require approximately one week ($T_{50} = 7.2 \pm 0.1$ days) to reach 50 % of the final germination percentage, and only 4 ± 0.9 days to go from 10% to 90% of the final germination percentage, once seeds were mechanically scarified to break dormancy.

4.5. Discussion

This study indicated that *C. sericea* displayed physical seed dormancy, imposed on the seeds by a water-impermeable seed coat. Mechanical scarification proved to be the most effective method to break dormancy. Once the seed coat became more permeable, germination improved significantly, and became more rapid and uniform. This result corresponds favourably with work done on other forage legume species (Bewley 2001, Uzun and Aydin 2004, Patané and Gresta 2006, Can *et al.* 2009, Mondoni *et al.* 2013, Rodrigues-Junior *et al.* 2014, Naim *et al.* 2015). Mechanical scarification, however, may be very time consuming, especially if a large number of seeds are required. Wet heat scarification methods therefore, have been proposed as an alternative to mechanical scarification, especially when large quantities of seeds are required. Several authors have indicated that wet heat scarification resulted in a significant improvement in germination of many legume species (Mondoni *et al.* 2013, Rodrigues-Junior *et al.* 2014, Naim *et al.* 2015). In this study, however, the boiling water treatment did not improve germination ability but rather resulted in significant seed mortality. This could possibly be due to the temperature being too high, or the duration of exposure being too long, and thus, injuring or killing the embryos, resulting in the significant mortality observed (McDonnell *et al.* 2012). Similar results were found for *Lessertia frutescens* by Shaik *et al.* (2008) who exposed seeds to hot water (80 °C) for 10 and 30 minutes, respectively. These authors found that applying these treatments improved germination from an initial 10% to 50% and 65%, respectively at the two different durations, 10 and 30 minutes, respectively. It is therefore possible that germination after hot water treatments could differ with different exposure times to different temperature treatments (Baskin and Baskin 1997, Shaik *et al.* 2008, McDonnell *et al.* 2012), and therefore, should be investigated further for *C. sericea*.

4.6. Conclusion

In conclusion, in ecosystems where out of season rainfall might occur, seed dormancy prevents the germination of seeds outside of the normal growing season (Bewley and Black 1994, Do Canto *et al.* 2013). However, under agricultural conditions, the objective is to have all seeds sown establish rapidly and uniformly when conditions become favorable. Due to the short rainfall season experienced within the semi-arid rangelands of South Africa (Jordaan *et al.* 2013), rapid seedling establishment early in the growing season when rainfall events are more common, could significantly increase seedling survival. Our results indicate that mechanical scarification was the most effective method evaluated to break dormancy of *C. sericea*. However, further research is needed to determine more efficient means to scarifying larger quantities of seeds, especially due to the fact that wet heat scarification was found to not be a viable option for *C. sericea*.

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CHAPTER 5

The effects of temperature, water availability and seed burial depth on seed germination and seedling establishment of *Calobota sericea* (Fabaceae)

5.1. Abstract

Calobota sericea is a perennial legume species from South Africa that has the potential to be implemented as a fodder crop in water-limited agro-ecosystems. Apart from dormancy breaking requirements, no information regarding the germination and seedling establishment requirements of *C. sericea* is currently available. Therefore, the aim of this study was to determine the germination and establishment ability of *C. sericea* at different temperatures, water potentials and seed burial depths. In the first trial seeds were incubated at constant temperatures of 5 °C to 30 °C at 5 °C increments and in the second trial, seeds were incubated at 20 °C, at water potentials ranging from 0 to - 1.0 MPa. The seedling emergence trial (third trial) consisted of a complete randomized block greenhouse trial where seeds were sown at five burial depths (1 cm to 5 cm), and seedling emergence was recorded daily. Seeds were found to germinate best at temperatures ranging between 10 °C and 20 °C, but still had a germination percentage greater than 80 % at 5 °C. *Calobota sericea* seeds were also found to require a water potential of not lower than – 0.3 MPa to reach a germination percentage of 60 %, below which germination was severely reduced. Seedling emergence was found to be highest at burial depths of 2 cm to 4 cm. After establishment, seedlings generally displayed a decrease in shoot height with increasing burial depth, but an increase in seedling mass. More resources, in terms of mass, were allocated to early root development as opposed to leaves and stems. Results from the current study therefore suggests that *C. sericea* seeds can be planted between 2 and 3 cm deep, during early winter season, when temperatures are lower, and rainfall more prevalent and less erratic.

Keywords: fodder crops; perennial legume; livestock feed; forage legumes

5.2. Introduction

5.2.1. Temperature and osmotic requirements for germination

The success with which seeds germinate and establish is closely related to the favorability of their surrounding environmental conditions (Bewley and Black 1994). Several bioclimatic and edaphic factors such as water, oxygen, light, temperature, soil pH and seed burial depth, influence both the success and the rate of seed germination (Bewley and Black 1994, Cristaudo *et al.* 2007, Qu *et al.* 2008, Gresta *et al.* 2010). Among these factors, temperature and moisture availability, as well as the depth at which seeds are buried, are considered some of the most important (Bewley and Black 1994; Chen and Maun 1999; Guo *et al.* 2001; 2009; 2010; Fenner and Thompson 2005; Maraghni *et al.* 2010; Windauer *et al.* 2011; Singh *et al.* 2013; Thompson and Ooi 2013; Hu *et al.* 2015; Naim and Ahmed 2015; Parmoon *et al.* 2015; Patané *et al.* 2016). This is especially true within semi-arid and arid environments where the water needed for seed germination and subsequent seedling growth is limited and available only for short periods (Hu *et al.* 2015). Within these water-limited areas in which seeds have to survive, they generally cannot persist for extended periods on the soil surface. This is primarily due to out of season rainfall events, which could trigger seed germination with no subsequent rainfall, which would result in seedling desiccation and death or the threat of predation (Thompson *et al.* 1993; Grime 2001). Consequently, within these water-limited environments, successful seedling establishment depends not only on rapid germination, but also on the ability of seeds to germinate under low water availability and steadily increasing temperatures (Fischer and Turner 1978; Windauer *et al.* 2011), and how well the seeds can establish from deeper burial depths.

For germination to commence, after the release of dormancy, seeds have to imbibe water from the soil (Bewley and Black 1994; Studdert *et al.* 1994; Singh *et al.* 2013). Soil water potential can influence seed germination either directly through changes in water content and hydraulic conductivity in the soil or, indirectly through physiological processes that occur during imbibition and seed germination (Lindstrom *et al.* 1976; Singh *et al.* 2013). In most seeds, germination takes place only when a critical moisture level is attained in the seed. The base water potential that is required for germination has been found to vary greatly among species, but is closely related to the environmental conditions in which the species occur (Evans and Etherington 1990; Fenner and Thompson 2005). For instance, seed germination of species adapted to drier environments are usually affected less by water stress than those adapted to wetter environments (Evans and Etherington 1990). In general, however, when the water potential of the germination medium decreases from the optimum, for a specific species, germination may be delayed or inhibited according to the extent of the water potential decline (Hegarty 1978; Singh *et al.* 2013; Patané *et al.* 2016).

In arid and semi-arid ecosystems, the adverse effects of water constraints on seed germination may be further aggravated by the exposure of seeds to temperatures that are either below or above the optimum germination temperatures (Patané and Tringali 2011; Patané *et al.* 2016). Temperature is the major determinant of germination rate, once seed dormancy has been released (Fenner and Thompson 2005; Baskin and Baskin 2014; Hu *et al.* 2015). Three 'cardinal temperatures' (minimum, optimum and maximum temperatures) generally characterize germination responses to temperature. The minimum or base temperature (T_b) and maximum or ceiling temperature (T_c) are those temperatures below and above which germination will not occur, respectively, while the optimum temperature (T_o) is the one at which germination is most rapid (Bewley and Black 1994; Bradford 2002; Hu *et al.* 2015). These 'cardinal temperatures' for germination are generally related to the environmental conditions to which the species are adapted in their native environments. These cardinal temperature ranges generally match the most favorable times for seed germination and subsequent seedling growth and development (Hu *et al.* 2015). Previous studies have shown large variations in the 'cardinal temperatures' among species as well as within species (Steinmaus *et al.* 2000; Phartyal *et al.* 2003; Hardegree 2006). These differences however, can often be related to ecological or geographical factors (Ascough *et al.* 2007; Luna *et al.* 2012; Hu *et al.* 2015). These 'cardinal temperatures' and base water potentials for germination are important when describing the patterns of germination in environments in which seeds have to germinate. They can provide a measure of yield, and are frequently used as indices to screen germplasm and rank potential temperature and water stress responses of different species (Covell *et al.* 1986; Ellis *et al.* 1986; Bradford 2002; Trudgill *et al.* 2005; Hardegree 2006; Hu *et al.* 2015).

5.2.2. Seed burial depth requirements for seedling establishment

When all requirements for seed germination are met, the depth at which seeds are buried becomes one of the most important factors that influences successful seed germination, seedling emergence and the rate of seedling emergence (Barnett 1977; Bewley and Black 1994; Chen and Maun 1999; Guo *et al.* 2001; 2009; 2010; Fenner and Thompson 2005; Thompson and Ooi 2013). The depth at which seeds are buried often results in seeds being subjected to different germination conditions (Borchert *et al.* 1989; Bewley and Black 1994). Seed burial, in contrast to seeds on the soil surface, may offer benefits to seed germination and successful seedling establishment. These benefits include a reduction in air exposure, maintenance of high levels of humidity, protection against extreme temperatures and reduced predation (Forcella *et al.* 2000). In many plant species shallow seed burial depths result in improved seed germination and subsequent seedling establishment (Bewley and Black 1994; Maun 1998; Benvenuti *et al.* 2001a,b; Müller *et al.* 2018). Deeper seed burial depths however, may have inhibitory effects on seed germination, seedling emergence and the rate of seedling emergence (Bewley and Black 1994; Maun 1994). Species-specific traits such as seed size and the amount of internally stored seed reserves determines the success with which seedlings will establish from different burial depths (Benvenuti 2007; Müller *et al.* 2018). Several studies have shown that larger seeds can establish from greater seed burial depths, which can be attributed to the larger quantities of internally stored food reserves found in these seeds that can facilitate upward growth towards the soil surface (Bewley and Black 1994; Bewley 1997; 2001; Baskin and Baskin 1998; Mennan and Ngouajio 2006; Müller *et al.* 2018).

Plant species that are able to establish from greater seed burial depths, are usually those that show some plasticity in their early seedling development in response to their environment. The successful emergence of seedlings from greater burial depths is attributed to the ability of seedlings to allocate more resources to the effective elongation of the coleoptile or hypocotyl (Maun and Riach 1981; Maun and Lappiere 1986; Redmann and Qi 1992; Wu *et al.* 2011). Because seedlings emerging from deeper seed burial depths must grow through a thicker layer of soil to reach the soil surface, they usually emerge later than seeds emerging from shallower burial depths (Cussans *et al.* 1996; Benvenuti *et al.* 2001a,b; Ren *et al.* 2002; Müller *et al.* 2018). Seedlings emerging from deeper burial depths usually also show an initial reduced above ground seedling growth due to the depletion of food reserves used to facilitate stem elongation in order for their shoot apices to reach the soil surface (Bewley and Black 1994; Li *et al.* 2006). This in turn, results in inferior competitiveness for limiting resources, which can lead to impaired biomass production and can also negatively impact the fitness of the plant in its later life stage (Cook 1980).

On the other hand, under certain environmental and bioclimatic conditions, deeper seed burial depths can improve seedling emergence and subsequent seedling survival and growth. In arid and semi-arid regions, and especially under rain-fed farming systems, deeper seed burial may be advantageous to seedling establishment and survival. This is because under dry conditions, seeds may germinate too quickly after the first rains when planted at too shallow depths and the seedling, after emergence, will suffer desiccation if the time between rainfall events are too long (Heckman *et al.*, 2002). The depth at which seeds are planted is therefore, usually a compromise between faster establishment from shallow burial depths, and better root development, moisture and nutrient availability associated with an increased seed burial depth (Bartholomew 2000). Previous studies have shown that there is an optimum seed burial depth range that will maximize seed germination, seedling establishment and subsequent seedling growth and survival. This optimum burial depth range however, differs between different plant species (Maun and Lappiere 1986, Huang and Gutterman 1998, 1999, 2000, Chen and Maun 1999, Ren *et al.* 2002, Huang *et al.* 2004, Zhu *et al.* 2004, Müller *et al.* 2018). For most commercially available plant species, or those deemed as economically and/or agronomically important, the optimum seed planting depth is well defined. However, for plant species still under investigation for their economic and/or agronomic importance, it is important to determine the optimum seed burial depth range under which these species can successfully, and rapidly establish.

Calobota sericea (Thunb.) Boatwr. & B.-E. van Wyk is a perennial legume which occurs primarily within the semi-arid rangelands of the Northern Cape Province of South Africa. The Northern Cape is characterized by large arid and semi-arid plains with a mean annual precipitation of 141 mm, but this ranges from 20 mm in the far West and up to 540 mm in the East. Temperatures vary from as low as -10 °C in winter to temperatures often exceeding 40 °C in the summer months (Palmer and Ainslie 2006, Jordaan *et al.* 2013). *Calobota sericea* has been found to do well within these rangelands and is currently being investigated as a fodder crop for use within water-limited agro-ecosystems within the Northern Cape and other areas experiencing similar bioclimatic conditions (Müller *et al.* 2017a,b). Very little is currently known about the eco-physiology of *C. sericea* and therefore, it is important to obtain a greater understanding of how the seeds of *C. sericea* will respond to different germination temperatures, different levels of water-limitation, and different seed burial depths, as these could play a major role in the establishment of these forages under the marginal conditions experienced within the targeted areas for pasture production.

The aim of this study was therefore to determine the 1) germination temperature optima; 2) germination base water potentials; and 3) the optimum sowing depth for *C. sericea*.

5.3. Materials and Methods

5.3.1. Seed collection and seed-lot properties

Seeds of *C. sericea* were collected from naturally occurring populations in the Leliefontein communal rangelands in the Northern Cape Province of South Africa in November 2016. The communal area consists of 10 villages, and the seeds were collected from a minimum of 75 plants per sampling location from the rangelands surrounding the Leliefontein, Tweerivier, Spoegrivier and Kharkhams villages (Fig. 5.1) after which all seeds collected were pooled to form a single seed-lot.

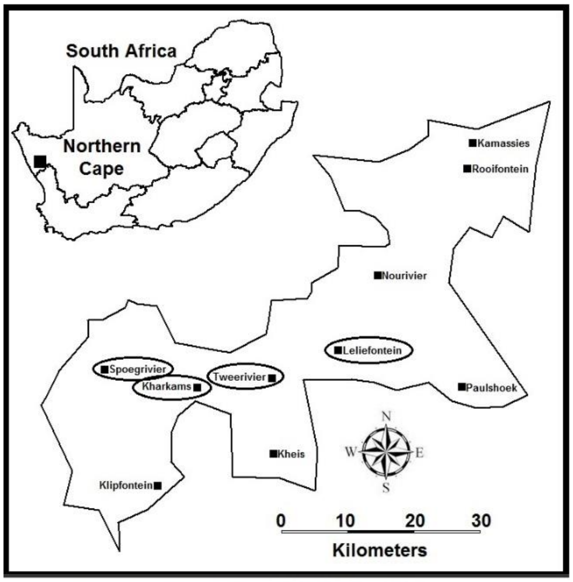

Figure 5.1: Leliefontein Communal Area in the Northern Cape Province of South Africa where seeds of *Calobota sericea* were collected from the four highlighted villages.

Seeds were regarded as mature when seed pods became brown and hard, and a number of seed pods on the parent plants had already opened and lost their seeds. After collection, seeds were removed from the seed pods by hand to reduce injury. Thereafter, a portion of the collected seeds were surface sterilized by soaking them in 1 % sodium hypochlorite (NaClO) (v/v) for 10 seconds followed by rinsing the seeds with deionised water (dH_2O). Germination characteristics of the seed-lot can be obtained from Müller *et al.* (2017b) and chapter 4. After sterilizing, following the results of Müller *et al.* (2017b) the seeds were mechanically scarified using an abrasive sand paper to remove the seed-coat imposed dormancy.

5.3.2. Seed germination potential at different temperatures

A total of 50 mechanically scarified seeds were sown on two sheets of moist filter paper in 120 mm glass Petri-dishes. These were placed in growth chambers set at constant temperatures of 5°C to 30°C at 5°C increments. Four replicates were prepared for each temperature treatment. All seeds were germinated in the dark, and the seeds were counted daily for germination. Seeds were considered to have germinated when a radicle of at least 5 mm was visible, and were removed from the Petri-dishes. The time (t_g) to different germination percentages (i.e. 10 % – 90 %) were calculated using equation 1 (Farooq *et al.*, 2004), for those temperatures where final germination percentage was greater than 50 %. Germination uniformity was then calculated as the time (days) taken to go from 10 % to 90 % of the final germination percentage

Equation 1: $t_g = t_i + \{[((N \times (g/100))-n_i) \times (t_j - t_i)]/(n_j - n_i)\}$ where: N is the final number of germinating seeds, g = germination percentile i.e. 10% - 90% and n_j and n_i are the cumulative number of seeds germinated by adjacent counts at times t_j and t_i, respectively, when $n_i < (N*(g/100)) < n_j$.

5.3.3. Seed germination at different water potentials

The effects of water potential on germination was examined by germinating a total of 50 *C. sericea* seeds at 20 °C in the dark, at water potentials of 0, -0.1, -0.2, -0.3, -0.4, -0.5, −0.6, -0.7, -0.8, −0.9 and -1.0 MPa. Polyethylene glycol 6000 (PEG) solutions were used to adjust the osmotic potential of dH_2O according to the method of Michael and Kaufmann (1973). For each treatment, four replicates of 50 mechanically scarified seeds were placed in 90 mm glass petri dishes on filter paper moistened with 2.5 mL of PEG solution or dH_20 (control). The petri dishes were closed to reduce evaporation. Seeds were transferred to new filter paper with new solution every 48 h to ensure relatively constant water potential in the treatments. Seeds were counted daily for germination and were considered to have germinated when a radicle of at least 5 mm long was visible. At each counting, all germinated seeds were removed from the petri-dishes.

5.3.4. Seedling establishment at different seed burial depths

A complete randomize block trial was conducted under greenhouse conditions. The greenhouse temperatures were set at 20°C daytime and 10°C night time temperatures, with natural day/night light cycles. Within the randomized block trial, a total of 15 seeds of *C. sericea* were planted in 9 cm diameter and 12 cm depth pots, at sowing depths (1, 2, 3, 4 and 5 cm), with three replicates. The entire block design was then further replicated three times. Seeds were sown in soils collected from where the seeds were harvested in the Leliefontein communal rangelands. Before planting the pots were watered to field capacity and were allowed to drain for 24 hours. Thereafter, the pots were watered once a week to capacity (until excess water dripped from the bottom of the pots) for the duration of the study.

5.3.4.1. Measurements

Emergence was recorded daily for 30 days after which the seedlings were removed from the pots. After removal, five randomly selected seedlings from each pot were separated into roots, stems and leaves. The stems were further divided into below ground stems, taken from the soil surface to where the roots start, and above ground stems, taken as all stems above the soil surface (Seiwa *et al.* 2002, Li *et al.* 2006, Wu *et al.* 2011). The roots and below ground stems were rinsed with dH_2O and blotted dry. Thereafter, the lengths of all plant components for all seedlings that emerged were measured using a digital caliper. The plant materials were then oven dried at 65°C until a constant mass was achieved. Dry mass of each plant component was determined, however, due to the small sizes of the plants these were grouped into composite samples of three plants resulting in five replicates of each treatment across all three trials.

5.3.5. Statistical Analyses

All data was statistically analyzed using IBM SPSS Statistics for Windows Version 22.0 (IBMCorporation, Armonk,NY, USA) and tested for normality using a Shapiro-Wilk test. Where necessary, data was subjected to log or ARCSINE transformations after which the data was subjected to a one-way analysis of variance (ANOVA) with a LSD Post-Hoc test. Log and ARCSINE transformed means were back-transformed for final illustrations. The emergence rate from different seed burial depths, were calculated as the time taken to reach 50% of the final emergence percentage (T_{50}), following the equation of Farooq *et al.* (2004). Resource allocation was calculated using the mass of each plant part, as a percentage of the final seedling mass.

5.4. Results

5.4.1. *The effect of temperature on seed germination*

Cumulative germination was highest at temperatures of 10, 15 and 20°C. Below and above these temperatures germination decreased significantly ($F_{(5,23)} = 167.569$, $p < 0.001$) (Fig. 5.2). At 5 °C the germination rate was significantly lower ($F_{(4,19)} = 16.890$, $p < 0.001$) than at the higher temperatures, which did not differ from each other. No difference in germination uniformity was found between temperatures ranging from 10°C to 20°C, but differed significantly ($F_{(4,18)} = 10.826$, $p < 0.001$) to germination uniformity at 5°C and 25°C (results not shown).

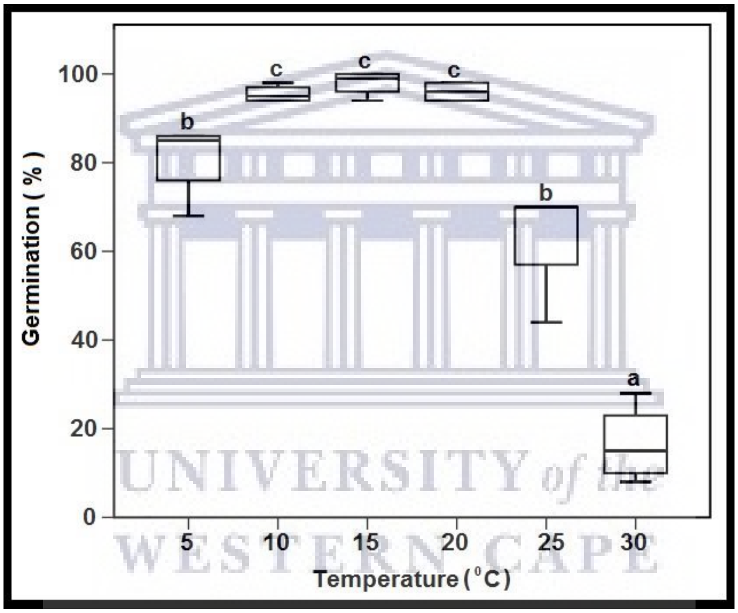

Figure 5.2: Seed germination of *Calobota sericea* under different temperatures. Boxes provide the median (line in the box), upper and lower quartiles (box), and the mean (middle of the box). Different letters indicate significant differences ($p < 0.05$) in germination between germination temperatures.

5.4.2. *The effect of water limitation on seed germination*

Cumulative germination was highest at water potentials of 0, -0.1 and -0.2 MPa after which germination decreased significantly ($F_{(10,43)} = 58.828$, $p < 0.001$) (Fig. 5.3). The final germination percentage exceeded 50% at water potentials ranging from 0 to -0.3 MPa, decreasing rapidly to no seeds germinating after -0.7 MPa (Fig. 5.3).

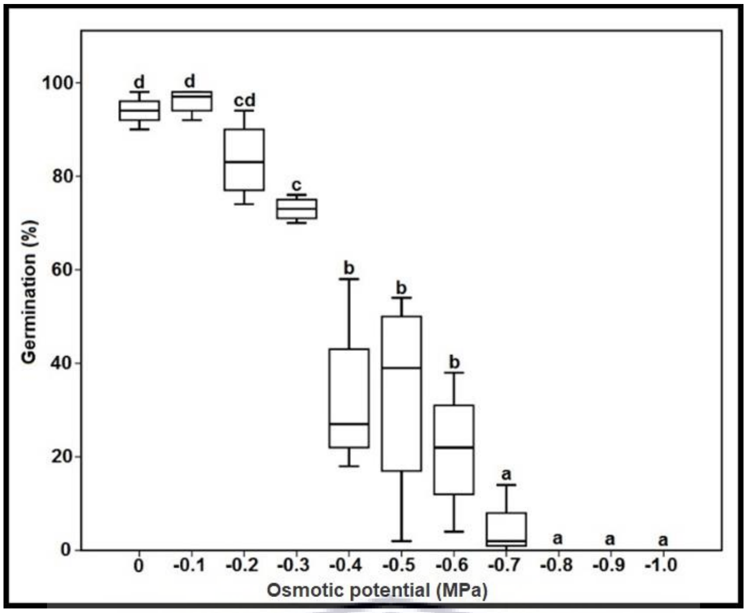

Figure 5.3: Seed germination of *Calobota sericea* at 20˚C under different osmotic potentials (MPa). Boxes provide the median (line in the box), upper and lower quartiles (box), and the mean (middle of the box). Different letters indicate significant differences ($p < 0.05$) in germination between different osmotic pressures.

The time taken to reach different germination percentiles (Table 5.1) increased significantly as the osmotic potential of the germination medium increased (i.e. became more negative). For *C. sericea* seeds collected from the Namaqualand area, a water potential of between 0 and -0.2 MPa resulted in 50% seed germination in approximately 4 days, whereas a water potential of -0.3 MPa was found to increase the time to 50% germination to approximately 11 days (Table 5.1).

Table 5.1: Mean time (days ± SEM) taken by *Calobota sericea* seeds to reach different germination percentiles, under different water availability treatments at 20˚C. Different letters indicate significant differences ($p < 0.05$) in germination between different water potentials (Ψ) at each germination percentile.

Germination percentiles (%)	**Ψ (MPa)**							**Significance**
	0	**-0.1**	**-0.2**	**-0.3**	**-0.4**	**-0.5**	**-0.6**	
10	3 ± 0.17^{a}	3 ± 0.12^{ab}	3 ± 0.07^{bc}	4 ± 0.14^{cd}	4 ± 0.10^{de}	6 ± 2.01^{e}	9 ± 0.45^{f}	$F_{(6,25)} = 19.080$; $p < 0.001$
20	3 ± 0.02^{a}	3 ± 0.20^{a}	4 ± 0.14^{a}	6 ± 1.02^{b}	6 ± 0.62^{b}	7 ± 1.77^{b}	14 ± 1.15^{c}	$F_{(6,25)} = 18.631$; $p < 0.001$
30	3 ± 0.02^{a}	3 ± 0.03^{a}	4 ± 0.14^{a}	6 ± 1.11^{b}	12 ± 6.34^{b}	9 ± 1.98^{b}	-	$F_{(5,20)} = 7.068$; $p = 0.001$
40	3 ± 0.02^{a}	3 ± 0.03^{a}	4 ± 0.19^{a}	8 ± 0.98^{b}	-	-	-	$F_{(3,15)} = 47.384$; $p < 0.001$
50	4 ± 0.02^{a}	4 ± 0.02^{a}	4 ± 0.14^{a}	11 ± 0.88^{b}	-	-	-	$F_{(3,15)} = 152.04$; $p < 0.001$
60	4 ± 0.02^{a}	4 ± 0.02^{a}	5 ± 0.16^{b}	13 ± 0.20^{c}	-	-	-	$F_{(3,15)} = 940.02$; $p < 0.001$
70	4 ± 0.02^{a}	4 ± 0.03^{a}	5 ± 0.12^{b}	13 ± 0.41^{c}	-	-	-	$F_{(3,15)} = 871.09$; $p < 0.001$
80	4 ± 0.02^{a}	4 ± 0.10^{a}	6 ± 1.50^{a}	-	-	-	-	$F_{(2,11)} = 2.537$; $p = 0.134$
90	8 ± 2.33^{a}	5 ± 0.40^{a}	-	-	-	-	-	$F_{(1,7)} = 1.850$; $p = 0.223$

5.4.3. Seed germination at different seed burial depths

5.4.3.1. *Seedling emergence and seedling mass*

A general trend of first increasing seedling emergence from 1 cm to 2 cm was observed (Fig. 5.4) thereafter, no difference was observed in seedling emergence from 2 cm to 4 cm, after which seedling emergence significantly decreased at a burial depth of 5 cm ($F_{(4,44)} = 5.940$, $p < 0.01$). Therefore, in order to obtain at least 60 % successfully emerged seedlings, seeds of *C. sericea* can be planted as shallow as 2 cm or to a depth of 4 cm, otherwise seedling emergence is significantly reduced. Seedling emergence rate (T_{50}) was calculated only for burial depths where at least 50 % seedling emergence were obtained i.e. 2 cm, 3 cm and 4 cm. At these seed burial depths, time to 50 % seedling emergence took 7 ± 0.3, 7 ± 0.4 and 8 ± 0.4 days, respectively, and did not differ significantly ($p \geq 0.05$) between the different burial depths (results not shown). Total seedling mass increased significantly ($F_{(4,24)} = 5.644$, $p = 0.003$) with increased seed burial depth. However, this was only observed after 2 cm burial depth. When divided into different plant parts, root mass was found to not differ between plants sown at 1 cm and

2 cm burial depth however, significantly increased below 2 cm burial depth ($F_{(4,24)} = 6.099$, $p = 0.002$). Leaf and stem mass did not differ significantly ($p \geq 0.05$) between the different seed burial depths (Fig. 5.4).

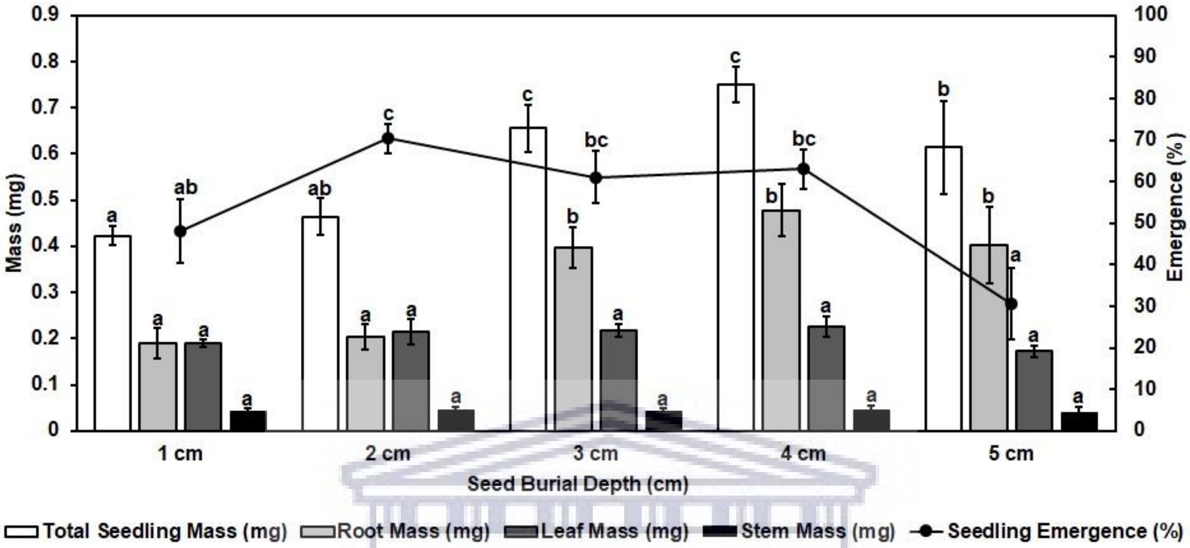

Figure 5.4: Seedling emergence (%) and seedling mass (mg) *of Calobota sericea* seedlings planted at different depths. Significant differences in seedling emergence, whole seedling, root, leaf and stem (below + above ground stems) mass between different seed burial depths are indicated with different letters. Comparisons were made for each plant component and emergence, across burial depths and not within burial depths.

5.4.3.2. *Seedling length and resource allocation (in terms of seedling mass)*

Root length tended to decrease significantly ($F_{(4,73)} = 3.156$, $p < 0.05$) with increased seed burial depth, however, total shoot length (above + below ground stems + leaves) did not differ significantly ($p \geq 0.05$) between the different burial depths. The above ground shoot length (leaves + above ground stems), however, decreased significantly after 3 cm burial depth, but did not differ between 1 cm to 3 cm burial depth ($F_{(4,73)} = 7.474$, $p < 0.001$) (Fig. 5.5A). Total stem length generally increased with increasing burial depth ($F_{(4,73)} = 5.478$, $p < 0.01$). However, when considering the above and belowground stems independently, the above ground stems were found to decrease with increasing seed burial depth ($F_{(4,73)} = 7.072$, $p < 0.001$) while the below ground stem lengths increased ($F_{(4,73)} = 14.990$, $p < 0.001$) (Fig. 5.5B). At shallow burial depth (1 cm and 2 cm), resource allocation (calculated as the % of each plant part i.e. roots stems and leaves contribute to the total plant) between the roots and leaves did not differ, however, from 3 cm burial depth, resource allocation was significantly more towards root growth and development than shoot (stems and leaves) growth (Fig. 5.6).

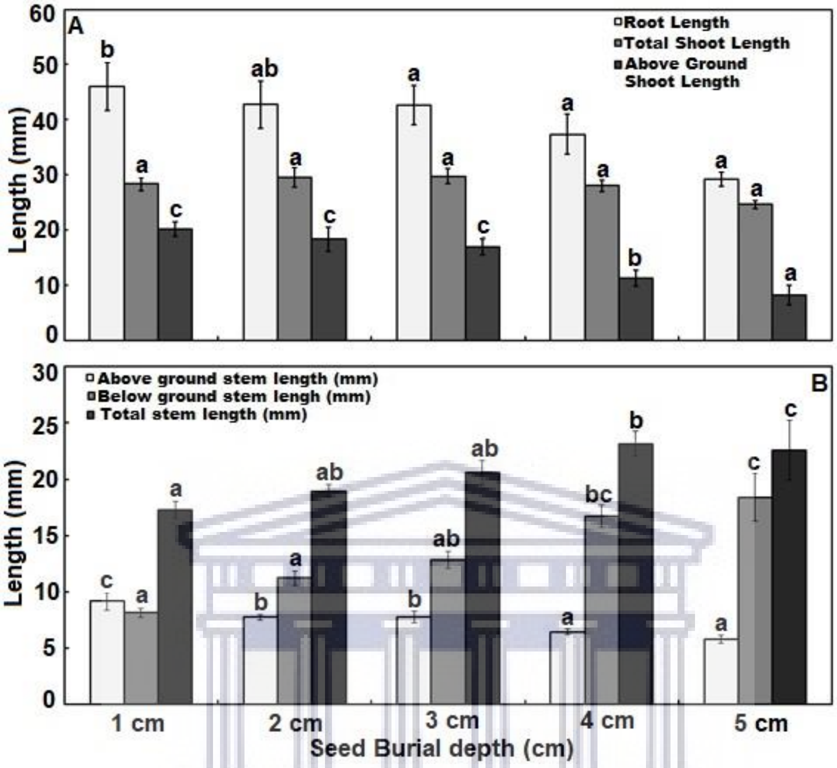

Figure 5.5: A: Root, total shoot (below ground stem + above ground stems with leaves), and above ground shoot length and B: below ground, above ground, and total stem length (mm) *of Calobota sericea* seedlings planted at different depths. Significant differences ($p < 0.05$) within root and shoot lengths between different seed burial depths are indicated with different letters.

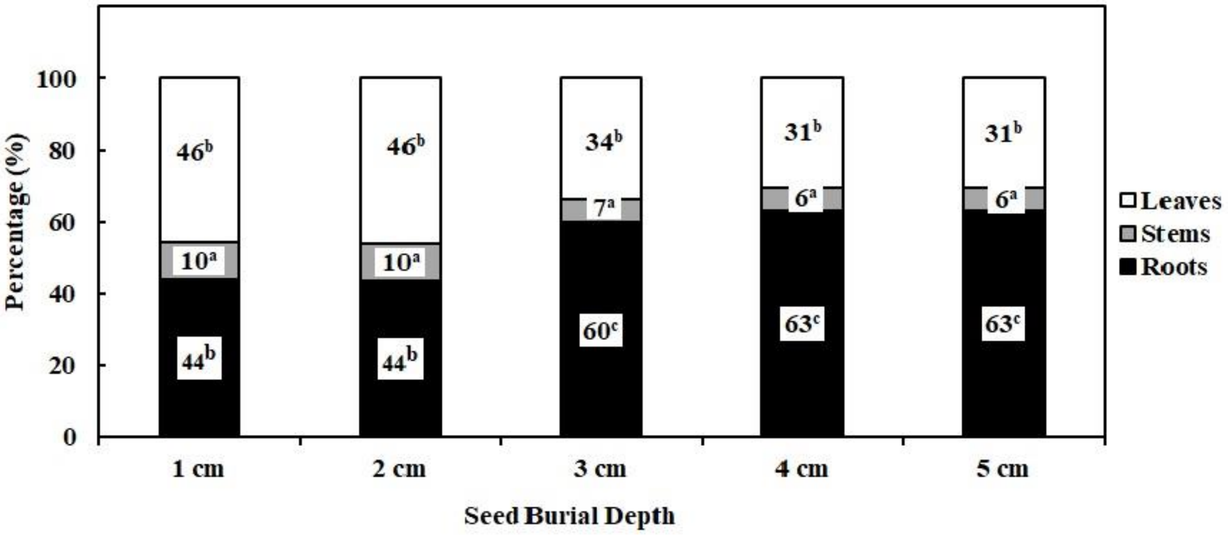

Figure 5.6: Resource allocation (%) in *Calobota sericea* seedlings planted at different depths. Significant differences ($p < 0.05$) in resource allocation within each burial depths are indicated with different letters.

5.5. Discussion

5.5.1. Seed germination at different temperatures and water potentials

Results from the current study indicated that the germination and establishment of *C. sericea* collected from the rangelands of Namaqualand is under strong environmental control, with narrow moisture requirement ranges of 0 to -0.3 MPa to reach at least 60 % germination, but a wider temperature requirement range of 5 °C to 20 °C to obtain a germination percentage of greater than 80 %. Previous work by Müller *et al.* (2017a) indicated that the distribution of *C. sericea* in South Africa is limited by both temperature and precipitation variables. This strong bioclimatic limitation to the germination and establishment of *C. sericea* could explain its current distribution pattern, which falls within the winter rainfall regions of the Northern Cape and Western Cape Provinces of South Africa (Müller *et al.*, 2017a, Boatwright *et al.*, 2018). These regions are known for their Mediterranean type climate, with cold and wet winters, and warm and dry summers. Many authors have indicated that the success, and rate, of germination at different germination temperatures and/or osmotic stress levels, can be related to the ecological and geographical conditions from where the seeds were collected (Ascough *et al.*, 2007, Luna *et al.*, 2012, Hu *et al.*, 2015). Within the Leliefontein communal rangelands, rainfall tends to be highest from May to August (14 to 27 mm), and this coincides with the lowest daily minimum temperatures (5 ± 0.3 °C). This could indicate why seeds of *C. sericea* collected from the Leliefontein communal rangelands could still germinate well at temperatures of 5 °C, but tended to only tolerate a relatively low water stress level, in this study ranging from 0 MPa to - 0.3 MPa.

The fact that *C. sericea* can germinate well at low temperatures suggest that it can germinate and establish early in the winter season when rainfall is usually the highest. This could possibly explain the relatively high soil water requirements of at least -0.3 MPa, found in this study, below which germination noticeably declines. The ability of *C. sericea* to potentially germinate and establish early, when winter temperatures are low, suggest that their seedlings will have longer access to winter water resources, thus, facilitating early growth and allowing for a longer growth period. This, in turn, results in more robust seedlings, with well-developed and established root systems that could allow them to access deeper sub-surface water resources when the above ground water resources dry up during summer. Further research is therefore needed to determine the impacts of low temperatures on the rate of seedling emergence and subsequent seedling growth.

5.5.2. Seedling emergence at different sowing depths

Seedling emergence at sowing depths of 1 cm and 5 cm, was found to be significantly lower than seedling emergence from 2 cm, 3 cm, and 4 cm. The poor emergence experienced at 1 cm sowing depth in this study, can be attributed to the top soil layer drying out more rapidly, resulting in the desiccation of the germinated seeds. Under water-limited conditions, where rainfall events are often far apart, and the top soil is often subjected to regular drying, deeper sowing depth (i.e. greater than 2 cm) is advantageous as it provides the seeds with increased moisture availability for longer periods. This in turn, allows the seedling to develop deeper root systems to access deeper water sources (Heckman *et al.*, 2002). Edaphic factors such as soil mass and texture could significantly influence seedling emergence from deeper sowing depths (Bewley and Black, 1994), however, at deeper sowing depths, seedling emergence above the soil surface is often related to the primary seed storage reserves available to the seedling to facilitate early seedling growth (Bewley and Black, 1994, Bewley, 2001). Seeds with greater storage reserves can produce longer hypocotyls thus, facilitating their emergence above the soil surface (Bewley and Black, 1994).

The reduced seedling emergence experienced at 5 cm sowing depth could be as a result of seeds exhausting their storage reserves while growing towards the soil surface. This is further substantiated in the present study where it was evident that as seed burial depth increased, there was an increase in the below ground stem lengths, and a decrease in the above ground stem lengths. Such plasticity in the response of seedling morphology suggests that *C. sericea* seedlings will allocate more resources to the upward growth of hypocotyls to ensure successful emergence from deeper sowing depths. These plastic responses in seedling morphology in response to the surrounding environmental conditions have also been reported in numerous other plant species (Forcella *et al.*, 2000; Benvinuti *et al.*, 2001a,b; Seiwa *et al.*, 2002; Li *et al.*, 2006; Ghaderi-Far *et al.*, 2010; Wu *et al.*, 2011).

Even though the length of the roots decreased with increasing sowing depth, the roots produced at the deeper sowing depths were significantly heavier than those produced from seeds at shallower sowing depths. Root systems that are found deeper below the soil surface generally have access to deeper water resources and nutrients such as nitrogen, which tends to leach into the deeper soil layers (Wasson *et al.*, 2012; Paez-Garcia *et al.*, 2015). At shallower sowing depths, plants have to produce longer root systems to actively mine for these resources, which, in turn, results in more resources being spent on the downward growth of roots, resulting in thinner, longer root systems that are lighter in mass. The size of a plant’s root system is a key trait that will influence the uptake of resources from the soil, and should be considered in relation to the size of the above ground plant parts. In this study, it was clear that at each of the sowing depths, significantly less resources were allocated to stem development. At shallower sowing depths i.e. 1 cm and 2 cm, resources were partitioned equally (not statistically different) between root and leaf production. At deeper (greater than 2 cm) sowing depths, significantly more resources were allocated to root development. This is believed to be an adaptation to early seedling development in unfavorable conditions. The initial allocation to root development will allow the seedlings access to deeper water and nutrient resources, which later, could allow for better shoot growth.

5.6. Conclusion

The distribution of *C. sericea* within the winter rainfall regions of the Northern Cape and Western Cape Provinces reflects the germination sensitivity of the species with regard to water requirements, and also the ability to germinate successfully at low temperatures. The current results therefore suggested that *C. sericea* can be planted early during the winter season, when temperatures are lower, and rainfall more prevalent and less erratic. *Calobota sericea* seeds could potentially also be planted to depths between 2 and 3 cm and still obtain a minimum stand of at least 60 %. Results from this study however, reflect the germination and seedling emergence requirements of seeds obtained from the Leliefontein communal rangelands and therefore, the results cannot necessarily be projected for seeds collected from other areas. However, the significant germination potential of *C. sericea* even at temperatures of 20 ˚C and 25 ˚C suggest its potential to be established within the summer rainfall regions of South Africa, where rainfall periods are often associated with high temperatures (Chapter 3). This should be evaluated further as this could allow for the expansion of the agronomic potential of *C. sericea* into these areas. In order to characterize the germination and establishment characteristics of the species, further research into the germination and seedling emergence requirements for seeds collected from different regions, with different bioclimatic conditions is still needed, in order to characterize the variability between different populations.

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CHAPTER 6

Morphological and physiological responses of *Calobota sericea* plants subjected to water-limitation and subsequent re-watering

6.1. Abstract

Calobota sericea is a native legume confined to the water-limited rangelands of South Africa, and has recently been prioritised for further characterisation for its pasture potential. In this study, we examined the growth characteristics of *C. sericea* under glasshouse conditions where water-limitation was imposed at different plant ages. Results indicate that preferential resource allocation to the roots, as well as reduced stomatal conductance and transpiration were early responses to water-limitation, irrespective of the age at which water-limitation was imposed, or the duration of water-limitation. Under water-limited conditions, increased production of protective pigments such as carotenoids and anthocyanins was also observed, which helped in recovery after re-watering. It was concluded that after re-watering, all negative impacts of water-limitation on morphology and physiology of *C. sericea* plants were generally returned to well-watered levels. This suggests that *C. sericea* plants employ a wide range of phenotypic adjustments in response to water-limitation, which makes the plants well adapted to areas with high rainfall variability.

Key words: drought stress, resource allocation, photosynthetic rate, stomatal conductance, drought resistance

6.2. Introduction

Large arid and semi-arid regions around the world are generally characterized by high variability and unpredictability in rainfall, as well as rainfall that is insufficient for arable crop production under rain fed conditions (Abu-Zanat *et al.* 2004; Belkheiri and Mulas 2013). Within these drylands, plants are often exposed to a variety of environmental stresses, with drought stress or water-limitation commonly regarded as the most significant under agricultural conditions, leading to significant reductions in agricultural productivity (Lambers *et al.* 2008; Walter *et al.* 2011). Due to these stresses, most of the available agricultural land in these drylands are rangelands that are primarily used for extensive livestock production. Such rangelands have limited options to sustainably increase agricultural productivity, especially where irrigation is not an option (Palmer and Ainslie 2006; Belkheiri and Mulas 2013; Jordaan *et al.* 2013). These limitations to improving agricultural productivity within these water-limited rangelands are expected to worsen under the predicted future bioclimatic conditions (IPCC 2007; Meissner *et al.* 2013).

Generally, for South Africa, it is predicted that the current unpredictability and variability in rainfall distribution and quantity will likely increase in the future. This will lead to further increases in marginal agro-ecological conditions, with increases in the duration and intensity of episodic drought events, resulting in further limitations to sustainably increase agricultural productivity (Kruger and Shongwe 2004; Benhin 2008; DEA 2013; Meissner *et al.* 2013). Therefore, in order to meet the future increase in the demand for livestock products in South Africa, the productivity of these water-limited rangelands will need to be improved. One of the ways to improve rangeland production is through better rangeland management, which includes the implementation of improved fodder flow programs that can adequately address the current dry season feed shortages occurring due to the seasonal deterioration of the natural veld (Müller *et al.* 2019a).

The current stock of commercial forage species suitable for these water-limited agro-ecological conditions in South Africa is limited (Dickinson *et al.* 2010; Truter *et al.* 2015). Recently, however, the Agricultural Research Council (ARC) of South Africa, along with various South African research institutions have started identifying and prioritizing native

South African legume species that can potentially be developed further as forage species for use within these marginal areas, especially where the current commercially available forages are not suitable. These native species are naturally adapted to the marginal agro-ecological conditions of these water-limited areas and therefore, have the potential to effectively be utilized in fodder flow programs that will allow for improved livestock production within these rangelands (Müller *et al.* 2017a; Trytsman *et al.* 2019; Chimphango *et al.* 2020). From these studies, *Calobota sericea* (Thunb.) Boatwr. & B.-E. van Wyk, a perennial legume species which occurs within the semi-arid rangelands of the Northern Cape and Western Cape provinces of South Africa (Boatwright *et al.* 2018), has been recognized as a species that should be evaluated for its agronomic potential within the semi-arid rangelands of Namaqualand, and other areas experiencing similar bioclimatic and edaphic conditions (Samuels *et al.* 2016; Müller *et al.* 2017a; 2019a).

Recent work has indicated that *C. sericea* already forms an important part of the fodder flow plan of communal farmers within the Leliefontein communal rangelands of South Africa (Samuels *et al.* 2016). Therefore, if managed properly, this species has the potential to significantly reduce feed and nutrient shortages during the dry season within these rangelands (Müller *et al.* 2019a). However, apart from the requirements for seed germination and seedling establishment (Müller *et al.* 2017b; 2019b), very little is currently known about the ecophysiology of plant-water relations in *C. sericea*. This, in turn, limits our understanding of how well the species will respond to the predicted increased variability in rainfall within these arid and semi-arid agro-ecosystems.

For a plant to withstand regular periods of water-limitation, it is important that they have the ability to compensate for periods of stress when the stress is relieved. A key trait for agronomic crops, therefore, is how quickly and effectively they can respond/recover from water-limitation. These adaptive responses of plants to water-limitation have been well documented for important agronomic crops such as potato, sorghum and maize (Obidiegwu *et al.* 2015; Wang *et al.* 2017), with some plants, such as soybeans, able to compensate for growth upon rehydration (Dong *et al.* 2019). This is often achieved through phenotypic plasticity. Phenotypic plasticity in plants can be defined as the capacity of a single genotype to generate alternative phenotypes based on shifts in environmental conditions. It is a mechanism by which plants can respond quickly to changes in their environment (Bradshaw 1965; 2006; Nicotra *et al.* 2010; Arnold *et al.* 2019). This ability of a plant to shift developmental processes in response to the environment is key to the success of plants in natural and agro-ecosystems (Nicrotra *et al.* 2010; Gray and Brady 2016). Determining whether *C. sericea* plants possess these recuperative abilities is therefore key to its implementation as a planted forage within semi-arid and arid agro-ecological areas. This, in turn, can provide an indication as to how quickly, and effectively, *C. sericea* plants can or will respond to, and recover from water-limitation, and give valuable information for future breeding and improvement initiatives.

The aim of the current study was to quantify the responses of *C. sericea* to different levels of water-limitation, at different ages. This was done by quantifying the responses to water-limitation, and subsequent re-watering at 2, 3 and 4 months after establishment. We aimed to quantify; 1) plant growth and resource allocation, 2) water relations, and 3) gas exchange and photosynthetic pigment content in the leaves of *C. sericea* plants. We ask the question whether there is any evidence of adaptive plasticity in morphological and/or physiological traits in response to water-limitation, which would help *C. sericea* plants to cope in water-limited growing conditions.

6.3. Materials and Methods

6.3.1. Seed collection and pre-germination treatments

Physiologically mature seeds of *C. sericea* were collected from naturally occurring populations in the semi-arid rangelands of Leliefontein in Namaqualand, South Africa in November 2016. Within the Leliefontein communal area seeds were collected from a minimum of 75 plants per sampling location from the rangelands surrounding the Leliefontein, Tweerivier, Spoegrivier and Kharkhams villages, after which all seeds collected were pooled to form a single seed-lot for the Leliefontein area (Müller *et al.* 2019b). After collection, the seeds were removed from the seed pods by hand to reduce injury to the seeds, after which a proportion of the seeds collected were mechanically scarified using an abrasive sand paper to remove the coat imposed seed dormancy (Müller *et al.* 2017b). Scarified seeds were pre-germinated in 90 mm petri-dishes on two layers of filter paper. Seeds were regarded as germinated when a radicle of ≥ 3 mm was visible. Seedlings were removed from the petri-dishes and transplanted into pots.

6.3.2. Experimental design

A complete randomized block pot experiment was conducted under greenhouse conditions. Within the trial, three drought treatments (well-watered control, water-limited and re-watered after water-limitation) were implemented three times (2, 3 and 4 months after establishment), with four water-limited periods (15, 30, 45 and 60 days). Pots (15 cm diameter, 40 cm deep) were filled with soil collected from the locations where seeds were collected. No chemical amendments were made to the soil prior to planting. Before planting, all pots were irrigated to saturated levels and allowed to drain for 48 hours. Three pre-germinated (radicle ≥ 3 mm) seeds were planted at a depth of 1 cm in each pot. The seedlings were allowed to grow for one month before a half-strength stock nutrient solution (Plant Food- Starke Ayres) was applied to the pots to avoid nutrient deficiencies. At six weeks after sowing, the pots were thinned to two uniformly sized plants per pot, resulting in a total of 6 plants per treatment. These pots were watered to saturated levels once a week for a further two weeks. At each plant age, before the drought treatments were imposed, all pots were watered to saturated levels, and allowed to drain to pot capacity. This was to ensure that none of the plants were stressed before the drought treatments were imposed. Thereafter, watering was withheld for drought stressed plants while well-watered pots were watered once a week. After each water-limitation period i.e. 15, 30, 45 and 60 days, drought stressed pots were re-watered to capacity once a week for 21 days before harvesting the recovered plants.

6.3.2.1. Measurements

The day before physiological measurements were made, all well-watered pots were watered and allowed to drain to field capacity. After 24 hours of watering, physiological measurements were conducted. Using one of the plants in each pot, the net photosynthesis (A), stomatal conductance (g_s), transpiration rate (E), and intercellular

CO_2 (C) of the largest, fully expanded compound leaf were measured between 12H00 and 14H00, using a Li-Cor 6400 xt portable open gas system with a red/blue light source (LI-COR Biosciences, Lincoln, NE, USA). The reference CO_2 concentration was maintained at 400 ppm, flow rate was 400 $\mu mol.s^{-1}$, and the light in the chamber was set at 400 photons $\mu mol.s^{-1}$. After measuring, the leaf was harvested and the leaf area immediately determined using a portable leaf area meter. The measurements obtained were thereafter adjusted for each leaf area measured. From the default measurements, photosynthetic water use efficiency (A/E) for each plant was calculated. Thereafter, all plant material was collected and separated into roots and shoots (stems + leaves) and the fresh mass (g) and root length (cm) determined. After fresh mass determination, the plant material was submerged in dH_2O for 24 hours at room temperature, after which the plant material was re-weighed to determine the turgor mass (g) of each plant organ (Turner 1981). The plant materials were thereafter oven dried at 60 °C until a constant mass was achieved. The dried material was re-weighed to determine the dry mass (g) of each plant component. Using this information, the relative shoot (RSWC) and root (RRWC) water content were determined as in equations 1 and 2.

Equation 1: $RSWC (\%) = [(SFW – SDW) \div (STW – SDW)] \times 100$

Equation 2: $RRWC (\%) = [(RFW – RDW) \div (RTW – RDW)] \times 100$

Where: SFW = shoot fresh mass, STW = shoot turgor mass, SDW = shoot dry mass, RFW = root fresh mass, RTW = root turgor mass, RDW = root dry mass

The other plant in the pot was used for pigment determination. The leaves of each of the plants were removed and cut into smaller pieces and mixed well after which a 0.5 g sample (fresh mass) was mashed in a mortar and pestle with 80 % acetone (v/v). The mixture was allowed to stand for 12h after which the extract was filtered through Whatman no. 1 filter paper. Absorbance of the filtrate using a uv-vis spectrophotometer at 470, 537, 647 and 663 mm were recorded and the concentrations of chlorophyll a, chlorophyll b, total chlorophyll, anthocyanin (Kong et al. 2017) and carotenoids (Pompelli et al. 2013) were calculated according to equations 3-7.

Equation 3: Chlorophyll a = $0.137 \times A_{663} – 0.000897 \times A_{537} – 0.003046 \times A_{647}$

Equation 4: $\text{Chlorophyll b} = 0.024 \times A_{647} - 0.004305 \times A_{537} - 0.005507 \times A_{663}$

Equation 5: $\text{Total Chlorophyll} = \text{Chlorophyll a} + \text{Chlorophyll b}$

Equation 6: $\text{Anthocyanin} = 0.0817 \times A_{537} - 0.00697 \times A_{647} - 0.002228 \times A_{663}$

Equation 7: $\text{Carotenoids} = [(1000 \times A_{470}) - (2.13 \times \text{Chlorophyll a}) - (97.64 \times \text{Chlorophyll b})] \div 209$

6.3.3. Statistical analyses

The Statistical Package for the Social Sciences Version 22 (SPSS Inc., Chicago, IL) was used to test the data for normality using a Shapiro-Willks test. When data was found to deviate from normality, the data were log-transformed to achieve normality. A one-way ANOVA was performed on all variables to determine whether significant differences ($p \leq 0.05$) were found between the different treatments, within each sampling time. Where significant differences were observed, a LSD post hoc test was performed to separate the means.

6.4. Results

6.4.1. Biomass production and resource allocation

Water-limitation was found to significantly influence the growth and development of *C. sericea*. Shoot mass (Fig. 6.1) significantly decreased in water-limited plants, irrespective of the duration of water-limitation, or the age at which water-limitation was imposed on the plants. With plants two and three months old, re-watering after water-limitation generally resulted in the shoot mass recovering to well-watered levels. However, when water-limitation was imposed on the plants at four months, although shoot mass increased from water-limited levels, recovery of shoot mass did not occur to well-watered levels. At 60 days of water-limitation when water-limitation was imposed on the plants at four months of age, no recovery after re-watering occurred.

Generally, root mass (Fig. 6.2) and root length (Fig. 6.3) in water-limited and re-watered plants was significantly heavier and longer that those of well-watered plants irrespective of the age at which water-limitation was imposed on the plants, or the duration of water-limitation. The exception to this was when water-limitation was imposed on the plants for 60 days at four months. Here, both root mass (Fig. 6.2) and root length (Fig. 6.3) significantly decreased from well-watered levels in both water-limited and re-watered plants, suggesting that at there was no recovery after re-watering.

When considering the root:shoot ratio (Fig. 6.4), significantly more resources were allocated to root production in water-limited plants, and this was true even after re-watering. The exception to this was when water-limitation was imposed on the plants for 60 days at four months where water-limited and re-watered plants had a significantly lower root:shoot mass ratio than well-watered plants.

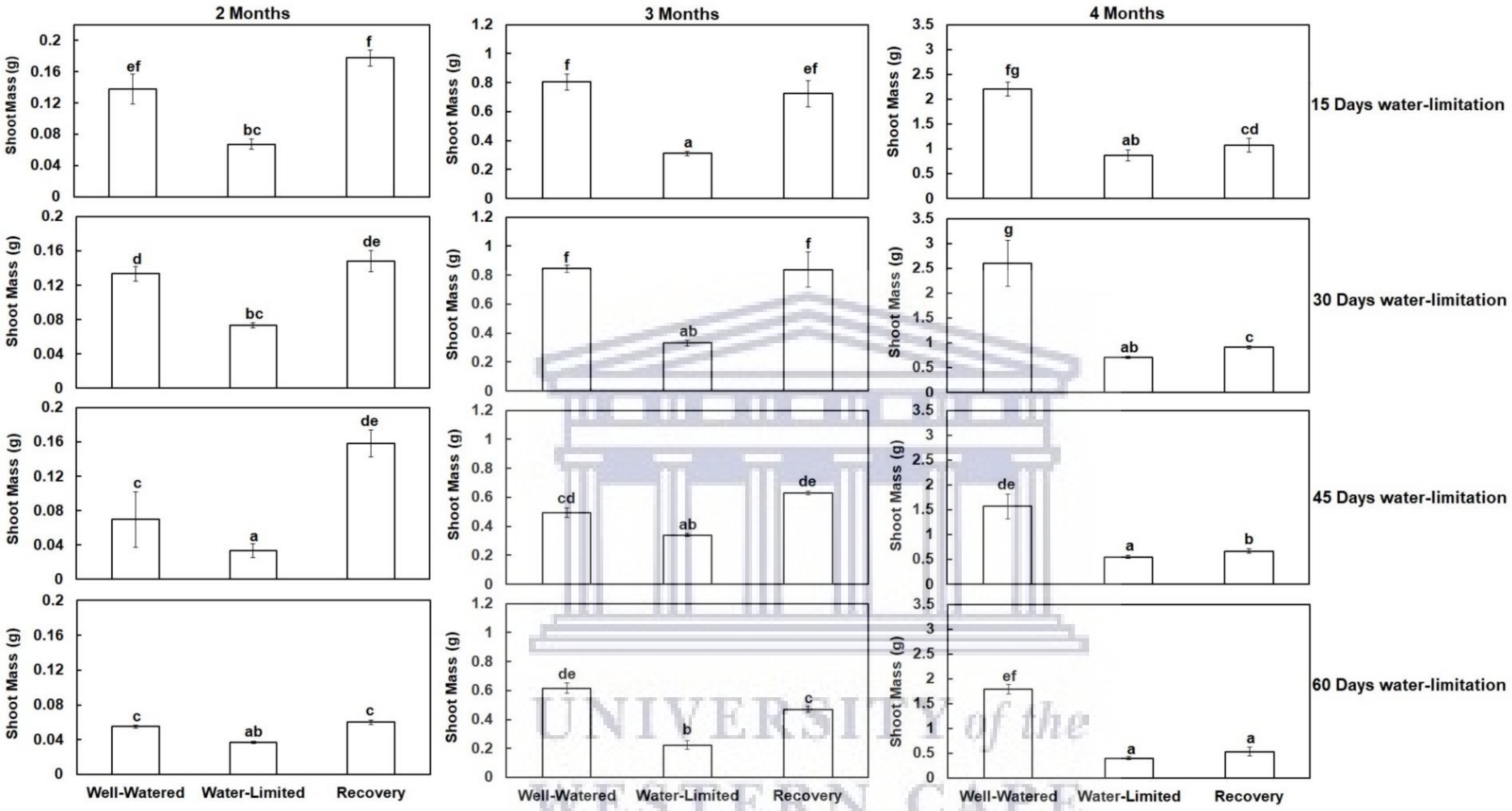

Figure 6.1: Shoot dry mass (g) of *Calobota sericea* plants at different ages under different durations of water-limitation and subsequent recovery after re-watering. Bars with the same letters are not statistically significantly different ($p \geq 0.05$) from one another. Comparison of treatments were made within a plant age.

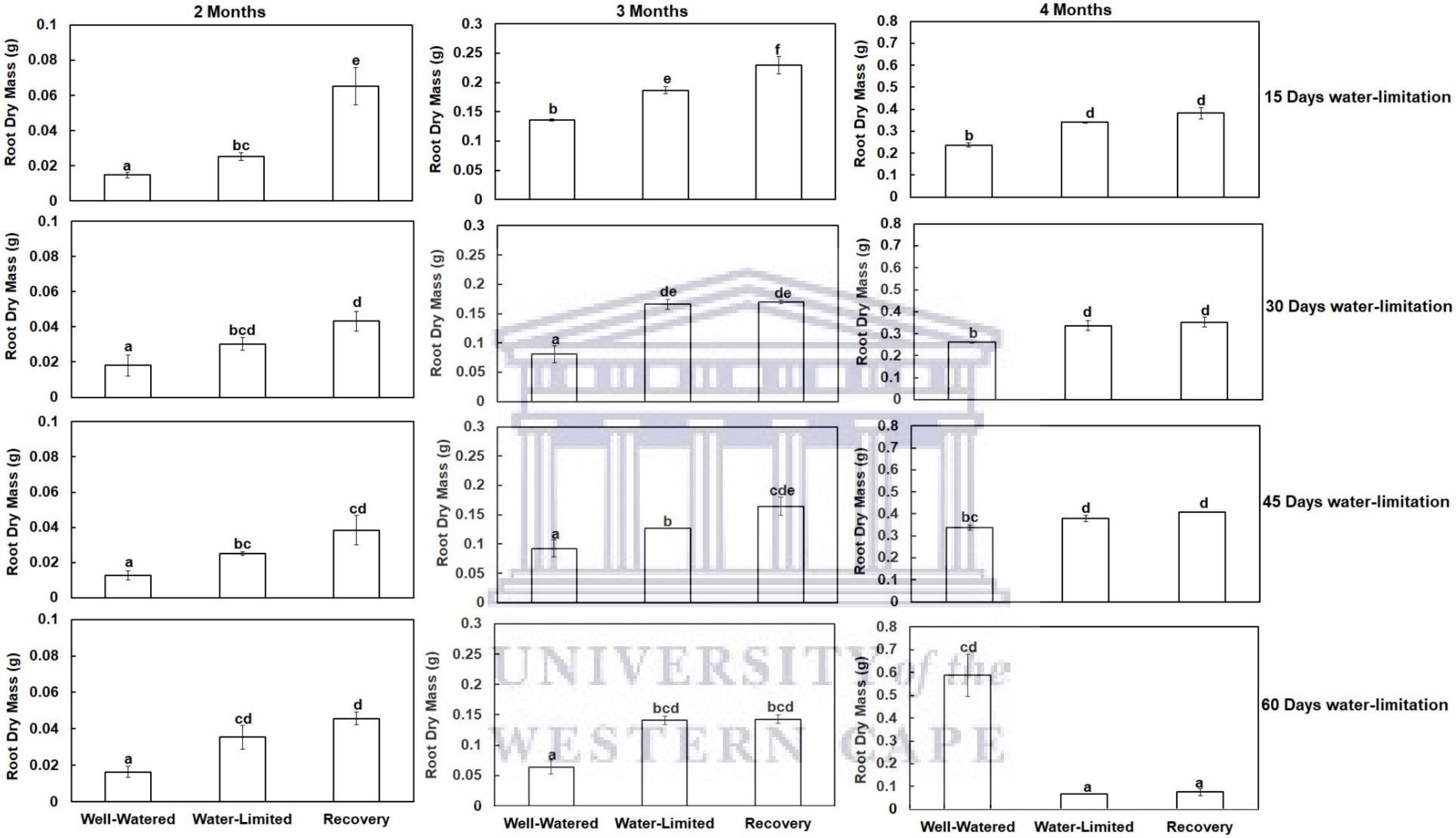

Figure 6.2: Root dry mass (mg) of *Calobota sericea* plants at different ages under different durations of water-limitation and subsequent recovery after re-watering. Bars with the same letters are not statistically significantly different ($p \geq 0.05$) from one another. Comparison of treatments were made within a plant age.

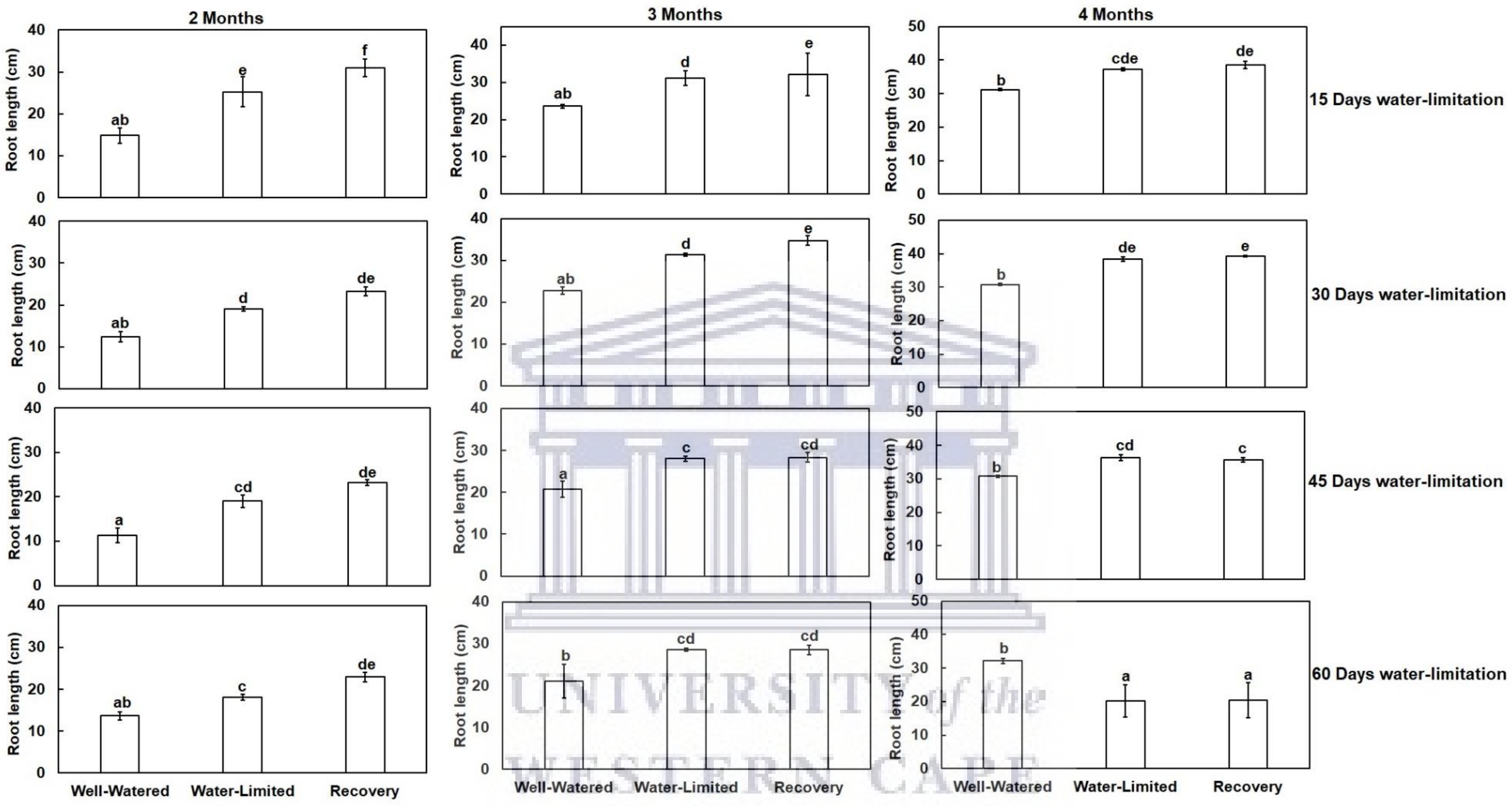

Figure 6.3: Root length (cm) of *Calobota sericea* plants at different ages under different durations of water-limitation and subsequent recovery after re-watering. Bars with the same letters are not statistically significantly different ($p \geq 0.05$) from one another. Comparison of treatments were made within a plant age.

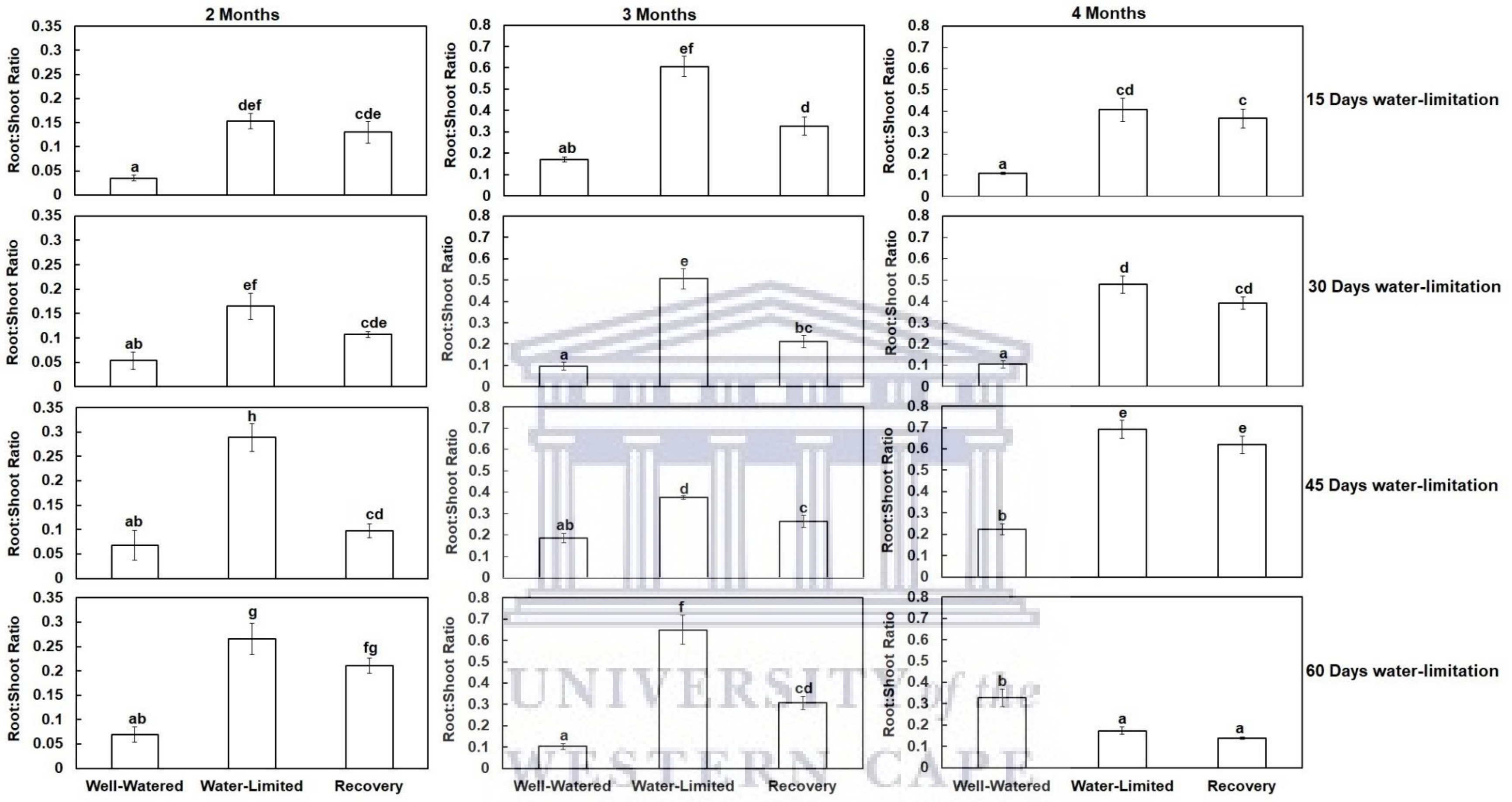

Figure 6.4: Root:Shoot mass ratio of *Calobota sericea* plants at different ages under different durations of water-limitation and subsequent recovery after re-watering. Bars with the same letters are not statistically significantly different (p ≥ 0.05) from one another. Comparison of treatments were made within a plant age.

6.4.2. Plant water status

The shoot water content (Fig. 6.5) decreased significantly from the well-watered levels in water-limited plants, but after re-watering, increased significantly from water-limited levels, irrespective of the age at which water-limitation was imposed on the plants, or the duration of water-limitation. Root water content (Fig. 6.6) however, when water-limitation was imposed on the plants at two and three months after establishment, did not differ between well-watered and water-limited plants for up to 45 days of water-limitation. When these plants were re-watered however, root water content significantly increased above both well-watered and water-limited levels. At 60 days of water-limitation, root water content decreased significantly from well-watered levels, but after re-watering, increased to levels greater than those in water-limited and well-watered plants. When water-limitation was imposed on the plants four months after establishment, root water content decreased significantly from well-watered levels, but after re-watering, increased significantly from water-limited levels. Both shoot (Fig. 6.5) and root (Fig. 6.6) water content, however, decreased significantly from well-watered levels when water-limitation was imposed on the plants for 60 days at four months. Even after re-watering, both shoot and root water content did not increase from water-limited levels, suggesting that no recovery occurred after re-watering.

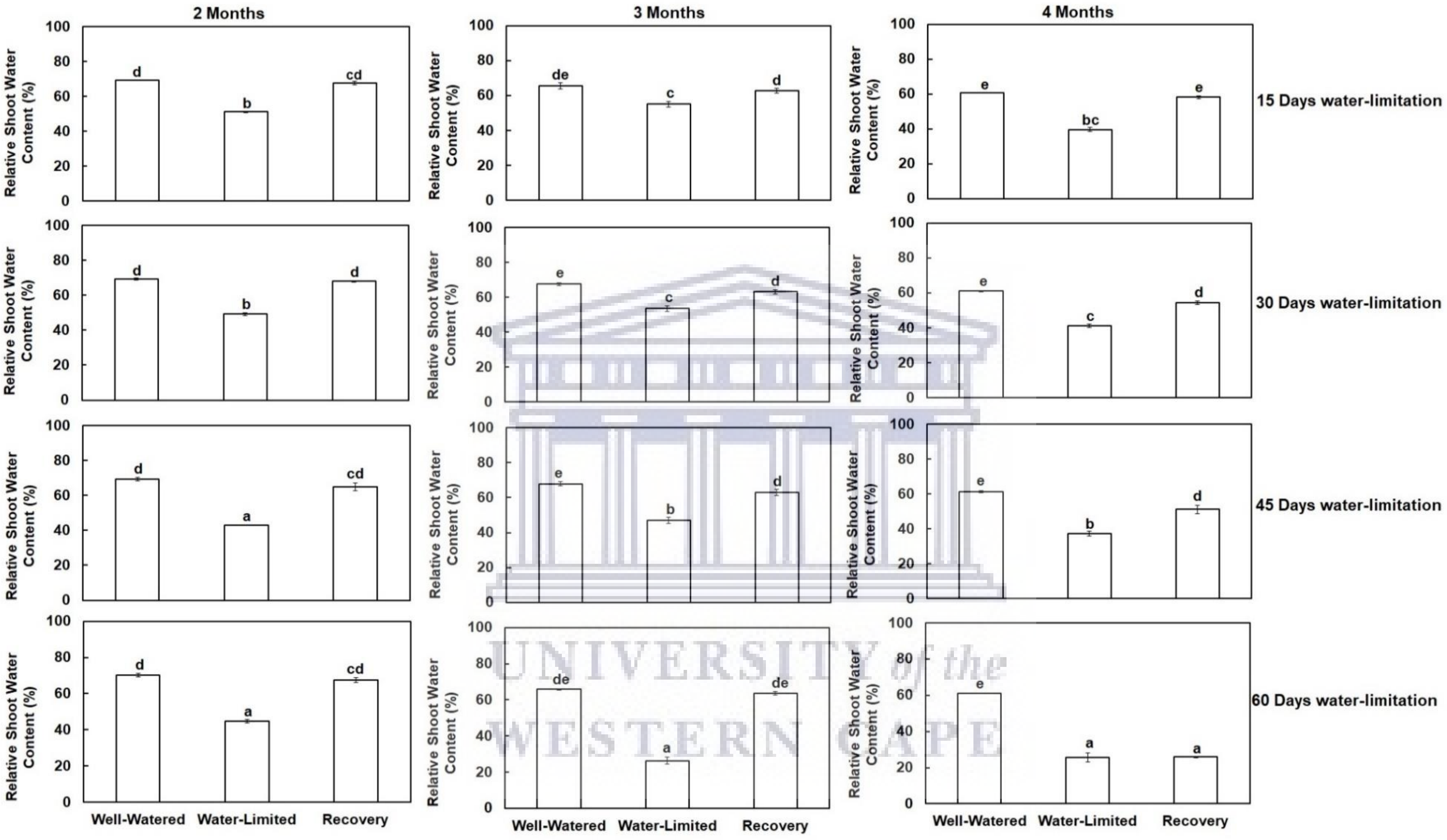

Figure 6.5: Shoot water content (%) of *Calobota sericea* plants at different ages under different durations of water-limitation and subsequent recovery after re-watering. Bars with the same letters are not statistically significantly different ($p \geq 0.05$) from one another. Comparison of treatments were made within a plant age.

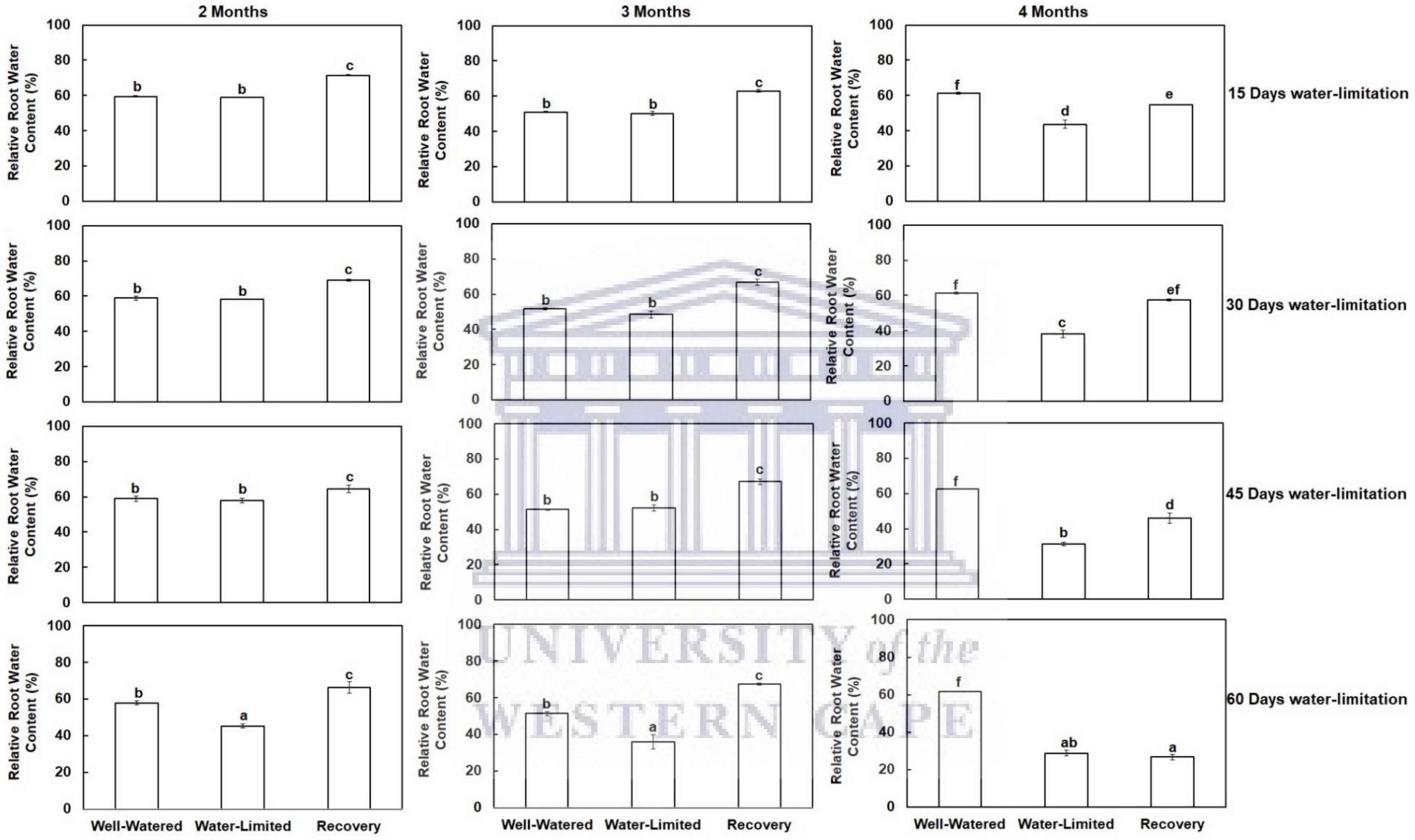

Figure 6.6: Root water content (%) of *Calobota sericea* plants at different ages under different durations of water-limitation and subsequent recovery after re-watering. Bars with the same letters are not statistically significantly different ($p \geq 0.05$) from one another. Comparison of treatments were made within a plant age.

6.4.3. Gas exchange

Stomatal conductance (Fig. 6.7), intercellular CO_2 (Fig. 6.8) and transpiration rate (Fig. 6.9) decreased significantly from well-watered levels in water-limited plants, irrespective of the age at which water-limitation was imposed on the plants and the duration of water-limitation. The decreased stomatal conductance and intercellular CO_2 concentrations also resulted in reduced photosynthesis (Fig. 6.10) in water-limited plants. When water-limitation was imposed on the plants for 15 and 30 days at two months after establishment, however, no significant differences in net photosynthesis was observed between the well-watered, water-limited and re-watered plants. At 45 and 60 days of water-limitation, and when water-limitation was imposed on the plants three and four months after establishment, irrespective of the duration of water-limitation, net photosynthesis decreased significantly from well-watered levels in water-limited plants. After re-watering however, stomatal conductance, intercellular CO_2, transpiration rate and net photosynthesis increased significantly. This was true for all water-limited periods at all ages at which water-limitation was imposed on the plants, except when water-limitation was imposed for 60 days at four months.

With the decrease in transpiration rate because of the closure of stomata in water-limited plants, PWUE (Fig. 6.11) in water-limited plants increased significantly in water stressed plants, irrespective of the age at which water-limitation was imposed on the plants, or the duration of water-limitation. The exception to this was when water-limitation was imposed for 60 days at 4 months. Re-watering after 15 and 30 days of water-limitation, at 2 months, resulted in a significant decrease in PWUE from water-limited levels. Re-watering after 45 and 60 days of water-limitation however, resulted in PWUE not differing between water-limited and re-watered plants. Similarly, re-watering after 15 days of water-limitation at three and four months after establishment, resulted in a significant decrease in PWUE from water-limited levels, but when re-watering occurred only after 30, 45 and 60 days of water-limitation, PWUE of the re-watered plants did not differ from water-limited levels.

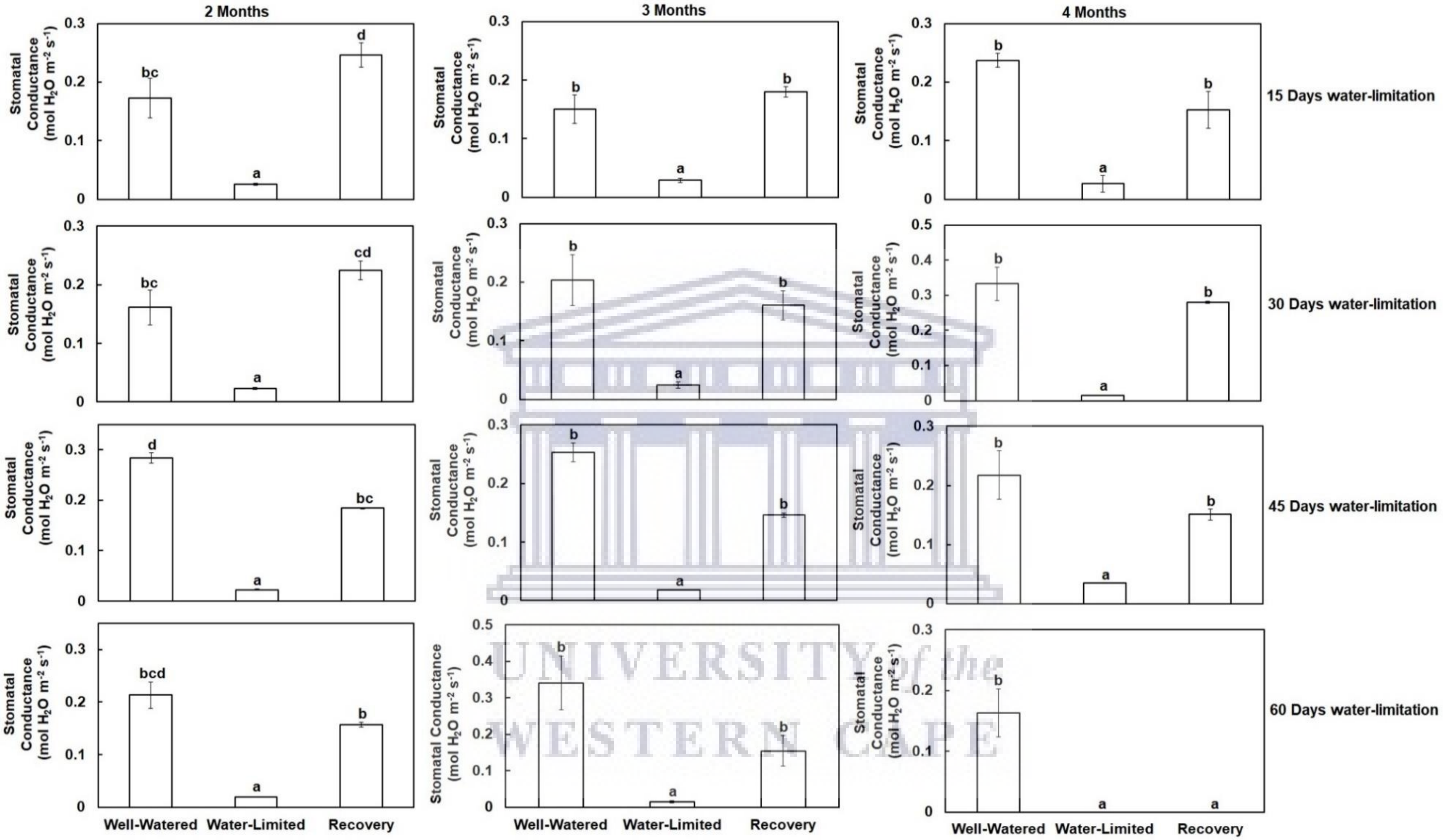

Figure 6.7: Stomatal conductance of *Calobota sericea* plants at different ages under different durations of water-limitation and subsequent recovery after re-watering. Bars with the same letters are not statistically significantly different ($p \geq 0.05$) from one another. Comparison of treatments were made within a plant age.

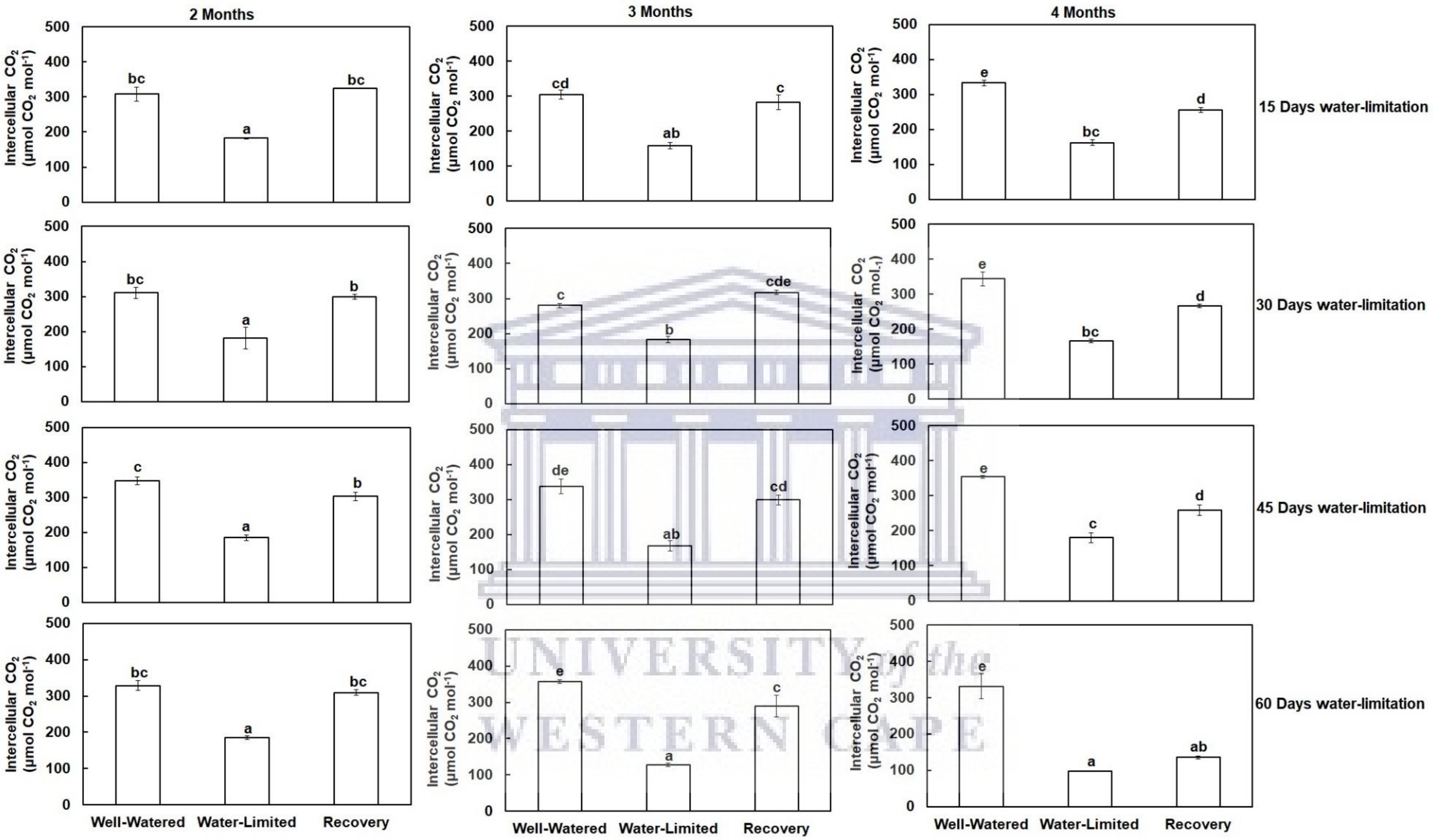

Figure 6.8: Intercellular CO₂ concentrations of *Calobota sericea* plants at different ages under different durations of water-limitation and subsequent recovery after re-watering. Bars with the same letters are not statistically significantly different ($p \geq 0.05$) from one another. Comparison of treatments were made within a plant age.

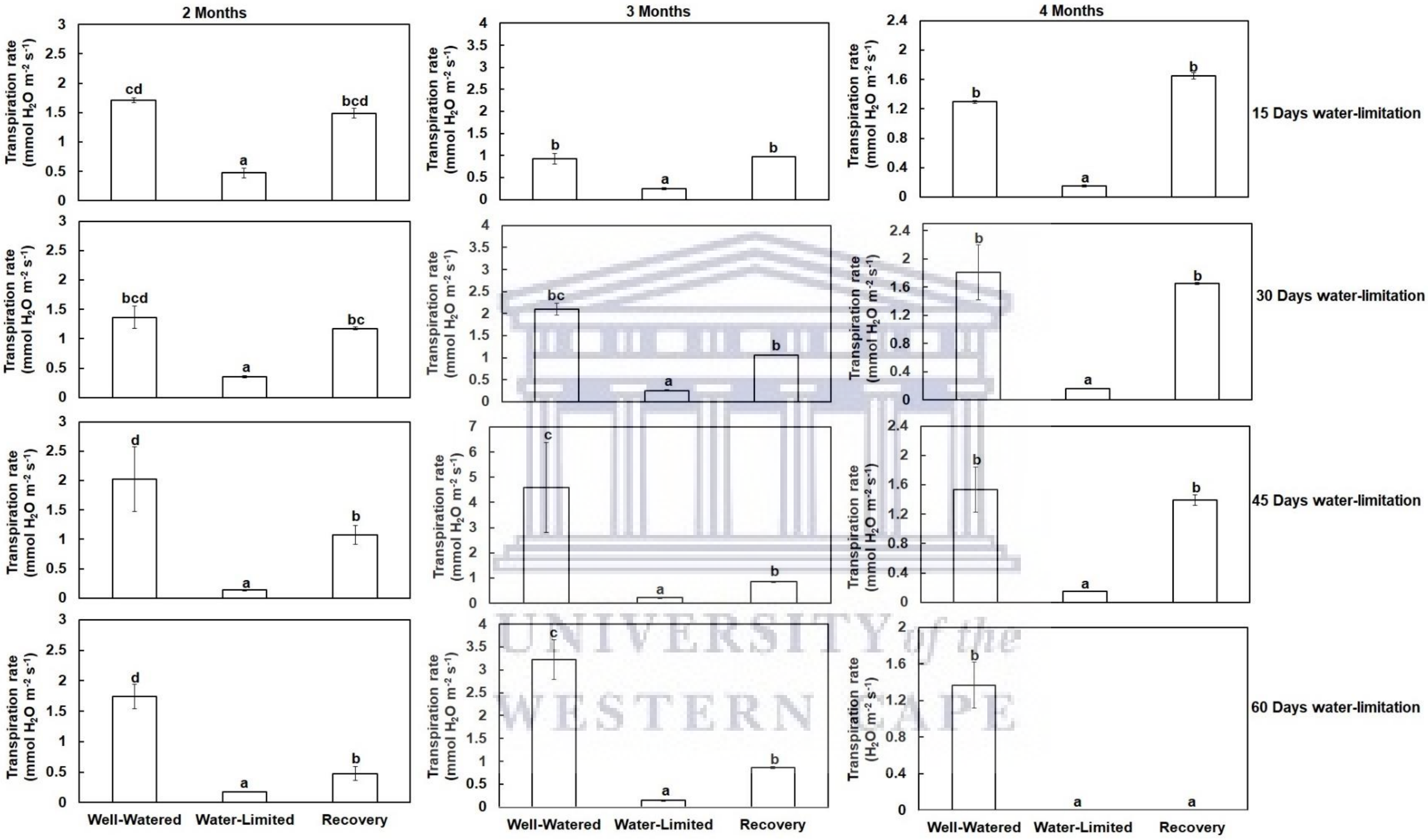

Figure 6.9: Transpiration rate of *Calobota sericea* plants at different ages under different durations of water-limitation and subsequent recovery after re-watering. Bars with the same letters are not statistically significantly different ($p \geq 0.05$) from one another. Comparison of treatments were made within a plant age.

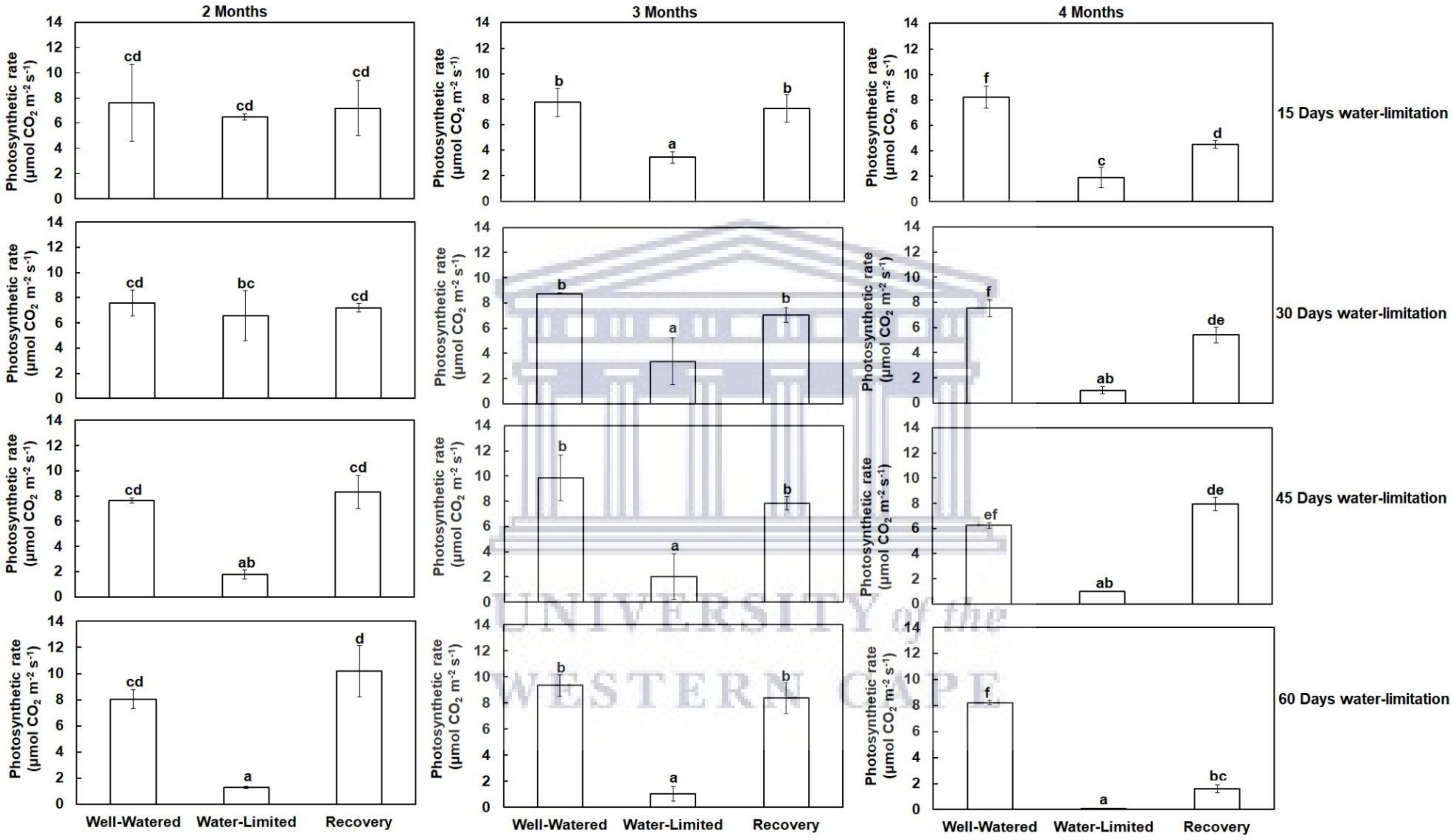

Figure 6.10: Photosynthetic rate of *Calobota sericea* plants at different ages under different durations of water-limitation and subsequent recovery after re-watering. Bars with the same letters are not statistically significantly different ($p \geq 0.05$) from one another. Comparison of treatments were made within a plant age.

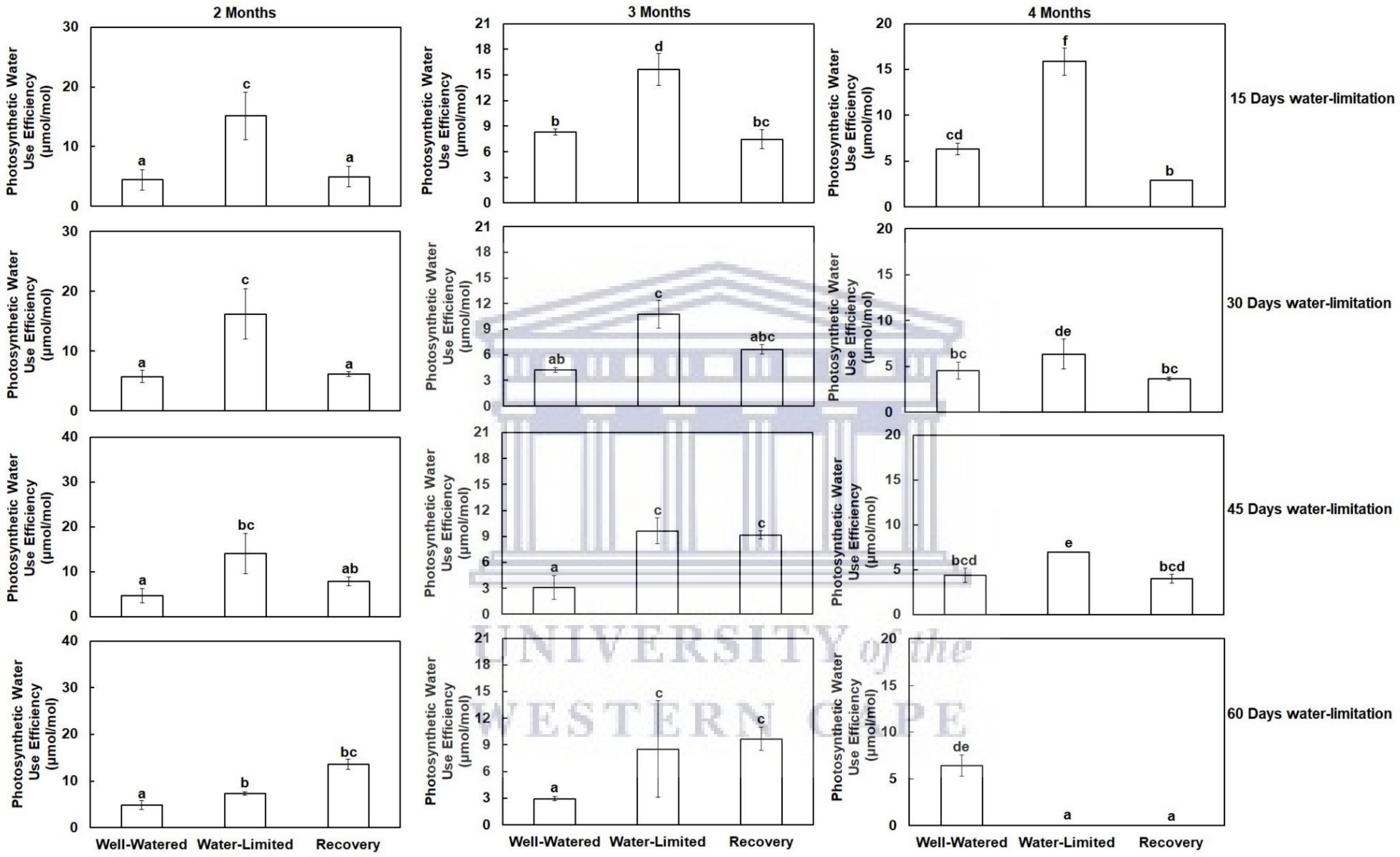

Figure 6.11: Photosynthetic water use efficiency of *Calobota sericea* plants at different ages under different durations of water-limitation and subsequent recovery after re-watering. Bars with the same letters are not statistically significantly different ($p \geq 0.05$) from one another. Comparison of treatments were made within a plant age.

6.4.4. Photosynthetic pigments

When water-limitation was imposed on the plants two months after establishment (Table 6.1), the chlorophyll content in water-limited plants was generally lower than in well-watered plants. After re-watering, chlorophyll (Chl a, Chl b and Total Chl) content returned to well-watered levels. Anthocyanin and carotenoid pigment content in these plants however, increased significantly in water-limited plants, irrespective of the duration of water-limitation.

When water-limitation was imposed on the plants three months after establishment (Table 6.2), chlorophyll content (Chl a and Total Chl) was found to generally be significantly lower in water-limited plants, but after re-watering, chlorophyll content in the plants recovered to well-watered levels. Chlorophyll b content however, did not differ between well-watered, water-limited and re-watered plants. Anthocyanin and carotenoid pigment content within these plants increased significantly from well-watered levels in water-limited plants. After re-watering, anthocyanin and carotenoid pigment content generally decreased with anthocyanin levels decreasing to well-watered levels, but carotenoid pigment content, although significantly lower, were still significantly higher than well-watered levels.

When water-limitation was implemented on the plants four months after establishment (Table 6.3), total chlorophyll content was found to decrease significantly from well-watered levels in water-limited plants, irrespective of the duration of water-limitation. After re-watering, the chlorophyll content increased significantly from water-limited levels. The exception was found in plants that were subjected to 60 days of water-limitation where no increases occurred. Anthocyanin and carotenoid pigment content in these plants was found to be significantly higher in water-limited plants as well as re-watered plants, irrespective of the duration of water-limitation.

Table 6.1: Photosynthetic pigment content in two months old *Calobota sericea* plant leaves subjected to different durations of water-limitation and subsequent re-watering. Mean concentrations with the same letters are not statistically significantly different (* $p < 0.05$) from one another.

Plant Age	Treatment		Chlorophyll a	Chlorophyll b	Total Chlorophyll	Anthocyanin	Carotenoids
2 Months	15 Days water-limitation	Well-watered	4.05 ± 0.486^{b}	0.17 ± 0.043^{b}	4.22 ± 0.526^{b}	0.03 ± 0.004^{a}	0.02 ± 0.016^{a}
		Water-limited	0.73 ± 0.172^{a}	0.02 ± 0.007^{a}	0.75 ± 0.178^{a}	0.16 ± 0.006^{c}	0.27 ± 0.022^{c}
		Recovery	2.70 ± 0.683^{b}	0.15 ± 0.029^{b}	2.85 ± 0.711^{b}	0.12 ± 0.008^{b}	0.13 ± 0.014^{b}
		$F_{(2,9)}$	11.477	7.654	11.245	72.706	48.506
		p	0.009	0.022	0.009	< 0.001	< 0.001
	30 Days water-limitation	Well-watered	1.29 ± 0.084^{ab}	0.05 ± 0.006^{a}	1.34 ± 0.090^{b}	0.07 ± 0.013^{a}	0.08 ± 0.006^{a}
		Water-limited	1.12 ± 0.134^{a}	0.02 ± 0.001^{a}	1.15 ± 0.133^{a}	0.23 ± 0.033^{c}	0.20 ± 0.002^{b}
		Recovery	2.29 ± 0.141^{b}	0.27 ± 0.018^{b}	2.56 ± 0.077^{b}	0.13 ± 0.001^{b}	0.19 ± 0.008^{b}
		$F_{(2,9)}$	27.447	119.904	39.644	26.104	42.500
		p	0.002	< 0.001	0.001	0.002	0.001
	45 Days water-limitation	Well-watered	1.97 ± 0.246^{b}	0.12 ± 0.008^{b}	2.09 ± 0.242^{b}	0.05 ± 0.008^{a}	0.09 ± 0.006^{a}
		Water-limited	1.27 ± 0.271^{a}	0.04 ± 0.009^{a}	1.31 ± 0.280^{a}	0.18 ± 0.023^{b}	0.20 ± 0.008^{b}
		Recovery	2.45 ± 0.080^{b}	0.12 ± 0.004^{b}	2.57 ± 0.077^{b}	0.12 ± 0.014^{b}	0.19 ± 0.006^{b}
		$F_{(2,9)}$	7.582	59.111	8.523	19.174	73.357
		p	0.023	< 0.001	0.018	0.002	< 0.001
	60 Days water-limitation	Well-watered	2.30 ± 0.307^{b}	0.14 ± 0.010^{b}	2.44 ± 0.311^{b}	0.02 ± 0.007^{a}	0.03 ± 0.003^{a}
		Water-limited	0.61 ± 0.129^{a}	0.06 ± 0.007^{a}	0.68 ± 0.123^{a}	0.16 ± 0.020^{b}	0.27 ± 0.020^{c}
		Recovery	2.61 ± 0.148^{b}	0.13 ± 0.025^{b}	2.74 ± 0.132^{b}	0.12 ± 0.008^{b}	0.17 ± 0.015^{b}
		$F_{(2,9)}$	26.339	6.254	28.940	28.896	77.782
		p	0.001	0.034	0.001	0.001	< 0.001

Table 6.2: Photosynthetic pigment content in three months old *Calobota sericea* plant leaves subjected to different durations of water-limitation and subsequent re-watering. Mean concentrations with the same letters are not statistically significantly different (* $p < 0.05$) from one another.

Plant Age	Treatment		Chlorophyll a	Chlorophyll b	Total Chlorophyll	Anthocyanin	Carotenoids
3 Months	15 Days water-limitation	Well-watered	1.43 ± 0.120^{b}	0.06 ± 0.004^{a}	1.49 ± 0.123^{b}	0.06 ± 0.010^{a}	0.12 ± 0.016^{a}
		Water-limited	0.67 ± 0.138^{a}	0.08 ± 0.015^{a}	0.75 ± 0.140^{a}	0.16 ± 0.029^{b}	0.51 ± 0.018^{c}
		Recovery	1.37 ± 0.222^{b}	0.05 ± 0.006^{a}	1.42 ± 0.227^{b}	0.05 ± 0.005^{a}	0.32 ± 0.010^{b}
		$F_{(2,9)}$	6.731	3.652	5.914	12.371	180.123
		p	0.029	0.092	0.038	0.007	< 0.001
	30 Days water-limitation	Well-watered	1.44 ± 0.182^{b}	0.06 ± 0.006^{a}	1.50 ± 0.187^{b}	0.05 ± 0.010^{a}	0.09 ± 0.008^{a}
		Water-limited	0.51 ± 0.163^{a}	0.07 ± 0.037^{a}	0.58 ± 0.200^{a}	0.12 ± 0.017^{b}	0.36 ± 0.024^{c}
		Recovery	1.50 ± 0.237^{b}	0.05 ± 0.010^{a}	1.55 ± 0.247^{b}	0.04 ± 0.016^{a}	0.16 ± 0.006^{b}
		$F_{(2,9)}$	8.047	0.312	6.655	12.511	95.550
		p	0.020	0.743	0.030	0.007	< 0.001
	45 Days water-limitation	Well-watered	1.76 ± 0.219^{b}	0.06 ± 0.010^{a}	1.82 ± 0.225^{b}	0.02 ± 0.006^{a}	0.09 ± 0.013^{a}
		Water-limited	0.39 ± 0.056^{a}	0.02 ± 0.004^{a}	0.41 ± 0.060^{a}	0.14 ± 0.012^{c}	0.38 ± 0.027^{c}
		Recovery	1.53 ± 0.065^{b}	0.03 ± 0.018^{a}	1.56 ± 0.053^{b}	0.10 ± 0.006^{b}	0.19 ± 0.023^{b}
		$F_{(2,9)}$	29.443	2.400	29.221	62.312	46.452
		p	0.001	0.171	0.001	< 0.001	< 0.001
	60 Days water-limitation	Well-watered	1.73 ± 0.125^{b}	0.03 ± 0.024^{a}	1.76 ± 0.147^{b}	0.01 ± 0.004^{a}	0.13 ± 0.012^{a}
		Water-limited	0.80 ± 0.044^{a}	0.04 ± 0.008^{a}	0.83 ± 0.052^{a}	0.17 ± 0.044^{b}	0.38 ± 0.013^{c}
		Recovery	1.52 ± 0.106^{b}	0.04 ± 0.010^{a}	1.56 ± 0.097^{b}	0.09 ± 0.017^{ab}	0.26 ± 0.004^{b}
		$F_{(2,9)}$	27.699	0.078	20.956	8.821	114.112
		p	0.002	0.926	0.004	0.023	< 0.001

Table 6.3: Photosynthetic pigment content in four months old *Calobota sericea* plant leaves subjected to different durations of water-limitation and subsequent re-watering. Mean concentrations with the same letters are not statistically significantly different (* $p < 0.05$) from one another.

Plant Age	Treatment		Chlorophyll a	Chlorophyll b	Total Chlorophyll	Anthocyanin	Carotenoids
4 Months	15 Days water-limitation	Well-watered	1.60 ± 0.073^{b}	0.04 ± 0.017^{a}	1.64 ± 0.090^{b}	0.003 ± 0.001^{a}	0.13 ± 0.018^{a}
		Water-limited	0.74 ± 0.077^{a}	0.03 ± 0.007^{a}	0.77 ± 0.083^{a}	0.18 ± 0.039^{b}	0.44 ± 0.014^{b}
		Recovery	1.59 ± 0.098^{b}	0.04 ± 0.009^{a}	1.63 ± 0.089^{b}	0.18 ± 0.002^{b}	0.45 ± 0.001^{b}
		F$_{(2,9)}$	39.220	0.381	32.615	15.497	14.957
		p	0.001	0.701	0.001	0.007	< 0.001
	30 Days water-limitation	Well-watered	0.90 ± 0.088^{b}	0.04 ± 0.003^{c}	0.94 ± 0.087^{c}	0.02 ± 0.009^{a}	0.09 ± 0.003^{a}
		Water-limited	0.21 ± 0.016^{a}	0.001 ± 0001^{a}	0.21 ± 0.017^{a}	0.17 ± 0.0001^{c}	0.40 ± 0.007^{c}
		Recovery	0.53 ± 0.147^{ab}	0.02 ± 0.003^{b}	0.55 ± 0.150^{b}	0.08 ± 0.019^{b}	0.23 ± 0.020^{b}
		F$_{(2,9)}$	19.271	52.160	21.384	73.067	76.406
		p	0.004	< 0.001	0.004	< 0.001	< 0.001
	45 Days water-limitation	Well-watered	1.30 ± 0.268^{b}	0.05 ± 0.005^{a}	1.35 ± 0.270^{c}	0.02 ± 0.007^{a}	0.08 ± 0.011^{a}
		Water-limited	0.38 ± 0.033^{a}	0.03 ± 0.012^{a}	0.41 ± 0.043^{a}	0.21 ± 0.019^{c}	0.29 ± 0.005^{c}
		Recovery	0.65 ± 0.045^{ab}	0.10 ± 0.008^{b}	0.74 ± 0.053^{b}	0.10 ± 0.003^{b}	0.18 ± 0.009^{b}
		F$_{(2,9)}$	8.940	12.652	8.806	63.700	141.857
		p	0.016	0.007	0.016	< 0.001	< 0.001
	60 Days water-limitation	Well-watered	1.18 ± 0.259^{b}	0.05 ± 0.002^{b}	1.23 ± 0.261^{b}	0.01 ± 0.002^{a}	0.16 ± 0.020^{a}
		Water-limited	0.07 ± 0.039^{a}	0.01 ± 0.006^{a}	0.08 ± 0.041^{a}	0.15 ± 0.032^{b}	0.49 ± 0.004^{b}
		Recovery	0.02 ± 0.006^{a}	0.003 ± 0.002^{a}	0.02 ± 0.007^{a}	0.11 ± 0.009^{b}	0.50 ± 0.007^{b}
		F$_{(2,9)}$	18.715	29.845	20.017	14.181	26.500
		p	0.003	0.001	0.002	0.005	< 0.001

6.5. Discussion

In this study, the morphological responses of *C. sericea* plants to water-limitation generally corresponded to what is found in the literature, in that there was a greater inhibition of shoot growth and a preferential allocation of resources to root growth (Pang *et al.* 2011; Foster *et al.* 2012; Poorter *et al.* 2012; Lotter *et al.* 2014; Eziz *et al.* 2017). This finding corresponds to the optimum partitioning theory (Bloom *et al.* 1985; Mao *et al.* 2012; Gargallo-Garriga *et al.* 2014; Eziz *et al.* 2017) in that *C. sericea* plants under water-limited conditions were found to allocate more resources towards the structures that are tasked with capturing the limited resource. In this study, after re-watering, shoot water content and biomass production generally recovered. Interestingly, even though shoot growth recovered after re-watering, the proportion of biomass allocated to the roots of these re-watered plants was still significantly higher than that of the well-watered plants. This suggests that the rapid recovery in shoot biomass after re-watering can partially be explained by the increased uptake of the now available water resources, by a better-developed and deeper root system. This, in turn, is partially responsible for rapid refilling of embolized xylem vessels, allowing for improved water movement through the plant (Holbrook *et al.* 2001; Lambers *et al.* 2008; Foster *et al.* 2015). The development of deeper and better-developed root systems by plants such as beans (Beebe *et al.* 2013; Fenta *et al.* 2014; Polania *et al.* 2017) under water-limited conditions has been shown to improve drought tolerance (Comas *et al.* 2013; Polle *et al.* 2019). The importance of root morphology in drought tolerance in plants has resulted in it becoming one of the targeted traits for plant breeders for improving water harvesting from deeper water resources from the soil (Polle *et al.* 2019). Therefore, the improved root traits developed by *C. sericea* plants under water-limitation may also result in better adaptation of these plants to subsequent water-limited conditions, which can be expected in semi-arid areas where rainfall variability is the major limiting factor for production (DEA 2013).

Results from this study also indicate that *C. sericea* plants subjected to water-limitation used multiple physiological adaptive responses to optimise the plant performance under the marginal conditions. Rapid stomatal closure, even under moderate durations of water-limitation in *C. sericea* plants could be the first line of defence against dehydration, resulting in reduced stomatal conductance and transpiration rate. Along with these, the reduced water availability and subsequent closure of the stomata resulted in decreased net photosynthesis and photosynthetic pigments, and therefore also carbon assimilation. This, in turn, could explain the significant reduction in biomass production under the water-limited conditions. These results generally correspond to the literature as many scientists believe that the first reaction of most plants to water-limitation is the closure of their stomata to prevent the loss of water through transpiration (Casson and Hetherington 2010; Anjum *et al.* 2011; Torres-Ruiz *et al.* 2013; Osakabe *et al.* 2014; Nemeskeri *et al.* 2015). Similarly, Mutava *et al.* (2015) revealed that under drought stress, reduced stomatal in soybean was responsible for reduced A. The rapid closure of the stomata in this study also resulted in a reduced E, which generally resulted in an increased PWUE in water-limited plants. Similar results were found by Kobata *et al.* (1996) and Tolk and Howell (2003) who found that reduced g_s in rice, and reduced evapotranspiration in sorghum were associated with higher water use efficiency (Blum 2005).

Chlorophyll content in *C. sericea* plants that were subjected to water-limitation were found to generally decrease from well-watered levels. A reduction in stomatal conductance has been shown to disrupt photosynthetic pigments because of the damage that occurs to the chloroplasts caused by reactive oxygen species (ROS) produced under drought stress. This, in turn, further reduces gas exchange and eventually leads to a reduction in plant growth and productivity (Anjum *et al.* 2011; Osakabe *et al.* 2014; Mutava *et al.* 2015; Pirasteh-Anosheh *et al.* 2016). The decrease in chlorophyll content is a commonly observed phenomenon under water-limited conditions (Bijanzadeh and Emam 2010; Din *et al.* 2011). Similar findings have been reported for other legume species such as mung bean (Batra *et al.* 2014), soybean (Makbul *et al.* 2011; Basal *et al.* 2020), chickpea (Mafakheri *et al.* 2010) and pea (Inaki-Iturbe *et al.* 1998). Results from the present study also indicated that under water-limited conditions, there was an increased production of protective pigments (carotenoid and anthocyanin). One of the ways that plants have evolved to protect themselves against ROS-induced damage under water-limited conditions, is the synthesis of protective pigments such as carotenoids and anthocyanin (Efeoglu *et al.* 2009; Batra *et al.* 2014; Basal *et al.* 2020). These pigments are believed to have contributed to the avoidance of severe damage to the photosynthetic machinery of the *C. sericea* plants during the water-limited conditions, and allowed for a faster recovery of the photosynthetic activity after re-watering (Hörtensteiner 2009; Frosi *et al.* 2017).

Re-watering of the *C. sericea* plants in this study generally resulted in recovery of all the photosynthetic parameters evaluated and a return to well-watered levels. After re-watering, transpiration rate and stomatal conductance returned to normal levels resulting in increased net photosynthesis. These recoveries to the photosynthetic machinery of *C. sericea* plants, along with the improved uptake of water through the deeper and better-developed root system, generally enabled shoot biomass to rapidly recover after the stress was removed. It is well known, however, that the extent of recovery after water-limitation can be limited by the intensity and duration of the preceding drought, before re-watering, a phenomenon called "pre-drought limitation" (Flexas *et al.* 2009; Wang *et al.* 2017). This was evident when water-limitation was imposed on the plants for 60 days, four months after establishment. Here, recovery after re-watering did not occur.

6.6. Conclusion

In this study, we examined the effects of reduced water availability and subsequent re-watering on the morphological and physiological traits of *C. sericea*. We specifically asked whether there was any evidence of adaptive plasticity in morphological and/or physiological traits in response to water-limitation and subsequent re-watering. Results from the current study indicated that *C. sericea* displayed a wide range of adaptive responses to water-limitation including increased allocation of resources to root growth, closure of stomata resulting in reduced loss of water through transpiration, and the development of protective pigments to aid in a faster recovery of the photosynthetic machinery after re-watering. Although *C. sericea* plants were significantly negatively influenced by water-limitation, rapid responses in both morphology and physiology allowed for rapid recovery once the stress had been removed. The extent to which the plants were able to cope with water-limitation, and whether or not they were able to recover after the stress was removed was, however, dependent of the age at which the stress was imposed on the plant, as well as the intensity/duration of the stress.

6.7. References

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CHAPTER 7

General conclusions and recommendations

Under future bioclimatic conditions, sustainable livestock production systems in semi-arid and arid agro-ecosystems in South Africa are at risk due to a lack of bioclimatically suitable forage crops for these water-limited areas (Dickinson *et al.* 2013, Truter *et al.* 2015). Furthermore, the predicted future climate change scenarios for South Africa (DEA 2013) will be coupled with reduced areas suitable for irrigated grazed pastures and fodder production (Schulze 2016), a rapidly increasing human population, and a subsequent increase in the demand for livestock products (Meissner *et al.* 2013). This, in turn, dictates the identification of improved fodder flow programs that can be used in current water-limited agro-ecosystems, and under future bioclimatic conditions. The work done for this thesis was mainly focused on selecting native forage species for further characterization and evaluation for their forage potential which subsequently can be implemented in these alternative fodder flow programs.

From the current study, after identifying a number of candidate legume species, one species was selected for further characterization, specifically focusing on the requirements for seed germination, seedling establishment, and plant survival under water-limited conditions. This final chapter therefore, examines the results of the preceding chapters, focusing on this one legume species (*C. sericea*), but puts the results obtained into a broader perspective. Although the body of work covered here is essential to determine whether a species should be considered for further evaluation, it lacks various components, which are important for agronomic characterization of the species, which would merit its inclusion into fodder flow programs for water-limited agro-ecosystems. Therefore, within this chapter, consideration will also be given to issues not examined by the prior research, but are deemed necessary and important to investigate further, and thus, may formulate the basis for further research.

7.1. Prioritization of native legume species

The initiative to identify alternative perennial leguminous forage species that can be implemented into fodder flow programs in semi-arid and arid South African agro-ecosystems was motivated by:

1. Knowing that due to rapid population growth and the predicted climate change scenarios for South Africa, vegetable, grain, and fruit production will most likely take priority over forage production systems under irrigated conditions and in suitable bioclimatic areas for rain fed agricultural systems. This, in turn, will most likely mean that livestock and fodder production will have to shift to more marginal areas characterized by water-limitation.
2. The lack of current commercially available perennial forage legume species suitable for use under dryland or minimum irrigated conditions within these water-limited agro-ecological areas of South Africa.
3. The lack of current forage species that can be used under the predicted hotter and drier South African agro-ecological conditions.
4. The recognition that the future demand for livestock products will most likely outweigh their supply if livestock production continues only using current livestock production systems.

It is therefore important to identify alternative forage species, already adapted to the marginal bioclimatic and edaphic conditions as predicted by future climate change scenarios. This in turn, will allow for inclusion of these bioclimatically adapted species into alternative fodder flow programs, and for future breeding focuses to be on improving the growth and nutritional aspects of these newly identified species rather than trying to breed exotic germplasm to become adapted to these semi-arid and arid livestock production areas.

During this research, 18 indigenous, perennial legume species were prioritized for further evaluation as potential forage crops within water-limited agro-ecological areas in South Africa. To date, from these original 18 species, seeds from a total of six prioritized species (*Calobota sericea* (SA-NFG 8732), *Crotalaria laburnifolia* (SA-NFG 1304), *Indigofera heterotricha* (SA-NFG 2275), *Lessertia diffusa* (SA-NFG 8730), *Lessertia frutescence* subs. frutescence (SA-NFG 8729) and *Lessertia incana* (SA-NFG 8316) have been collected and entered into the South African National Forage Genebank (SA-NFG) in Pretoria. These accessions are planned for characterization in 2021/2022. Several other collection trips are also planned for different areas within the succulent Karoo biome and also along an aridity gradient within the winter rainfall zone of South Africa, for collection of alternative ecotypes of these accessions, potentially containing different morphological and/or physiological characteristics than the already collected materials.

Furthermore, a renewed interest in annual legume species for the semi-arid, winter rainfall areas of South Africa has resulted in further research needs in identifying and prioritizing native annual legume species that can be used under the predicted future bioclimatic conditions. Under future bioclimatic conditions, these species will be used as alternative, better adapted, forages in the current, lucrative, pasture-crop rotation systems, like in the Western Cape province of South Africa, and others experiencing similar bioclimatic conditions around the world. Already in the initial screening of legume species that occur within the water-limited areas of the Northern Cape, four annual legume species (*Crotalaria effusa, Indigastrum argyroides, Lotononis falcata and Lotononis leptoloba*) were identified as potential alternative forages to be evaluated further (Chapter 2) with Trytsman *et al.* (2019) highlighting others that should also be considered.

7.2. Selection of *Calobota sericea* and current knowledge of its nutritional quality

From the 18 perennial legume species originally prioritized for further characterization and evaluation, *C. sericea* was one of the species that were already known to provide several benefits to livestock farmers within the semi-arid and arid rangelands of Namaqualand, South Africa. Prior studies has already shown that *C. sericea* plants contribute up to 16 % of the diets of grazing sheep and goats during the late dry season within the Leliefontein communal rangelands of Namaqualand (Samuels *et al.* 2016, Müller *et al.* 2019). One of the shortcomings of *C. sericea* plants harvested from natural populations is its low nutritional quality. Plant material collected from the Leliefontein communal rangelands of Namaqualand indicated that crude protein concentrations of *C. sericea* ranged between 6 % and 8 %, depending on when and where the plants were collected (unpublished results, Appendix 1). At these concentrations, if livestock diets were to be supplemented with *C. sericea* fodders, it would definitely mean that these animals would suffer from significant protein and mineral nutrient deficiencies (Meissner *et al.* 2000).

A preliminary investigation (unpublished results, Appendix 1) however, found that under minimum fertilized conditions crude protein concentrations in *C. sericea* leaves could be increased to concentrations ranging between 7 % and 15 % under well-watered conditions and between 6 % and 10 % under drought stress conditions. The crude protein concentration in the fodders however, was found to depend on the age of the plant when the materials were harvested, and the extent of the drought period, with older plant materials containing lower crude protein concentrations. These preliminary studies suggest that further evaluation into the fertilizer requirements of *C. sericea* is needed to determine the optimum fertilization regime for maximum yield and quality. Furthermore, trials to determine the best time to harvest and store *C. sericea* fodders are also underway to determine whether the physiological stage at which the plants are harvested from natural populations could influence the quality of the fodders produced. Within these trials, different storage techniques (hay or silage) will also be considered to determine the best means to conserve the highest quality *C. sericea* fodders for use during the dry season.

7.3. The impacts of climate change on the adaptation range of C. sericea

Due to the narrow distribution range of *C. sericea* (Boatwright *et al.* 2018), it was hypothesized that under future climate change scenarios for South Africa the species will most likely have significant range reductions, which would limit its agronomic potential. Results from this work (Chapter 3) supports the initial hypothesis in that *C. sericea* will most likely face a reduction in its current adaptation range under various future climate change scenarios, even though this will be with less than 2 % from its current distribution range. This modelled range reduction is predicted to result in significant loss (more than 5 %) of current *C. sericea* populations. Therefore, it was suggested that collection of plant genetic resources that fall within different adaptation ranges be collected in order to conserve as much genetic variability within the current *C. sericea* populations. These genetic resources will allow for the effective utilization and implementation of *C. sericea* fodders under different agro-ecological conditions under future bioclimatic conditions.

In order to collect and conserve as much of the current genetic variability within the current *C. sericea* populations, it is suggested that an eco-geographical study (Maxted and Guarino 2000) be conducted on the existing *C. sericea* populations. Eco-geographical studies refers to the process of gathering and synthesizing information on the ecological, geographical, taxonomic and genetic diversity of a species within a region of interest (Maxted and Guarino 2000). Results from these eco-geographic studies are predictive and can be used to assist in the identification of populations and areas of collection priority based on specific ecological variables. A preliminary eco-geographical investigation into *C. sericea* by the SA-NFG team has highlighted several collection priorities from unique populations across its current adaptation range which will be the focus of collection trips within the 2021-2022 growing season in the Namaqualand region of the Northern Cape, and Karoo region of the Western Cape.

Similarly, screening trials focusing on how well the remaining 17 prioritized perennial legume species are adapted to future bioclimatic conditions, as well as eco-geographical studies on these species are needed. This, in turn, will inform future collection, selection, evaluation and breeding activities for these species. Also, some of the species (*Cullen tomentosum, Indigofera alternans* subsp. *alternans, Indigofera heterotricha, Lessertia depressa, Lessertia pauciflora* var. *pauciflora, Senna italica and Lessertia frutescens* subsp. *frutescens*) initially prioritized, already have wide potential distribution ranges, with their potential ranges covering over 50 % of the total South African land surface (Chapter 2). Collection of these genetic resources, and identifying specific traits suitable for specific agro-ecological conditions, across South Africa, could lead to earlier adoption of these native species into breeding and crop improvement programs.

7.4. Requirements for dormancy breaking of Calobota sericea seeds

From this work, it was confirmed that germination of *C. sericea* seeds are constrained by physical seed dormancy because of an impermeable seed coat or testa. This dormancy could successfully be overcome following mechanical scarification using an abrasive sand paper. Although highly effective, increasing seed germination of *C. sericea* by more than 70 % (Chapter 4), and therefore can be used for small-scale plantings. The efficiency and commercial application of this scarification technique however, needs to be considered for large-scale operations.

Due to the small seed size of *C. sericea* mechanical scarification of large quantities of seeds will be very difficult and therefore, it is suggested that temperature fluctuations be considered as a means to break dormancy of large quantities of seeds for commercial distribution. Taylor (2005) highlights the progress made in Australia over the past 30 years towards breaking legume seed dormancy more effectively. Although this work of Taylor (2005) focusses on annual species, the techniques highlighted can also be tested for perennial species like *C. sericea* and other native legume species that are displaying physical seed dormancy.

The reasoning behind the use of temperature fluctuations to break seed dormancy comes from the fact that dormant seeds are exposed to significant diurnal temperature fluctuations, especially in the summer (dormant) months within the winter rainfall regions. It was found in Australia that during the day, in summer, soils could reach temperatures of more than 60 ˚C, while at night these temperatures can decrease to approximately 15 – 20 ˚C. This, in turn, produces a diurnal temperature fluctuation of approximately 40 – 45 ˚C (Taylor 2005). Aitken (1939) was the first who recognized the importance of temperature fluctuations on seed softening. Thereafter, Quinlivan (1961, 1966, 1968) further showed that increasing diurnal breadths from 15 ˚C to 70 ˚C generally increased the rates of seed softening. Later, Taylor (1981, 1993, 1996a,b) and Taylor and Revell (1999) showed that seed softening happened in two phases, the first phase (preconditioning) being a treatment at constant high temperature (> 50 ˚C), which softened some seeds, but most seeds remain dormant. The second phase is a treatment using appropriate diurnal temperature fluctuations, which leads to rapid softening of the remaining seeds.

Taylor and Revell (1999) showed the rate of preconditioning of yellow seradella seeds increased exponentially at constant temperatures between 30 ˚C and 70 ˚C. These authors showed that the rate of preconditioning doubled for every 5.2 ˚C rise, which resulted in 676 days at 30 ˚C for 50 % seeds to soften, and only 3 days at 70 ˚C. Preconditioning temperature requirements were found to differ between species and with some species, a separate preconditioning treatment was not needed as the diurnal fluctuation treatment simultaneously resulted in preconditioning and softening of the seeds (Taylor *et al.* 1991, Taylor 2005). An example of this can be seen in the diurnal temperature cycle of 60/15 ˚C in *Trifolium subterraneum* L. that results in soft seeds without an initial preconditioning temperature treatment phase. This 60/15 ˚C diurnal temperature fluctuation regime however, is unsuitable for the final stage of softening of seeds of *Medicago polymorpha* L. (Taylor 1996a) and *Ornithopus* *compressus* L. (Taylor and Revell 1999), but does result in the preconditioning of these species.

Just like in the Australian rangelands, soil temperatures during summer months in South Africa, when *C. sericea* seeds have been dispersed in the rangelands, differ greatly between day and night, causing large diurnal fluctuations. Due to the large diurnal temperature fluctuations expected in South Africa, it is believed that these temperature treatments could potentially also work for South African legume species. It is therefore, firstly important to quantify the amplitudes of the diurnal fluctuations in Namaqualand rangeland soils, by measuring the soil temperatures during the day and at night during the dry summer months. These readings should provide a good starting point when determining which preconditioning and/or diurnal temperature treatments to use for *C. sericea* seed softening. However, as seen in the examples above, these temperature treatments and their fluctuations, although highly successful for overcoming seed dormancy, can become very tricky. It is therefore suggested that further research into the temperature requirements for seed softening of *C. sericea* is needed to develop a cost effective means for large-scale dormancy breaking of *C. sericea* seeds.

7.5. Phenotypic plasticity in Calobota sericea

One of the major findings of this work was the phenotypic plasticity displayed by *C. sericea* at different developmental stages, which allows the plant to survive and/or overcome water-limitation at different growth stages. Phenotypic plasticity in plants gives them the ability to respond quickly to changes in their environments, and is key to the success of plants in natural and agro-ecosystems (Bradshaw 1965, 2006, Nicrota *et al.* 2010, Gray and Brady 2016, Arnold *et al.* 2019). At different stages of development, plants are often exposed to different stresses, or degrees of a particular stress, impacting differently on their survival. This is because the ecological niche requirements of plants are known to vary at different stages in their life cycle. This is primarily because the plant traits that determine the success with which a plant can survive a specific environment, changes as the plant develops (Grubb 1977). Therefore, it is important for fodder species, being developed especially for water-limited areas, to have a range of different plasticity responses to environmental changes as they develop. These responses, when characterized appropriately, can be selected for, when developing cultivars for specific agro-ecological conditions (Kusmec *et al.* 2018).

In *C. sericea*, phenotypic plasticity was observed in seed germination at different temperatures and osmotic treatments, as well as at seedling emergence from deeper seed burial depths (Chapter 5). At germination, it was found that seeds of *C. sericea* could germinate under a wide range of temperatures, but had a narrow range of osmotic requirements. The fact that seeds of *C. sericea* could germinate at temperatures of up to 30 ˚C substantiates the fact that as long as the water requirements for seed germination are met, the seeds of *C. sericea* plants would be able to germinate under the predicted future temperature increases in semi-arid and arid agro-ecosystems. The success of seedling establishment after germination however, will depend on follow up rain. Results from a preliminary study looking at seedling emergence under different soil moisture regimes, showed that seedling emergence/establishment was greater than 70 % up to a soil moisture content of 52 % of field capacity, but decreased to just over 30 % when soil moisture content reached 43 % of field capacity (unpublished results, Appendix 2). All of the established seedlings generally survived until soil moisture content reached approximately 14 % of field capacity after which rapid seedling mortality was observed to less than 10 % survival when soil moisture content reached 6 % of field capacity. This was true, irrespective of what soil moisture conditions the seeds were initially established in (unpublished results, Appendix 2).

When looking at seedling establishment from different seed burial depths, it was found that *C. sericea* seeds were able to establish from depths of up to 4 cm, despite their small seed size. This trait of *C. sericea* makes it very well adapted to establish under conditions with high rainfall variability where after the initial germinating rains, follow-up rains are delayed. Here, deeper seed burial may be advantageous to seedling establishment, allowing seeds and emerging seedlings, longer access to deeper water resources as the top soil layers rapidly dry after the germinating rains. At these deeper seed burial depths *C. sericea* seedlings were found to allocate more resources to the upward growth of the hypocotyls, to ensure successful seedling emergence from the deeper sowing depths. This trait was found to not be present in seeds sown at shallower burial depths (Chapter 5). At shallow seed burial depth, and as the top soil layers were drying, it was also found that significantly more resources were allocated to deeper root growth. This in turn, allows for *C. sericea* seedlings to actively mine for subsurface water and nutrient resources as the top soil dries.

After successful seedling establishment, during early seedling growth of *C. sericea*, it was also observed that heliotropism could potentially play a significant role in the efficiency that *C. sericea* plants deals with water-limitation, specifically in water conservation by the plants (Appendix 3). Heliotropism, or leaf movements, is the ability of plant leaves to track the movements of the sun. These leaf movements along with stomatal movement can improve plant production under different stress conditions (Blad and Baker 1972, Ehleringer and Forseth 1980, Meyer and Walker 1981, Kao and Tsai 1998, Chaves *et al.* 2003, Raeini-Sarjaz and Chalvi 2008). This is because the plant water status and its energy balance can be modified by the movement of leaves and/or stomata. Stomata primarily have the regulatory function of controlling the uptake of CO_2 for photosynthesis over transpirational water losses (Raeini-Sarjaz and Chalvi 2008). Leaf movements on the other hand, optimize the leaf energy balance by changing the amount, and intensity of light hitting the leaves, thereby changing leaf latent heat flux and leaf temperature (Forseth 1990, Raeini-Sarjaz and Chalvi 2008). In *C. sericea*, paraheloitropic leaf movements, or light-avoiding leaf movements were observed in water-limited plants (Appendix 3). In comparison to leaves of well-watered plants which were horizontally angled, this type of leaf movements minimizes light interception (Yu and Berg 1994) and avoids excessive heating of the leaves (Ehleringer and Forseth 1980), and therefore, reduces leaf temperature and transpiration rate (Shackel and Hall 1979, Ehleringer and Forseth 1980, Yu and Berg 1994). Several researchers have indicated that paraheloitropic leaf movements is an enhancing factor for increasing plant water-use efficiency (WUE) (Ehleringer and Forseth 1980, Forseth and Ehleringer 1983, Raeini-Sarjaz *et al.* 1997, Bielenberg *et al.* 2003, Raeini-Sarjaz and Chalvi 2008).

At irradiances above photosaturation, instantaneous WUE, which is the ratio between photosynthetic rate and transpiration rate, might decrease for horizontally angled leaves, while in the case of paraheloitropic leaf movement, WUE should be enhanced due to a reduction in light interception to below photosaturation level (Gamon and Pearcy 1989). The reduction in CO_2 assimilation as a result of closing stomata and paraheliotropism however, needs to be investigated further. Reduced photosynthesis due to lower CO_2 assimilation as a result of paraheliotrophic leaf movements can significantly reduce aerial biomass production under water-limitation. Therefore, the specific effects of these leaf movements on biomass production should be investigated further. This should especially be done under re-occurring short term periods of water-limitation in *C. sericea* seedlings to quantify its impacts on early biomass production, and how well, and how quickly, seedlings can recover after re-watering.

The older plants get, the more regularly they are expected to be exposed to periodic water-limitation as the seasons' progress, under semi-arid and arid agro-ecosystems. This is expected to worsen under future climate change conditions (DEA 2013). Therefore, it is important to understand how these plants will respond to, and their recuperative potential after, these frequent, short-term water-limited periods. Within this study in *C. sericea* plants experiencing water-limited conditions at two, three and four months after establishment, it was shown that *C. sericea* plants are able to change their resources allocated to different plant parts, based on the availability of water. It was shown that under water-limited conditions, *C. sericea* plants will allocate more resources to root growth, reducing shoot development, but were able to rapidly allocate more resources to shoot growth when the stress condition was reversed (Chapter 6). This was found to generally result in shoot biomass yields equivalent to those of well-watered plants within just 30 days after re-watering. This is a trait deemed essential when developing a fodder species for water-limited agro-ecosystems, under rain-fed, or minimum irrigated conditions, and areas with significant rainfall variability.

The protective properties of phenotypic plasticity displayed by *C. sericea* plants were evident in how fast these plants were able to generally recover from drought stress, after re-watering. This was due to a combination of morphological adjustments, such as increased allocation of resources to root growth for more efficient water-harvesting from sub-surface water, and physiological adjustments, such as reduced photosynthetic rate, and transpiration rate, and increased stomatal limitation, resulting in increased water-use efficiency, and an increased production of protective photosynthetic pigments such as anthocyanin's and carotenoids. These features together, after re-watering, allowed *C. sericea* plants to generally recover to well-watered levels within 30 days after re-watering.

The most important physiological adjustments displayed by *C. sericea* that helps with this rapid recovery, is the early reduction in stomatal conductance and reduced transpiration rates. Within this study, *C. sericea* plants were subjected to one water-limited period, irrespective of the duration, and one re-watering period. Measurements here were taken at the end of the water-limited or re-watering period. At each age at which water-limitation was imposed on the *C. sericea* plants, there was an increased duration of water-limitation, from 15 to 60 days. Therefore, enough information was gathered to conclude that *C. sericea* plants had an early reduction in transpiration rate, even under moderate (15 days) drought stress conditions, which suggest that the plants have a conservative subsequent use of water, during the water-limited period. Under long-term water-limitation, this approach is beneficial because it allows the plant to use less water while maintaining most of its physiological functions, even if it is at the expense of increased growth rates, but they are able to rapidly recover after re-watering. In this study, water-limitation for 15 days were regarded as moderate levels of drought stress. In nature however, water-limitation could occur for shorter durations within this 15-day period.

An alternative scenario that should be investigated further, and that is deemed to be more beneficial under short-term water-limitation is when stomatal closure only occurs later during the drought stress conditions, and plants consume as much water as possible, while the water is available. This means that stomatal closure only occurs later during the drought period, resulting in higher CO_2 assimilation than when stomata are closed early during the water-limited period. This, in turn, means that the plant can produce as much biomass as possible during the time when water is still available. Under short-term water-limited conditions such as variable irrigation frequencies or controlled deficit irrigation, this approach is much more favorable, resulting in increased plant biomass. In this study, it was clear that the duration between re-watering events had a significant impact on the recovery of the plants. Therefore, the responses of *C. sericea* to shorter periods of water-limitation and subsequent re-watering needs to be investigated further to effectively quantify its ability to grow and produce sufficient amounts of biomass for livestock production.

7.6. Conclusion

This study highlights the potential and techniques needed to identify and characterize native species with the potential to be included into fodder flow programs in water-limited agro-ecological areas. The selection of native species that naturally occur within water-limited ecosystems provides an alternative to the conventional breeding and development of existing commercially available exotic forage germplasm for specific agro-ecological conditions. When considering conventional forage breeding practices compared to the implementation of native forage species, it is clear that the process of selecting native forage species is different to domestication and breeding of exotic, well known forages, with the aim of developing cultivars that are adapted to water-limited conditions of South Africa. The selection of native species from current water-limited areas in South Africa means that little or no efforts will be needed to improve the climatic adaptability of these species for use within their native water-limited ranges. If the native species are to be implemented in novel environments however, significant breeding for adaptability will still be needed. Within their native ranges, breeding and improvement of agronomic characteristics such as improved biomass production, indehiscence in seedpods, nutritional quality, etc. will still be needed to produce highly productive forage cultivars.

The time required for breeding a drought tolerant variety/cultivar can take more than 10 years, depending on the breeding techniques used (per communication ARC Cedara forage breeding division). Therefore, by selecting native species that are already adapted to their native ranges drastically reduces the time needed to implement the species, as is, in alternative fodder flow programs. However, further research will still be needed to understand the mechanisms employed by these native species that enables them to survive these harsh conditions. In addition, studies into the agronomic requirements (germination, seedling establishment, fertilizer requirements, and harvesting times) for these species will still be needed, as well as studies into how these native forages will influence livestock production i.e. animal health, quality of the products produced and the socio-economic impact on the farmers using the new fodder flow program.

The promising preliminary results obtained in this study however, verifies the potential of *C. sericea* as a species that can survive significant degrees of water-limitation, and its ability to recover rapidly once the stress is relieved. The potential of this species is also attributed to its wide range of adaptive responses to drought and its potential for expanding its agronomic use into novel agro-ecosystems with further breeding and improvement. This makes it a good candidate for inclusion into semi-arid and arid fodder flow programs. However, unlike existing exotic commercial forages, which are already known to be of high forage value and the agronomic requirements for high productivity, further evaluation into the agronomic requirements of *C. sericea*, which includes best practices such as fertilization requirements, harvesting times, forage storage techniques and the effects of supplementary feeding of *C. sericea* fodders to livestock are needed. These research questions will be answered in the evaluation phase (Figure 1.1) of the project, which will commence in 2020/2021 through an RMRD-SA funded research grant.

7.7. References

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<u>Appendix 1</u>: Nutritional quality of *C. sericea* biomass collected from the Namaqualand rangelands and those grown under minimum fertilization

Table 1: Nutritional quality of *C. sericea* shoots collected from the Leliefontein communal rangelands in Namaqualand, South Africa

	Wet Season	**Dry Season**
Condensed Tannins (mgSTE/g)	**1.1**	**0.2**
Total Phenolics (mgGAE/g)	**9.8**	**4.9**
NDF (%)	**43**	**74**
ADF (%)	**35**	**66**
Hemicellulose (%)	**8.7**	**7.3**
Crude Protein (%)	**8**	**6**
Dry Matter Digestibility (%)	**62**	**37**
Total Digestable Nutrients (%)	**64**	**41**
Metabolisable Energy (Mcal/kg)	**8.5**	**4.3**
Digestible Forage Energy (Mcal/kg)	**2.8**	**1.8**
Net Energy for Lactation (Mcal/kg)	**1.4**	**0.9**

Table 2: Crude protein content in fertilized* *C. sericea* plants harvested 15, 30, 45 and 60 days after drought when drought was imposed at 2, 3 and 4 months after establishment.

		2 Months	**3 Months**	**4 Months**
15 Days Drought	Well-watered	14 ± 0.6	14 ± 1.3	7 ± 0.2
	Drought	10 ± 0.7	8 ± 0.8	7 ± 0.4
	Re-watered	11 ± 1.1	10 ± 1.6	7 ± 0.2
30 Days Drought	Well-watered	15 ± 0.4	12 ± 1.8	7 ± 0.01
	Drought	10 ± 0.2	7 ± 0.3	6 ± 0.2
	Re-watered	11 ± 1.0	10 ± 0.1	8 ± 0.6
45 Days Drought	Well-watered	15 ± 2.1	13 ± 0.9	7 ± 0.1
	Drought	9 ± 1.5	7 ± 0.1	7 ± 0.1
	Re-watered	11 ± 1.3	10 ± 0.9	7 ± 0.2
60 Days Drought	Well-watered	15 ± 1.4	13 ± 1.2	7 ± 0.4
	Drought	9 ± 0.8	7 ± 0.3	7 ± 0.1
	Re-watered	11 ± 1.6	11 ± 0.7	7 ± 0.2

* The seedlings were allowed to grow for one month before a full-strength stock nutrient solution (Plant Food- Starke Ayres) was applied once to the pots.

Appendix 2: Seedling emergence under different soil moisture regimes

Table 1: Maximum seedling emergence (%) and total seedling mortality (%) after eight days of water-limitation. Significant differences ($p < 0.05$) in maximum seedling emergence is indicated by different superscript letters. The soil moisture content (%) depicts the soil moisture content when the seeds were planted.

Soil Moisture content (%)	Maximum seedling emergence (%)	% Seedling mortality
100	98 ± 2^{c}	92 ± 2
70	97 ± 2^{c}	95 ± 3
52	74 ± 10^{b}	93 ± 1
43	32 ± 4^{a}	100 ± 0

Table 2: Seedling emergence *Calobota sericea* in relation to soil moisture content (%) at planting.

Soil Moisture Content (%)	Emerged Seedlings (%)							
100	0 ± 0^{a}	35 ± 5^{b}	84 ± 4^{c}	98 ± 2^{d}	98 ± 2^{d}	98 ± 2^{d}	85 ± 7^{c}	8 ± 2^{a}
70		0 ± 0^{a}	65 ± 5^{b}	93 ± 1^{d}	96 ± 2^{d}	97 ± 2^{d}	78 ± 7^{c}	5 ± 3^{a}
52			0 ± 0^{a}	36 ± 7^{b}	70 ± 10^{c}	74 ± 10^{c}	29 ± 3^{b}	5 ± 1^{a}
43				0 ± 0^{a}	25 ± 5^{b}	32 ± 4^{b}	5 ± 1^{a}	0 ± 0^{a}
Soil Moisture (% of pot capacity)	100 ± 0^{h}	70 ± 1.4^{g}	52 ± 0.6^{f}	43 ± 1.4^{e}	31 ± 2.1^{d}	14 ± 1.3^{c}	11 ± 1.0^{b}	6 ± 0.5^{a}

Appendix 3: Paraheliotrophic leaf movements in *Calobota sericea* under well-watered (A) and water-limited (B) conditions