Potential implications of climate change for Rooibos (*A. linearis*) production and distribution in the greater Cederberg region, South Africa.

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ABSTRACT

Wild plants assist in supporting human livelihoods worldwide, both within traditional systems of medicine, and as economically useful plants. Indigenous to the Fynbos biome in the north-western part of the Western Cape, South Africa is the leguminous shrub, *A. linearis* (rooibos), which is extensively used as ethnomedicine by local communities, while also commercially grown and exported for the herbal tea market. Being a range-restricted species, climate change poses a threat to wild plants and their dependent communities, as well as the sustainability of the rooibos industry. Climate mediated impacts on rooibos are mostly substantiated by anecdotal evidence from commercial growers and local communities and have traditionally been insufficiently addressed. This study integrates predictive modelling and empirical data to provide important insights into rooibos' plant physiological functioning in the presence of climatic and environmental constraints. The aim is to determine whether there is evidence of climate change over the rooibos distribution area, how these climate anomalies are expected to affect the species distribution and to perform experimental studies by testing plant physiological functioning of *A. linearis* under changing climate conditions.

Analysis of climate parameters important for rooibos production (rainfall frequency and intensity, temperature extremes and wind speed) have shown that plants will experience a shorter period of water availability during winter, and prolonged exposure to summer conditions (high temperatures and water stress) in the coming decades. Under these conditions, climate envelope modelling suggests that wild and cultivated rooibos types are at risk to lose between 49.8% and 88.7% in the extent of the bio-climatically suitable localities, most notably along the western and northern periphery of the rooibos production area by 2070.

Plant physiological responses (growth analysis, gas exchange parameters and leaf carbon and nitrogen isotope ratios) to the assessed climate anomalies were measured in experimental studies at glasshouse and field scale. Specific adaptation mechanisms (increasing water use efficiency, developing a higher level of sclerophylly and altering the allocation of plant reserves) which helped seedlings to survive short term drought in the glasshouse were not able to offset more severe conditions in field settings. Finally, a comparison of wild and cultivated tea has shown an apparent adaptive advantage of wild tea to tolerate increased aridity with greater water economy, and more reliance on biological nitrogen fixation for N nutrition, indicating a potentially less severe scenario of range contraction for wild types than initially indicated. This study provides a more robust prediction of rooibos plant responses to climate change factors to enable more effective adaptive planning and conservation management in a changing climate.

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CONTENTS

CHAPTER 1: GENERAL INTRODUCTION

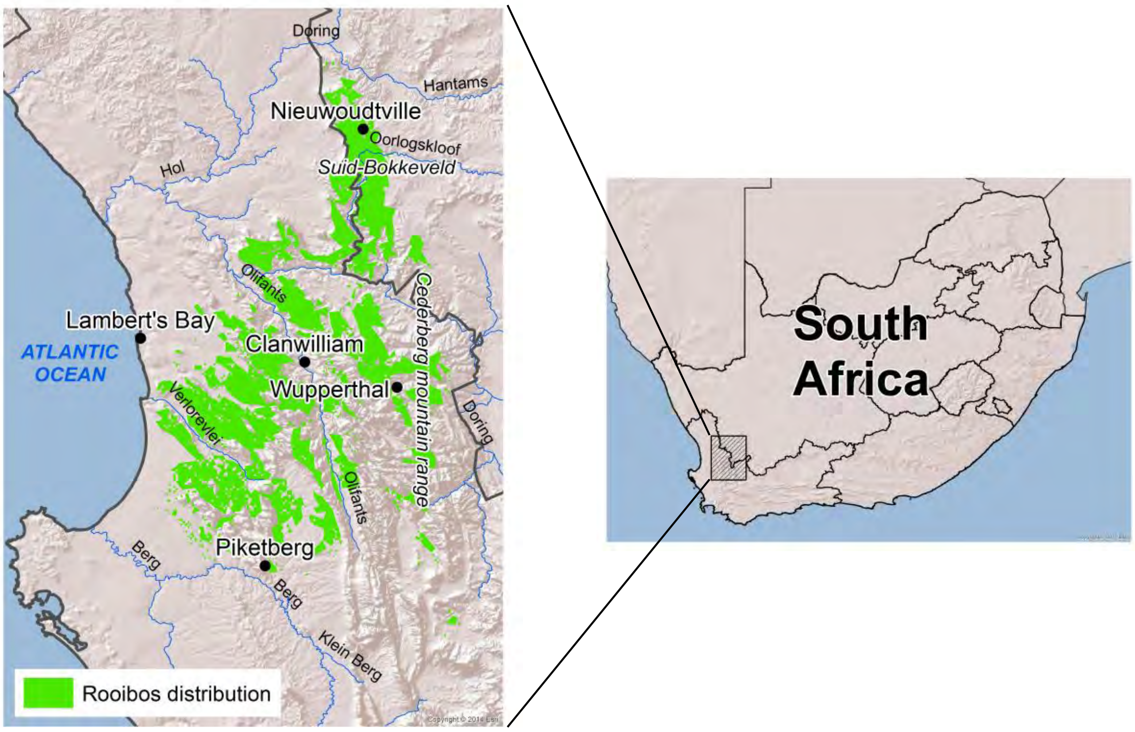
1.1 Background

Globally, there has been a major resurgence in interest in traditionally used medicinal plants due to growing recognition of their therapeutic, ecological and economic significance (Street and Prinsloo, 2013). Although the Cape Floral Region (CFR) of South Africa has an extraordinarily high plant species density, diversity and endemism it has comparatively few species of medicinal value compared to other biomes (Goldblatt and Manning, 2000; Rebelo *et al.,* 2006). Nevertheless, the most extensively studied and economically important medicinal species in South Africa is arguably the leguminous shrub *Aspalathus linearis* (Burm. F) Dahlg., Fabaceae (Dahlgren, 1968) which is endemic to the CFR.

This species occurs naturally in the greater Cederberg region of the Western and Northern Cape Province of South Africa where it has been used for centuries as traditional medicine. *Aspalathus linearis* made an effective transition from wild resource to important cultivated crop in the early 20th century (van der Bank *et al.,* 1995), but is still harvested in the wild by local people for niche markets abroad. The leaves and twigs of the plant are used to produce an herbal beverage known as "*rooibos*" tea which is internationally recognized for its health-promoting properties (Baba *et al.,* 2009; Kawano *et al.,* 2009; Dludla *et al.,* 2013; Hong *et al.,* 2014). Due to its unique soil and climatic requirements rooibos has not been successfully cultivated in other parts of the world. All demand is supplied from a single production area that is approximately 200 x 100 kilometres in extent. Moreover, rooibos is the only known source of aspalathin which is a significant antioxidant with antimutagenic activity and therefore adds to the uniqueness and novelty of this herbal tea (Joubert and Schulz, 2006).

Rooibos production has traditionally encompassed the mountainous part of the Fynbos biome but has progressively expanded into lowland areas in the south and the west of the CFR where wild rooibos does not occur. The Cederberg region, being one of the traditional mountainous growing regions, is a semi-arid environment where conventional agricultural production is limited by low and erratic rainfall, high evapotranspiration rates and nutrient poor soils (Cowling *et al.,* 1997; Goldblatt and Manning, 2000). The area is bounded by the Atlantic Ocean on its west side and the Cederberg mountain range in the east while Nieuwoudtville in the Northern Cape Province and the Berg River form its northern and southern boundaries (Fig. 1). Its Mediterranean climate is characterised by a strong seasonal pattern of rainfall, with marked aridity during summer months (December-February) and most of the annual rainfall falling during the winter period (June-August). However, the topographically diverse nature of the region ensures that the climate is by no means uniform. Rainfall varies from as little as 150 mm per annum on the coast and lowlands to over 1000 mm on the interior plateaus of the Cederberg mountain range (over 2000 m.a.s.l.). Winter rainfall is mainly associated with rain bearing mid-latitude systems, while summer convective precipitation events contribute to annual rainfall in the far north east. It is a drought prone environment with regular and recurrent dry spells (Rouault and Richard, 2003). According to the IPCC (2013) Mediterranean ecosystems such as the CFR are particularly exposed and vulnerable to the impacts of climate change. Not only is biodiversity at high risk but also the rural livelihoods which are dependent on rain-fed crops, such as rooibos tea.

Figure 1 Map of the Cederberg region, showing the natural distribution of rooibos in the north western part of the Western Cape province, South Africa.



1.2 Habitat suitability and climate change

1.2.1 Aspalathus linearis' ecology and distribution

Aspalathus linearis belongs to the genus Aspalathus (Fabaceae, Tribe Crotalarieae) which consists of 279 species (Dahlgren, 1988). Although taxonomically treated as one species, wild populations of *A. linearis* vary significantly from one another. Van Heerden *et al.,* (2003) distinguish seven main rooibos tea 'types' based on distinct morphological and chemical differences. These types also differ, in fire survival strategy, vegetative and reproductive morphology, isozyme patterns and flavonoid composition (Malgas *et al.,* 2010). Of particular interest is the congeneric occurrence of obligate seeder (hereafter seeder) and sprouter life-histories, of which both belong to the *A. linearis* species (Schutte *et al.,* 1995). For both types, resprouters and seeders, seed germination is triggered by fire (Brown *et al.,* 1993). However, sprouters are also able to regrow from an underground lignotuber after fire, while seeders are killed by fire and can only re-establish from the soil-stored seed bank after fire. Seeder life histories (i.e. non-sprouters) dominate in Fynbos (Le Maitre and Midgley, 1992), and this feature has been ascribed to the reliable winter rainfall in this region (Ojeda, 1998; Cowling *et al.,* 2005; Ojeda *et al.,* 2005). These findings have particular relevance to the present study since the mode of regeneration may determine the extent to which changing climate conditions will affect the species.

The species is ant dispersed and its hard-shelled, fire-stimulated seeds germinate in the early winter months after the passage of the first rain bearing cold fronts, whereas commercially collected seeds need special smoke treatment and acid scarification to reduce their impermeability and increase germination success (Kelly and Van Staden, 1985). The seeds are sown in well-prepared seedbeds during the late summer months of February and March. This is the only phase during the lifecycle of the plant when supplementary watering is applied and hence the critical and stressful stages of seed germination and seedling emergence are artificially moderated. During early stages of development, cultivated rooibos may, therefore, not be limited to the same extent by rainfall onset and distribution compared to wild types. During the winter months of June to August, when seedlings have reached a height of 10-20cm they are transplanted into cultivated fields. Plants are especially vulnerable to environmental constraints during these early life-stages (Harper, 1977; Kitajima and Fenner, 2000) and in Mediterranean ecosystems, most seedling mortality occurs in the first dry season of their life cycle. Hence, sufficient late winter and spring rainfall is critical to ensure initial establishment of seedlings during winter and will enhance the ability of plants to survive the first summer drought (Richards & Lamont, 1996). Approximately 18 months after plants have first been established; they are pruned and then harvested annually during late summer and early autumn (January to April). By contrast, it has been established that certain wild rooibos types are relatively slow-growing plants and should be harvested only every two years (Louw, 2006). Commercial plantations have an average lifespan of 7 years and peak production is reached after three years (Cheney and Scholtz, 1963).

Despite conventional crop production in the Fynbos biome being severely limited by environmental constraints, indigenous rooibos production is particularly well adapted to local soil and climate conditions. *Aspalathus linearis* is a nodulating legume which is able to form symbiotic relationships with both rhizobial bacteria, (specifically *Bradyrhizobium* species (Staphorst and Strijdom, 1975; Dakora, 1998) and arbuscular mycorrhizal fungi. The species grows mainly in nutrient poor, highly acidic and well-drained, sandstone-derived soils (pH 3–5.3) typical of the mountainous areas in the area (Muofhe and Dakora, 2000). Yet, *A. linearis* and its associated microsymbionts have managed to establish a functional N_2-fixing symbiosis which can tolerate the extremities of soil acidity and low nutrient stress by fixing high levels of N (annual contribution of 105.0–128.0 kg N fixed ha^{-1}, Muofhe and Dakora, 1999). Both establishment and activity of the legume Rhizobium symbiosis have been found to be extremely sensitive to drought stress (Sprent, 1972; Kirda *et al.,* 1989). The plant therefore fulfils a very important nitrogen fixing, ecological role as pioneer plant after fire.

1.2.2 Climate change in the Cape Floral Region

Analyses of historical rainfall data for the latter part of the 20th century for the Western Cape indicate that there are few spatially coherent or statistically significant trends in the annual rainfall (Kruger, 2006; New *et al.,* 2006). However, studies of frequency and intensity related characteristics of rainfall distribution have revealed some trends. Midgley *et al.,* (2005) found evidence of a shift towards later winter rainfall in the western part of the CFR, whilst Hewitson *et al.,* (2005) found divergent seasonal trends during the latter part of the 20th century for the Western Cape. There were positive trends in rainfall during March – May, which was mostly explained by the increase in intensity of rain events, whereas June-August exhibited a drying trend.

By contrast, there is more confidence and consistency in time series analyses of historical temperature trends across the CFR. In a recent study on South African temperature trends (MacKellar *et al.,* 2014) for the period 1960–2010, significant increases in maximum temperature of up to 0.027 °C/year were found in all seasons over the Western Cape, while increases in minimum temperature were mostly smaller (0.011 °C/year to 0.021 °C/year). In a study by Kruger and Sekele (2013) which focussed on extreme temperature indices (1962-2009) it was found that there was a significant increase in hot extremes and decrease in cold extremes, especially towards the western and northern interior of the country, which include parts of the north-eastern section of the rooibos production area.

Climate change and extensive habitat transformation are considered key pressures on biodiversity in the Fynbos and Succulent Karoo biomes (Midgley *et al.,* 2002, 2003; Rouget *et al.,* 2003c; Latimer *et al.,* 2004). The consensus among Global Climate Models (GCMs) is that Mediterranean climate regions will become warmer and drier and hence are particularly vulnerable to climate change. Globally, the extent of the Mediterranean climate, which stretches across five continents and supports five biodiversity hotspots, is projected to contract by 7.2%, mainly due to warming in winter, and to a lesser extent also as a result of a decrease in total annual precipitation (Klausmeyer and Shaw, 2009).

Consistent with these projections of climate change for Mediterranean climates, the IPCC's 4th assessment report indicates a reduction of 15-20% in annual mean rainfall and an increase in temperature of 1-2 ˚C for the period 2040–2060 over the north-western parts of the Western Cape which coincides with the rooibos production area. Annual average temperatures may increase up to 5°C for the period 2080–2100 (Malherbe *et al.,* 2013) while rainfall in the Western Cape is expected to further decline towards the end of the 21st century (Engelbrecht *et al.,* 2009, Engelbrecht *et al.,* 2011). The projected negative rainfall anomalies may attain values that are well outside those associated with the present-day climatological regime (DEA, 2013). Engelbrecht *et al.,* (2009) and Malherbe *et al.,* (2013) ascribe these future drying trends over the south-western Cape to strengthening of mid-level anti-cyclones over southern Africa, and a strengthening of anti-cyclonic circulation to the south of the country. Both of these occur in conjunction with a southward displacement of the westerly wind regime. Other studies (Hewitson *et al.,* 2005; Midgley *et al.,* 2005; Hewitson & Crane, 2006) also suggest the occurrence of more extreme events, leading to increased frequency and intensity of drought. Given that the area is already water-limited, an annual average temperature increase of 2 to 4°C, coupled with less rainfall, could significantly impact the hydrological and climate regime of the region.

1.2.3 Rooibos and climate change

Since rooibos is a rain-fed crop, such climate projections are expected to have important consequences for plant performance and yield. Published studies (Archer *et al.,* 2008; Archer *et al.,* 2009; Malgas *et al.,* 2010) regarding the projected climate change impacts on rooibos tea are partially supported by anecdotal evidence of the effects of existing climate variability on rooibos stands. Archer *et al.,* (2008) engaged local farmers through participatory action research (PAR) to monitor climate conditions and document the associated effects on rooibos stands. Late onset of winter rains, increased frequencies of dry spells, and heat stress cause severely diminished yields and affect the quality of the rooibos products. These findings have fuelled further research into using climate scenarios to determine the risk of exceeding certain critical plant physiological thresholds (as suggested by rooibos agricultural representatives) under a future climate regime. In this regard, Archer *et al.,* (2009) used statistically downscaled climate scenarios to show that there is an increasing likelihood in the future of exceeding the temperature threshold (32˚C) for heat stress. The expected increase in temperatures and altered rainfall patterns may therefore modify existing spatial distribution patterns and suitability of wild and commercial rooibos.

According to Gerard (2011), rooibos has stringent habitat requirements and its distribution seems to be mainly driven by abiotic factors and water availability in particular. Since climate exerts a dominant control over the natural distribution of species, it is expected that climate change will have a significant effect on species geographic ranges (Pearson and Dawson, 2003). Endemic species may be particularly threatened by anthropogenic disturbances and climate change due to their small range and narrow ecological requirements (Midgley *et al.,* 2003). Wild populations of *A. linearis* have a narrow geographic range within the Fynbos biome and are largely confined to the Cederberg Mountains at elevations between 450 and 1000 m.a.s.l. If climatic factors such as temperature and precipitation in a region change beyond a species' evolutionary adaptation potential, then distribution changes of the species may be inevitable. Evidence suggests that some species have already responded to recent warming by shifting their geographic distributions toward higher latitudes and elevations (Parmesan *et al.,* 1999; Thomas *et al.,* 2004; Hickling *et al.,* 2006; Chen *et al.,* 2011).

Species distribution models (SDM's) use environmental variables in conjunction with species location information to characterize species' niches and map their geographic ranges. In combination with climate scenarios SDM's can provide valuable information regarding future shifts in climate envelopes. Based on SDM's, early estimates of the potential impacts of drier and hotter conditions on species distribution in the CFR show that the extent of the Fynbos biome could be reduced by between 51% and 65% (depending on the particular future climate scenario) (Midgley *et al.,* 2002). Up to one third of species of Proteaceae may need to migrate to completely novel geographic ranges in order to persist. More recent predictions of change for the climate envelope associated with the Fynbos biome suggest, however, a less radical change than the initial assessments, although a similar pattern emerges. Based on outputs from 15 global circulation models, DEA (2013) predict that the eastern and northern sections of Fynbos are likely to undergo more change, with the climate envelopes in these areas becoming more like Albany Thicket or Succulent Karoo, respectively. These results may therefore have important implications for rooibos distribution and production potential, especially along the north-eastern periphery of the production area which lies within the transition zone between the Fynbos and the Succulent Karoo biomes.

Although these models are extremely useful as a first cut assessment of species range changes, they make many basic ecological assumptions when extrapolating current correlations between species distributions and the environment into the future (Sinclair *et al.,* 2010). The use of experimental field and glasshouse studies in conjunction with SDM's can greatly improve confidence in these models. However, the majority of studies using species distribution models to address climate change impacts on biodiversity do not attempt to support their predictions through direct experimental evaluations. There is therefore a need to combine distribution models and experimental work to gain a more complete understanding of plant physiological responses and ability of rooibos to adapt to a future climate.

1.3 Economic and cultural significance of rooibos production

The consumption of rooibos as a herbal infusion can probably be traced back to the traditional knowledge of the Khoi-San people that have lived as herders and gatherers in the

Cederberg area since around 1200 AD (Mountain, 2003). Some literature suggests that local inhabitants being descendants of the Khoi-San communities used the plant to treat ailments during the 17th and 18th centuries. More recently, in the early 20th century, Watt and Breyer-Brandwijk (1932) listed rooibos as a South African medicinal plant, but no indication of specific applications were given. This shows that the use of rooibos as a traditional medicine have long been part of South African folklore, while anecdotal evidence of its medicinal properties has become widespread since the 1960's.

The modern day production process of the tea is largely based on the traditional techniques developed by local inhabitants over several generations. One particular tradition that local people take pride in is the collection of the tiny rooibos seeds by hand. Although commercial seed collection has evolved over time, seed collection by hand is seen as a rewarding activity and provides locals with an additional source of money (Leclercq *et al.,* 2009).

The commercialization process of the tea began in 1904, but it was only in the 1930's that a few individuals from Clanwilliam managed to successfully propagate the first crop from seeds collected in the Pakhuis area of the southern Cederberg (Cheney and Scholz, 1963; Morton, 1983). At present the 350 to 550 Rooibos farmers in South Africa produce approximately 12 000 tons of rooibos tea per year on an area of 36,000 ha under cultivation. Local consumption accounts for between 4500 and 5000 tons, while the rest is destined for international markets, with Germany being the main export destination. Conventional prices for Rooibos fluctuate greatly depending on climate conditions and the area planted. Rooibos production, processing and packaging is a labour-intensive process and the industry is therefore one of the largest employers of people from the rural provinces of South Africa, providing both permanent and seasonal employment opportunities.

Apart from the appeal of the beverage for health reasons, rooibos is of particular interest since a growing proportion of the commodity is sourced from resource-poor small-scale farmers situated in isolated rural areas around Nieuwoudtville and Wupperthal (Nel *et al.,* 2007). These are two of the more economically marginalized areas of South Africa due to past racial inequalities, land access difficulties, geographical remoteness and the low agricultural potential of land. These resource poor farmers are often particularly vulnerable in that they do not have sufficient resources and access to timely information to deal with the adverse effects of climate change. Until recently, small-scale rooibos producers used to receive scandalously low prices for their harvest (Heiveld, 2008a). However, with the assistance of local and international non-governmental organizations, these two communities were organized into cooperatives (named Heiveld and Wupperthal respectively) which enabled them to have more bargaining power, better access to information, and to share capital. Both communities also produce wild tea that is harvested from near pristine areas and marketed separately as a niche product. The Wupperthal cooperative now has 170 members and produces from 80 to 100 tons of Rooibos while the Heiveld cooperative has 42 members producing about 45–55 tons of Rooibos annually. Both cooperatives use production techniques where indigenous knowledge has been firmly embedded in the harvesting and processing of the tea. Rooibos has very recently received geographic indicator status (GI) and protection due to the ecologically important environment within which the plant is cultivated, its limited geographical range and strong link with local farmers' heritage. Therefore, predictions that the Fynbos and Succulent Karoo biome are particularly vulnerable to climate change not only indicate a high probability of extensive species loss, but also pose a challenge to the region's ability to support livelihoods dependent on dryland cultivation.

1.4 Objectives of this study

The majority of rooibos tea research in South Africa focuses on its biochemical composition and associated health benefits, while the remainder address root physiology, pollination ecology, pathology and ecological significance. As such, the overarching aim of this study is to examine and describe the potential for climate change to induce distributional shifts for *A. linearis*, as well as to broaden scientific knowledge on the eco-physiological response and adaptive traits of the species to climate stresses.

Within this overarching aim, several clearly distinguished objectives of the thesis can be identified:

i. To establish whether there is any evidence of recent climate change over the rooibos production area and how this coincides with projected climate change.

ii. To explore how future climate scenarios might affect the geographical distribution and habitat suitability of wild and cultivated *A. linearis*.

iii. To empirically test the predictions of distribution models and assess rooibos' ability to survive under different climate conditions.

iv. To investigate physiological adaptation strategies in wild and cultivated *A. linearis* to seasonal water deficits.

These objectives are addressed in chapters 2 to 5 and are presented as four manuscripts that have been submitted to or published in peer-reviewed journals. Consistent with the requirements for a Ph.D. thesis the lead author was responsible for all data acquisition, analysis and interpretation as well as drafting, revising and preparing the manuscripts to be published. The co-authors in the papers acted in their capacity as supervisors, overseeing the writing process.

Chapter 2 is a manuscript entitled "Recent climatic trends and future change: potential implications for rooibos production in the Greater Cederberg region of South Africa" which has been submitted to the *Journal of Arid Environments*. In this chapter, I undertake a comprehensive analysis of three meteorological variables (historical rainfall, temperature and wind speed) for the period 1961- 2010 across the south west coastal region of South Africa. This addresses the first objective of the thesis, i.e., to determine whether there is evidence of climate change already ocurring over the rooibos production area. Based on the most important rooibos-specific indicators as suggested by local farmers and scientific literature, I systematically evaluate trends in historical data to identify evidence of climate change. Indices relating to changes in the extremes of rainfall and temperature are also evaluated. Lastly, an ensemble of dynamically downscaled climate models is assessed to determine whether future climate change scenarios for the Western Cape can be reconciled with the observed trends. These findings are then discussed in terms of its implications for rooibos production and survival.

Chapter 3 is a paper entitled "Modelling the distribution of *Aspalathus linearis*' (Rooibos tea): Implications of climate change for livelihoods dependent on both cultivation and harvesting from the wild" and published in *Ecology and Evolution 4(8), 1209–1221, April 2014, DOI:10.1002/ece3.985*. **Chapter 3** focuses on the spatial distribution of wild and cultivated *A. linearis* under scenarios of climate change and the subsequent implications for livelihoods, conservation, and adaption planning. This chapter thus covers the second objective of the PhD thesis. The same climate variables and scenarios assessed in **Chapter 2**

are used in **Chapter 3** to develop an estimate of the realized niche of wild rooibos and the current geographic distribution of areas suitable for commercial production. Using presence only occurrences of rooibos and relevant environmental variables, a maximum entropy (Maxent) species distribution model is employed to develop the current bio-climatic envelope for wild and cultivated rooibos. This model for the current conditions is then projected using an ensemble of climate change scenarios to assess possible range/suitability shifts under future climate conditions.

Chapter 4 of the thesis comprise a paper entitled "Physiological responses of a Fynbos legume, *Aspalathus linearis* to drought stress" published in the *SA Journal of Botany 94, 218–223, Sep 2014, http://dx.doi.org/10.1016/j.sajb.2014.07.005*. The climatic limits and requirements identified in **Chapter 3** are used in **Chapter 4** to inform the location of a field trial and design of a glasshouse study to test the predictions of the species distribution model and assess plant functional responses to water limitation. This chapter therefore addresses the third objective of the thesis. Using seedlings of *A. linearis*, the effects of drought on plant performance was tested by measuring growth, photosynthetic performance and leaf carbon and nitrogen isotope ratios ($\delta^{13}C$ and $\delta^{15}N$).

Chapter 5 is a paper entitled "Seasonal variation in the N nutrition and C assimilation in wild and cultivated *Aspalathus linearis* (Rooibos tea)" published in the *Australian Journal of Botany 62(1), 65-73, April 2014 http://dx.doi.org/10.1071/BT13237*. In **Chapter 5** the last objective of the thesis is addressed and therefore seeks to establish whether there are significant differences between wild and cultivated rooibos in their ability to tolerate adverse environmental conditions. To test these possibilities, I evaluated changes in leaf gas exchange rates, nutrient cycling and natural leaf abundances of $\delta\ ^{15}N$ and $\delta\ ^{13}C$ for wild and cultivated *A. linearis* at the extremes of temperature and rainfall conditions during summer and winter.

The synthesis (**Chapter 6**) discusses and synthesises the implications of the thesis findings and how the individual areas of research contribute to an understanding of how climate change may affect rooibos producers, as well as the significance for wild plant populations and conservation. The limitations of the present study are highlighted, and areas of promising research which can significantly move the research forward in the future are discussed.

CHAPTER 2: CLIMATE TRENDS

Recent climatic trends and future change: potential implications for rooibos production in the Greater Cederberg region of South Africa.

Abstract: Commercial rooibos production is amongst the most important economic activities in the arid west coast region of South Africa. Being a rain-fed crop, cultivation of this indigenous shrub is particularly vulnerable to rainfall variability and high temperatures. This study aims to investigate historical trends and future simulations of 3 meteorological variables (temperature, rainfall and wind speed) to establish whether there is any evidence of climate change (as it relates to rooibos production) over the west coast region of South Africa. Data from 26 weather stations covering the time period 1961-2010, were examined using the Mann-Kendall non-parametric trend test, the Sen's nonparametric method and the Stardex extreme indices software. The results indicate that there is little significant trend in annual and seasonal rainfall, but there are some distinctive trends in the characteristics of rainfall. Negative trends in total rainfall during winter were accompanied by increases in the length of dry spells. For the temperature indices, a significant warming trend in summer and autumn maximum temperatures (0.046 ˚C y^{-1}) was associated with an increase in frequency of hot extremes. Climate change scenarios mostly coincided with observed temperature trends while only the observed decrease in winter rainfall and wind speed was consistent with future rainfall projections. These changes would decrease water availability in winter and further intensify or prolong summer drought with important consequences for early regeneration phases of the plant's life cycle. A decrease in wind speed and increase in minimum temperatures during winter could however reduce the risk of wind and frost damage in rooibos seedlings.

2.1 Introduction

The semi-arid west coast region[1] of South Africa forms part of the Cape Floristic Region (hereafter CFR) which is an internationally recognized biodiversity hotspot (Hannah *et al.,* 2002; Cowling *et al.,* 2003). On a biome scale, the major part of the CFR is classified as Fynbos, but also includes parts of the Succulent Karoo biome in the far northern region of the Western Cape province. The leguminous shrub *Aspalathus linearis* (Dahlgren, 1968) is indigenous to the CFR and used to produce an herbal beverage better known as rooibos tea. Wild populations of *A. linearis* have a narrow geographic range within the CFR and are largely confined to nutrient poor and well-drained, sandstone-derived soils (pH 3–5.3) typical of the mountainous areas in the area (Muofhe and Dakora, 2000). Within these edaphic constraints its climatic distribution is dictated particularly by the combination of winter rainfall and hot dry summers with an annual rainfall of between 450 and 1000mm (Dahlgren, 1968). Commercial cultivation occurs over a wider geographical range than the natural distribution and often includes marginal areas where the climatic conditions are less favourable. Both wild and cultivated types of *A. linearis* are harvested for local and international markets and provide employment to many unschooled and semi-schooled workers in the rural Cederberg region. As a medicinally useful shrub, the rooibos plant also fulfils an important role in the cultural heritage of local communities. It therefore contributes to both the social and economic stability of the area.

Climate variability and change have however been identified as important threats to the unique biodiversity of the Fynbos and Succulent Karoo biome (Hannah *et al.,* 2002; Midgley *et al.,* 2003, 2005). Regional climate models predict increasing aridity for the south western part of South Africa (i.e. higher temperatures, lower rainfall, and greater potential evapotranspiration), as well as greater frequency of extreme weather conditions (Hewitson and Crane, 2006; Engelbrecht *et al.,* 2011; Malherbe *et al.,* 2013). An experimental investigation by Agenbach (2006) on the potential effects of climate change on the Fynbos-Succulent Karoo boundary concludes that rising temperatures and prolonged drought periods will adversely affect productivity in Fynbos. DEA (2013) indicate that along the north-eastern distribution limits of the Fynbos biome, the region may become climatically more suitable for conditions which are currently associated with the Succulent Karoo and Albany Thicket biomes. A study by Archer *et al.,* (2008) has revealed specific impacts of current climate variability on rooibos through engaging in participatory action research with small scale farmers. Farmers typically reported that the late onset of winter rain, prolonged dry spells and heat stress cause severely diminished yields and quality of rooibos.

2.2.1 Study area

The rooibos production area is mainly located in the north-western CFR, which is characterised by marked summer drought, with lower annual rainfall and winter temperatures compared to the rest of the CFR. According to DAFF (2011) almost 450 commercial producers operate from Nieuwoudtville in the north to Piketberg in the south, while the fewer than 200 small-scale farmers are concentrated in the remote rural areas of Wupperthal and the Suid-Bokkeveld area south of Nieuwoudtville (Fig. 2.1). The area includes low lying coastal areas and interior plateaux, varying in altitude from the Cederberg mountain peaks in the north east (over 1800 m.a.s.l), to the low-lying Sandveld area in the south west (150 m.a.s.l.). The topographical diversity contributes to the high local scale variability and complexity of rainfall patterns. Winter rainfall is mainly brought about by rain bearing mid-latitude systems, whereas convective activity during the summer months bring about thunderstorm associated rainfall. There is marked spatial variability in the mean annual rainfall, with a range of 180mm – 600mm over the study area. Maximum temperatures can exceed 40°C during summer and fall to below 0°C in some areas during winter (Fig. 2.1).

Figure 2.1 Location of the rooibos production area and weather station distribution in the semi-arid west coast region of South Africa.

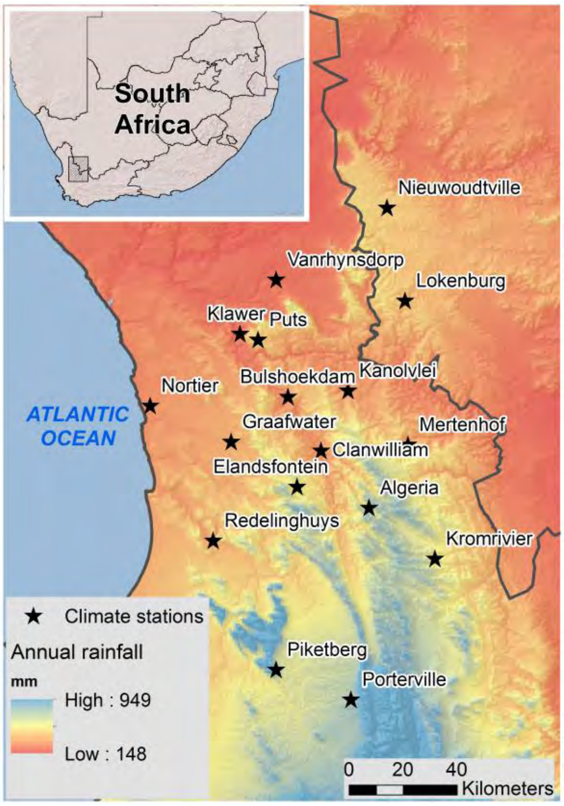
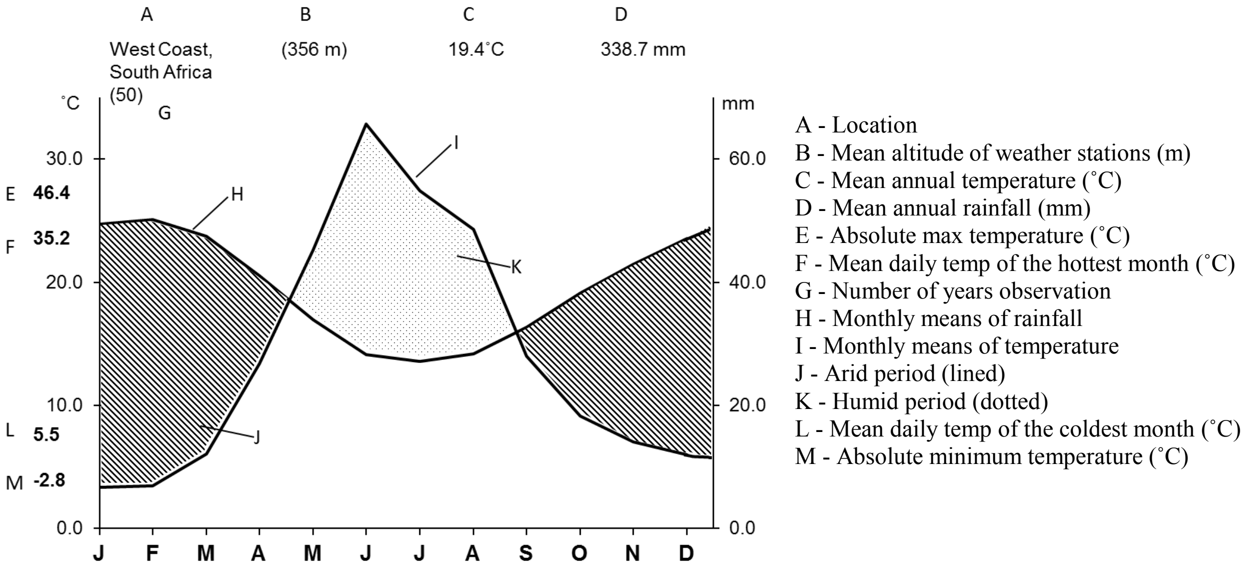


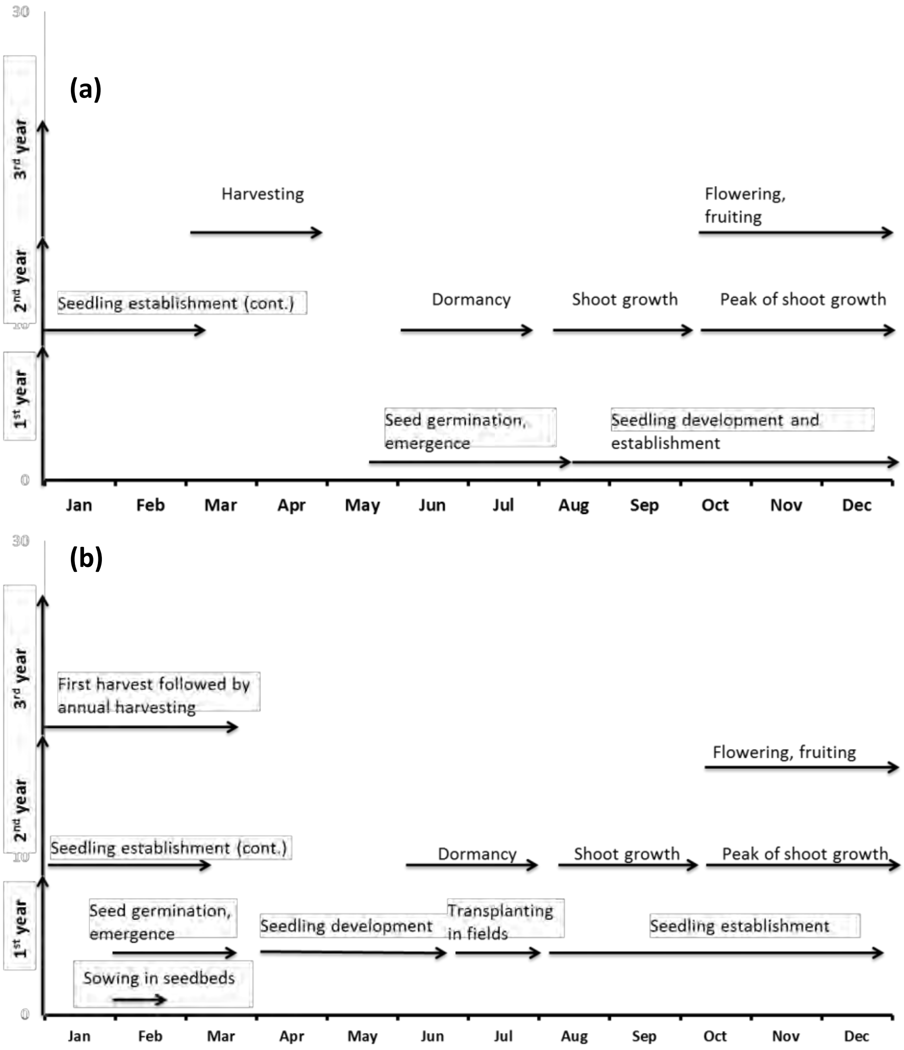
Figure 2.2 Walter climate diagram illustrating the regional averaged long term monthly rainfall and temperatures across all weather stations (1961-2010) in the study area.



2.2.2 Rooibos plant life cycle

A. linearis displays different growth forms and are able to regenerate either through re-growth exclusively from seed (seeders) or from both seeds and re-sprouting from its roots after fire (sprouters) (Louw, 2006). These two regeneration strategies are common amongst plant species growing in a fire-prone environment (Bond & van Wilgen, 1996; Keeley *et al.,* 2012). For both types, seed germination is triggered by fire while sprouting types are also able to survive fire by regrowth from an underground lignotuber (Van Der Bank *et al.,* 1999). Seeder plants however, are killed following a fire and depend completely upon fire-activated seed banks to regenerate. Cultivated plants are seeders whereas certain wild types of *A. linearis* are slower growing sprouters (Hawkins, 2010). In wild plants, fire stimulated seeds germinate in the early winter months after the passage of the first rain bearing cold fronts (Fig. 2.3A). These critical stages of seed germination and seedling emergence are artificially overcome in commercially propagated rooibos when seeds are sown during February/March in well prepared, irrigated seed-beds (Fig. 2.3B). During early stages of development, cultivated rooibos may therefore not be limited to the same extent by rainfall onset and seasonal distribution compared to wild types. During the winter months of June to August, seedlings are transplanted into cultivated fields and do not receive any additional water. Hence, sufficient late winter and spring rainfall is critical to ensure initial establishment of seedlings during winter and will enhance the ability of plants to survive the first summer drought (Richards & Lamont, 1996). The onset of the rainy season and distribution of winter and late winter rainfall is therefore critical in the recruitment of non-sprouting wild and cultivated plants. Apart from rainfall, strong wind is an important stress factor during the regeneration phase, since young plants and germinating seeds are very sensitive to wind damage. The reproductive cycle of flowering and fruiting activity occurs from October to January, while the active growth phenophase for rooibos including leaf elongation and shoot growth commences in spring. Shoot growth continues to increase towards midsummer after which it gradually decline, being followed by a period of dormancy during the winter months (Louw, 2006).

Figure 2.3 The timing of important phenophases in the early life cycle of (a.) wild and (b.) cultivated *A. linearis* plants.



2.2.3 *Climatic trends and variability*

The ability to anticipate alterations in the climate patterns, and trends associated with global warming, is critically important in developing adaptation strategies for resource management

(Lawler, 2009). Previous studies exploring historical climate trends over the Western Cape generally indicate significant warming trends and complex changes in rainfall. Hewitson and Crane (2005) developed a gridded interpolation of rainfall from station data over South Africa for the period 1950 to 1999. A historical rainfall trend analysis based on this dataset revealed distinct seasonal patterns for the Western Cape (Hewitson *et al.,* 2005). During late summer/early winter (March – May) most of the region indicated positive trends whereas mid-winter (June-August) exhibited a drying trend. Increases in rainfall were mostly explained by the increase in intensity of rain events and were most noticeable in mountainous regions. Hoffman *et al.,* (2009) and Warburton and Schulze (2005) did not detect any decreasing trends in annual rainfall for weather stations in the winter rainfall region for the latter part of the 20th century, though this could be explained by the variation in seasonal rainfall totals as shown by Hewitson *et al.,* (2005), where increases and decreases during different seasons cancel each other in the annual totals. Fauchereau *et al.,* (2003) reported greater inter-annual rainfall variability during the last few decades of the 20th century over southern Africa, whereas Kruger (2004, 2006) assessed trends in extreme rainfall and temperature indices over South Africa using Stardex methods. The latter reported increases in total annual rainfall during 1910 -2004 over large parts of the Western Cape, although mostly statistically non-significant. Annual mean temperatures, however, showed statistically significant increases of 0.15°C/decade over the Western Cape for the period 1960-2003, along with an increased occurrence of hot day events. According to Midgley *et al.,* (2005) considerable warming has taken place over the South Western Cape during the period 1967-2001 with significant increases in annual mean maximum and minimum temperatures.

The aim of this study is to deduce the potential impact of climate variability and change on rooibos tea production through three approaches; 1.) analyse variability and trends in historical rainfall and temperature station data, within the rooibos production area, using rooibos-specific indicators proposed by farmers, 2.) assess predictions of future climate change for the region and 3.) assess the implications of observed trends and future climate change for rooibos production.

Table 2.1 Weather stations located in the semi-arid west coast region of South Africa

Station	Longitude	Latitude	Altitude	Variable	Period
Algeria	19.06	-32.37	517	Rainfall	1961-2010
Bulshoekdam	18.79	-32	93	Rainfall	1961-2010
Elandsfontein	18.82	-32.3	457	Rainfall	1961-2010
Graafwater	18.6	-32.15	205	Temp, Wind	1961-2010
Kanolvlei	18.99	-31.98	180	Rainfall	1961-2010
Klawer	18.63	-31.79	42	Temp, Wind	1961-2010
Kromrivier	19.28	-32.54	1045	Rainfall	1961-2010
Lokenburg	19.18	-31.68	640	Rainfall	1961-2010
Mertenhof	19.19	-32.16	381	Rainfall	1961-2010
Nieuwoudtville	19.12	-31.37	731	Rainfall	1961-2010
Nortier	18.33	-32.03	98	Temp, Wind	1961-2010
Piketberg	18.75	-32.91	274	Rainfall	1961-2010
Porterville	19	-33.01	142	Rainfall, Temp, Wind	1961-2010
Puts	18.69	-31.81	592	Rainfall	1961-2010
Redelinghuys	18.54	-32.48	61	Rainfall	1961-2010
Vanrhynsdorp	18.75	-31.61	115	Rainfall	1961-2010

2.3 Data and methods

2.3.1 Historical trend analyses

Through interviews with farmers, local stakeholders and sourcing literature, indices significant for rooibos production were identified to analyse past and future changes in the climate. An inventory of rainfall and temperature stations across the study area was obtained from the South African Weather Service and Institute for Soil, Climate and Water (Agricultural Research Council, South Africa). Climate change signals have become most apparent during the second half of the 20th century (IPCC, 2013) and stations were selected based on the amount of data and completeness during the 1960-2010 period (only stations recording 95% of daily data were selected). Whilst quality control procedures are undertaken by the Agricultural Research Council and the South African Weather Services, missing values and temporal inhomogeneity of data were screened before being used in the subsequent analysis. The locations and attributes of stations meeting these criteria are indicated in figure 2.1 and table 2.1 respectively. From the available list, only 4 stations (Table 2.1) could be selected with more than 30 years of continuous reliable temperature and wind speed data for the period 1961 - 2010. Daily rainfall data were extracted for 13 stations (Table 2.1) over a 50 year period from 1961-2010. For these stations, rainfall, temperature and wind speed data length (30 years or more) is considered long enough to warrant valid statistics of trend analysis in climate change research (Burn and Elnur, 2002).

The Mann-Kendal (MK) and Sen's slope methods (Mann, 1945; Sen 1968) were chosen for this study to detect any trends in the temperature, rainfall and wind speed time series. These methods are well suited for non-parametric climate data analysis and limit the influence of outliers on the slope. The purpose of the MK test (Hipel and McLeod, 2005) is to statistically assess if there is a monotonic upward or downward trend of the variable of interest over time, while a significance level α is also utilised for testing either an upward or downward monotone trend (a two-tailed test). The Sen's slope estimator was used with the MK test to determine the magnitude of trend in the data. Sen (1968) developed the non-parametric procedure for estimating the slope of trend in the sample of N pairs of data:

$$Qi = \frac{x_j - x_k}{j - k} \quad \text{for } i = 1, \ldots, N, \qquad (1)$$

where x_j and x_k are the data values at times j and k $(j>k)$, respectively.

In addition, any trends in frequency and intensity of extreme temperature and rainfall events were analyzed with the *Stardex* diagnostic software tool (http://www.cru.uea.ac.uk/projects/*Stardex*) which calculates a standard set of extreme statistics. The *Stardex* extremes indices routine analyses intensity and frequency related seasonal rainfall and temperature characteristics which are important in rain-fed crop production. It uses a Fortran subroutine to process daily temperature and precipitation time series station data. A least squares linear regression is applied to fit linear trends for each index, while significance of the trend is calculated using a Kendall-tau significance test.

2.3.2 Rainfall analysis

Basic descriptive statistics were calculated to evaluate rainfall characteristics for each station. These statistics include annual minimum rainfall, annual maximum rainfall, annual mean rainfall, standard deviation and coefficient of variation (Table 2.2). Trend analysis by means of the Mann Kendal test (MK) (Mann, 1945; Kendall, 1975) was then applied to determine annual rainfall trends and trends in rainy days per year. Rain days were subsequently correlated with annual rainfall to determine whether annual rainfall is dependent on the number of rain days or possibly on isolated heavy rainfall events. To further disaggregate existing annual rainfall trends, which may include opposing trends in seasonal rainfall characteristics, the Mann Kendal test was applied to detect trends in average seasonal and monthly rainfall. The Sen's slope estimator (Sen, 1968) was used to estimate the slope of all calculated trends.

The set of *STARDEX* rainfall-related indices were computed to examine whether any seasonal trends of rainfall extremes could be identified. These include trends in 90th percentile of rain day amounts, greatest five day total rainfall, the percentage of total rainfall coming from events greater than the long-term 90th percentile and the number of consecutive dry days.

2.3.3 Temperature and wind run analysis

Temperature (T minimum and T maximum) and wind data were analyzed with the Mann Kendal and Sen's slope statistical test on a seasonal and monthly basis to detect any trends in maximum and minimum temperatures. The *STARDEX* indices were also used to assess any trends in seasonal temperature extremes. These include amongst others the 90th percentile of maximum temperatures, the 10th percentile of minimum temperatures, heat wave duration and the percentage of days maximum temperature is greater than the 90th percentile.

2.3.4 Simulations of future climate

An ensemble of dynamically downscaled models which assume the A2 emissions scenario were assessed to investigate future rainfall, temperature and wind speed change over the study area for the period 2041-2070. The A2 storyline describes a heterogeneous world with a development path where emissions of the major greenhouse gases grow rapidly during the 21st century (Nakicenovic, 2000). It was selected because the current trajectory of emissions is already ahead of the higher end emissions scenarios and the A2 scenario is closer to the observed evolution of CO_2 concentrations during the last 10 years (IEA, 2013). Additionally, the uncertainty due to choice of scenario is mostly apparent for climate projections in the second half of the century. For the A2 scenario, six coupled global climate models (CGCMs), which were dynamically downscaled for Southern Africa by means of the conformal-cubic atmospheric model (CCAM) (see Engelbrecht *et al.,* 2011 for more details), were obtained from the Council for Scientific and Industrial Research. The downscaling followed a multiple nudging procedure, using the sea-ice and bias-corrected sea-surface temperatures (SSTs) of the CGCMs (CSIRO Mk 3.5, GFDL2.1, GFDL2.0, HadCM2, ECHAM5 and Miroc-Medres) to first drive 200 km quasi-uniform resolution global simulations of CCAM, followed by subsequent downscaling of the simulations to 50 km resolution over southern Africa (Engelbrecht *et al.,* 2011). The CCAM simulations are provided in a grid format with a horizontal resolution of about 0.5°. From the 6 coupled climate models seasonal rainfall, temperature and wind speed (SRES A2 scenario) were calculated for a control period (1961-2000), as well as for the near future simulation period (2041-2070) across the study area. Seasonal anomalies of rainfall, temperature and wind speed for each grid cell within the study area were then derived between the future simulation period and the control simulation period (1961-2000). Results are represented as a best estimate (50th percentile) for the respective variables.

2.4 Results

2.4.1 Observed trends

Rainfall totals, seasonality and variability

Lowest average annual rainfall (1961-2010) was observed at Vanrhynsdorp (160.8 mm) while Algeria displayed highest average annual rainfall (768.82 mm) (Table 2.2). Standard deviations ranged between 56.3 for Vanrhynsdorp and 208 for Algeria, implying that the variability of annual rainfall increases as mean annual rainfall decreases. The average coefficient of variation (CV) for the stations was 30%, though Mertenhof had the highest CV (36%) and Piketberg the lowest (24%) which indicates a relatively reliable rainfall regime. The Mann Kendal trend analysis of annual rainfall indicated that all stations except Algeria experienced an increase in annual rainfall (Table 2.2). However, statistically significant trends ($p<0.05$) were observed for only two stations, namely Lokenburg and Redelinghuys (both increasing trends). Rainfall changes over 50 years ranged between a maximum increase of 73 mm at Redelinghuys and drying of 50 mm at Algeria. The trends in rainfall totals were at least partly explained by corresponding positive/negative trends in rain days.

The application of the MK test to seasonal rainfall revealed a negative, yet insignificant trend for most stations during June, July and August (peak of the rainfall season) and a consistently strong positive trend (all stations agree) for the months of March, April and May (Table 2.3 and Fig. 2.5A). An apparent spatial trend that emerges from the seasonal analysis is the significant positive trends during December, January and February for the two stations at the highest elevations in the north of the study area.

To further elucidate these results, monthly rainfall trends were calculated. A distinct and persistent positive trend was observed during May and July for all stations with 5 of these being statistically significant during May and only one during July (Table 2.4 and Fig. 2.4). The opposite trends were found in the months of June and August (though none of these negative trends are statistically significant). The increases in seasonal totals for March, April and May were mainly attributed to the strong increase in rainfall during May where the average increase over 50 years across all stations was 22 mm. Decreases during June, July and August were the effect of persistent, though not significant, drying during June and August, the effect of which is reduced by the positive trend during July. Rainfall decreased on average for all stations by 14 mm during June and 10.8 mm during August while it increased by 13.6 mm in July.

Table 2.2 Annual mean rainfall (R_e), annual minimum rainfall (R_m), annual maximum rainfall (R_x), standard deviation of annual mean rainfall (SD), coefficient of skewness (C_s), coefficient of kurtosis (C_k), coefficient of variation (C_v), trend in annual rainfall (rainfall trend), mean number of rainy days per year (MRD) and trend in rainy days (RD trend) for the period 1961-2010. Re, Rm, Rx, and SD are in mm. Rainfall trend and RD trend represent Sen's estimator of the slope of the trend line. Values marked in bold indicate trends statistically significant at the 95% confidence interval according to the Mann-Kendall test.

	R_e mm	**R_M mm**	**R_X mm**	**SD mm**	**C_K**	**C_S**	**C_V**	**Rainfall trend mm a^{-2}**	**MRD**	**RD trend days/year**
Nieuwoudtville	356.7	186.8	589	104	-0.39	0.6	0.29	0.60	43.60	**0.20**
Vanrhynsdorp	160.8	63	285.4	56.3	-0.88	0.04	0.35	0.39	32.00	0.00
Lokenburg	263.2	136	492.3	78.6	0.2	0.67	0.3	**1.35**	37.10	0.00
Puts	365.5	203.5	693.5	108.7	0.34	0.62	0.3	0.04	40.60	**-0.35**
Kanolvlei	186.1	84	390.5	58.4	1.68	0.74	0.31	0.25	33.30	0.03
Bulshoekdam	240.5	92.4	496.9	85.3	1.14	1.04	0.35	0.83	38.80	-0.11
Mertenhof	245.5	112.4	519.6	87.6	0.72	0.98	0.36	0.30	33.20	-0.15
Elandsfontein	512.5	313.5	843.1	128.4	0.51	0.88	0.25	0.77	57.30	-0.08
Algeria	768.8	440.4	1412.4	208.4	1.04	0.86	0.27	-1.30	56.40	-0.11
Redelinghuys	284.1	126.3	508	83.6	0.2	0.5	0.29	**1.50**	46.10	-0.14
Kromrivier	357.4	163.7	685.3	118.9	0.07	0.51	0.33	0.63	46.70	-0.25
Piketberg	441.6	221.8	721.8	103.9	-0.05	0.2	0.24	0.66	50.60	0.18
Porterville	505.94	340.1	733.6	99.05	-0.05	0.6	0.2	1.21	43.60	**-0.23**

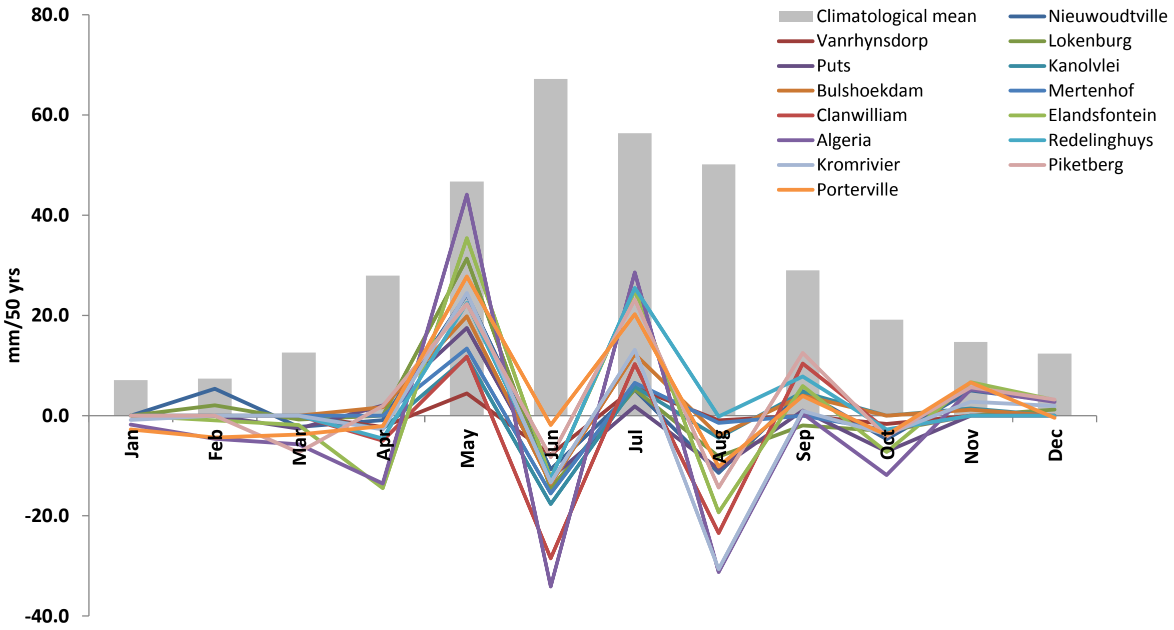
Table 2.3 Trends of seasonal rainfall time series for stations in the semi-arid west coast region of South Africa (1961-2010). Values for each season represent change in total seasonal rainfall per year (mm a^{-2}). Values marked in bold indicate trends statistically significant at the 95% confidence interval according to the Mann-Kendall test.

Station	**DJF**	**MAM**	**JJA**	**SON**
	mm a^{-2}			
Nieuwoudtville	**0.21**	0.50	-0.32	0.08
Vanrhynsdorp	0.06	0.11	-0.06	-0.14
Lokenburg	**0.18**	**0.65**	-0.20	-0.09
Puts	-0.09	0.16	-0.41	0.02
Kanolvlei	0.03	0.18	-0.17	-0.01
Bulshoekdam	-0.01	**0.59**	-0.09	0.27
Mertenhof	0.00	0.25	-0.37	-0.14
Elandsfontein	-0.11	0.37	0.00	0.27
Algeria	-0.44	0.15	-1.09	-0.26
Redelinghuys	0.11	0.37	-0.13	0.29
Kromrivier	0.02	0.58	-0.29	-0.04
Piketberg	-0.03	0.23	0.07	0.29
Porterville	-0.18	0.33	0.35	0.22

Table 2.4 Trends in monthly rainfall time series for stations in the semi-arid west coast region of South Africa (1961-2010). Values for each month represent change in total monthly rainfall per year (mm a^{-2}). Values marked in bold indicate trends statistically significant at the 95% confidence interval according to the Mann-Kendall test.

Station	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
	mm a^{-2}											
Nieuwoudtville	0.00	**0.11**	-0.05	-0.02	0.48	-0.21	0.10	-0.23	0.10	-0.09	0.10	0.06
Vanrhynsdorp	0.00	0.00	0.00	-0.05	0.09	-0.16	0.11	-0.02	0.00	-0.03	0.00	0.00
Lokenburg	0.00	0.04	-0.02	0.00	**0.63**	-0.29	0.11	-0.16	-0.04	-0.06	0.00	0.02
Puts	0.00	0.00	-0.05	0.04	0.35	-0.25	0.04	-0.21	0.02	-0.14	0.00	0.00
Kanolvlei	0.00	0.00	0.00	-0.05	0.23	-0.35	0.12	-0.09	0.09	0.00	0.03	0.00
Bulshoekdam	0.00	0.00	0.00	0.03	0.40	-0.27	0.25	-0.08	0.08	0.00	0.02	0.00
Mertenhof	0.00	0.00	0.00	0.00	0.27	-0.31	0.13	-0.03	0.00	**-0.06**	0.00	0.00
Elandsfontein	0.00	-0.02	-0.04	-0.29	**0.71**	-0.25	0.50	-0.39	0.12	-0.15	0.13	0.06
Algeria	-0.04	-0.09	-0.11	-0.27	0.88	-0.68	0.57	-0.63	0.01	-0.24	0.10	0.06
Redelinghuys	0.00	0.00	0.00	-0.09	**0.45**	-0.25	**0.51**	0.00	0.16	-0.06	0.00	0.00
Kromrivier	-0.02	0.00	0.00	-0.06	**0.49**	-0.27	0.26	-0.61	0.02	-0.08	0.06	0.04
Piketberg	0.00	0.00	-0.15	0.04	0.45	-0.17	0.46	-0.29	0.25	-0.08	0.11	0.06
Porterville	-0.06	-0.09	-0.08	-0.04	0.56	-0.04	0.41	-0.20	0.08	-0.08	0.13	-0.01

Figure 2.4 Trends of monthly rainfall time series for stations in the semi-arid west coast region of South Africa (1961-2010). Values for each month represent change in total monthly rainfall over 50 years (mm/50). The climatological mean monthly rainfall across the study area is indicated by the shaded bars.



Rainfall extremes

A few core indices were selected from the 31 generated by the *Stardex* software based on their ability to highlight changes in the frequency and intensity of rainfall. The 90th percentile of rain day amounts (pq90), greatest 5-day total rainfall (px5d) and simple daily intensity (rain per rain day, PINT) provide a good measure of trends in intensity (Table 2.5) whereas consecutive dry days (CDD), mean dry spell lengths (PDSAV) and the percentage of total rainfall coming from events greater than the long-term 90th percentile (pfl90) are important frequency and distribution measures (Table 2.6).

Table 2.5 Seasonal indices of rainfall intensity for stations in the semi-arid west coast region of South Africa (1961-2010). Values for each season represent trends per year. Values marked in bold indicate trends statistically significant at the 95% confidence interval using a Kendall-tau significance test.

Station	90th percentile of rain day amounts (mm)					greatest 5-day total rainfall (mm)					simple daily intensity (mm)				
	DJF	MAM	JJA	SON	ANN	DJF	MAM	JJA	SON	ANN	DJF	MAM	JJA	SON	ANN
Nieuwoudtville	0.00	-0.01	0.05	0.02	0.01	**0.20**	0.29	-0.04	-0.04	0.02	**0.06**	0.04	-0.01	-0.04	0.00
Vanrhynsdorp	0.00	0.15	0.00	0.14	**0.04**	**0.20**	-0.02	0.07	-0.03	0.14	0.12	0.02	0.00	-0.02	0.01
Lokenburg	0.00	0.14	0.01	0.16	**0.12**	**0.26**	**0.27**	0.06	-0.04	0.14	**0.12**	**0.12**	0.06	-0.01	**0.06**
Puts	0.00	0.13	0.06	0.16	**0.11**	-0.02	0.10	-0.11	0.14	0.10	**0.08**	**0.09**	0.05	**0.09**	**0.08**
Kanolvlei	0.00	0.09	-0.04	2.70	-0.01	0.10	0.08	-0.02	-0.06	0.02	0.01	0.02	0.01	-0.03	0.01
Bulshoekdam	0.00	0.20	-0.01	0.15	0.07	0.03	0.29	0.12	0.04	0.19	0.04	0.06	0.02	0.01	0.02
Mertenhof	0.00	**0.40**	-0.04	0.75	**0.09**	0.01	0.19	-0.20	0.01	-0.17	0.03	**0.10**	0.01	-0.02	**0.04**
Elandsfontein	0.46	0.11	0.03	0.00	0.06	-0.12	0.12	-0.03	0.13	0.01	0.01	0.03	0.01	-0.01	0.01
Algeria	1.83	0.03	-0.02	-0.04	-0.05	-0.38	0.09	-0.21	0.03	-0.04	-0.01	0.03	-0.01	0.00	0.00
Redelinghuys	0.12	0.24	**0.17**	0.03	**0.19**	0.30	0.18	0.27	0.15	0.31	0.18	**0.06**	**0.08**	0.06	**0.08**
Kromrivier	0.00	0.17	0.06	-0.10	0.04	0.03	0.24	-0.40	-0.09	-0.51	0.06	0.07	-0.02	0.01	0.01
Piketberg	0.00	0.15	-0.01	0.02	0.05	0.02	0.08	0.12	0.06	0.26	-0.04	0.02	-0.02	-0.01	-0.01
Porterville	0.95	**0.34**	**0.21**	0.10	**0.26**	0.00	0.11	0.16	0.18	0.15	0.14	**0.13**	**0.11**	**0.09**	**0.10**

Table 2.6 Seasonal indices of rainfall frequency and distribution for stations in the semi-arid west coast region of South Africa (1961-2010). Values for each season represent trends per year. Values marked in bold indicate trends statistically significant at the 95% confidence interval using a Kendall-tau significance test.

Station	% rainfall from events > 90th perc (%)					consecutive dry days (d)					mean dry spell length (d)				
	DJF	MAM	JJA	SON	ANN	DJF	MAM	JJA	SON	ANN	DJF	MAM	JJA	SON	ANN
Nieuwoudtville	0.004	0.002	0.000	-0.002	0.000	-0.188	0.036	0.074	-0.078	-0.192	-0.188	-0.020	0.012	-0.018	-0.010
Vanrhynsdorp	**0.008**	0.002	0.000	0.000	0.002	-0.228	0.022	0.080	0.194	-0.128	-0.372	0.002	0.032	0.054	0.064
Lokenburg	**0.005**	**0.006**	0.000	-0.002	**0.004**	-0.166	0.090	0.064	-0.024	-0.108	-0.160	-0.034	0.016	0.086	0.022
Puts	0.000	0.004	0.000	0.004	0.002	0.310	0.204	**0.172**	0.136	**0.444**	0.320	0.050	0.032	0.072	**0.074**
Kanolvlei	0.002	0.004	0.002	0.000	0.002	-0.326	-0.196	-0.004	-0.160	**-0.423**	-0.284	-0.156	-0.016	-0.070	-0.058
Bulshoekdam	0.002	**0.006**	0.000	0.002	0.002	0.256	-0.230	0.136	-0.042	-0.066	0.258	-0.068	0.026	0.036	0.010
Mertenhof	0.000	0.002	0.000	-0.002	0.002	-0.114	0.020	0.187	0.226	0.032	-0.102	-0.108	0.020	0.138	**0.045**
Elandsfontein	-0.002	**0.005**	0.000	-0.002	0.000	-0.178	-0.010	**0.158**	-0.004	0.016	0.014	-0.016	0.012	-0.056	0.006
Algeria	-0.004	0.002	-0.002	-0.004	0.000	0.278	0.004	0.114	-0.018	0.060	**0.326**	-0.004	0.012	-0.018	0.010
Redelinghuys	0.012	**0.005**	0.004	**0.007**	**0.004**	0.106	0.088	**0.218**	**0.308**	0.190	0.166	0.036	0.030	0.130	0.072
Kromrivier	0.000	0.004	-0.002	-0.002	-0.002	0.066	0.036	0.112	0.124	0.042	0.164	0.006	0.014	-0.040	0.024
Piketberg	-0.002	0.004	-0.002	0.002	0.000	-0.136	-0.046	0.102	-0.232	-0.092	-0.106	0.004	0.006	-0.090	-0.002
Porterville	0.006	**0.007**	**0.006**	**0.006**	**0.006**	**0.300**	0.138	**0.070**	-0.108	-0.002	**0.282**	0.038	**0.022**	-0.012	0.038

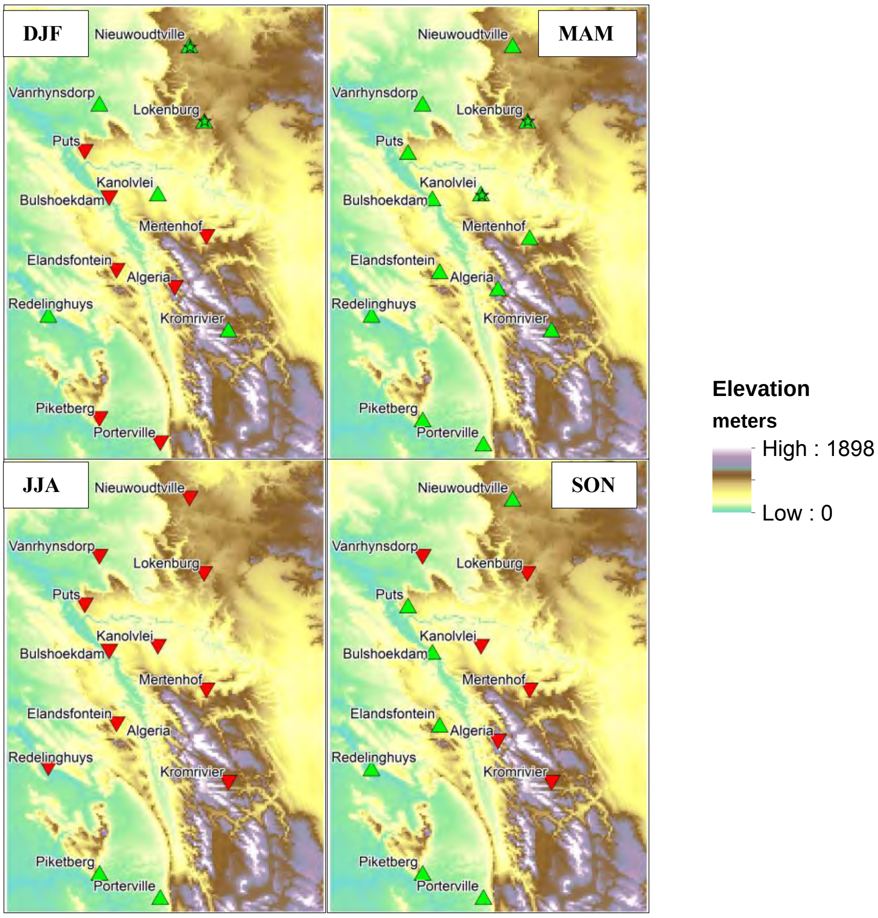
Indices related to the intensity of rain events displayed a clear increasing temporal trend across the study area during March, April and May (Table 2.5). During this late summer - early winter period, two stations had significant positive trends in the 90th percentile of rain day amounts (pq90), while a further five stations exhibited significant positive trends in simple daily intensity (PINT). An increasing proportion of total rainfall received during this March, April and May period is also coming from events greater than the long-term 90th percentile as indicated by the five significant positive trends (Table 2.6). The increase in intensity of rain events during March, April and May is congruent with the seasonal rainfall totals, which indicated a general increasing trend in total rainfall for all stations during these months.

The same spatial trend of wetter conditions during December, January and February, for stations in the northern part of the study area is evident in the rainfall intensity indices. During December, January and February, Lokenburg and Nieuwoudtville, which are situated on the Bokkeveld plateau at the north-eastern edge of the winter rainfall area, had significant positive trends in simple daily intensity and greatest 5-day total rainfall (Fig. 2.5B).

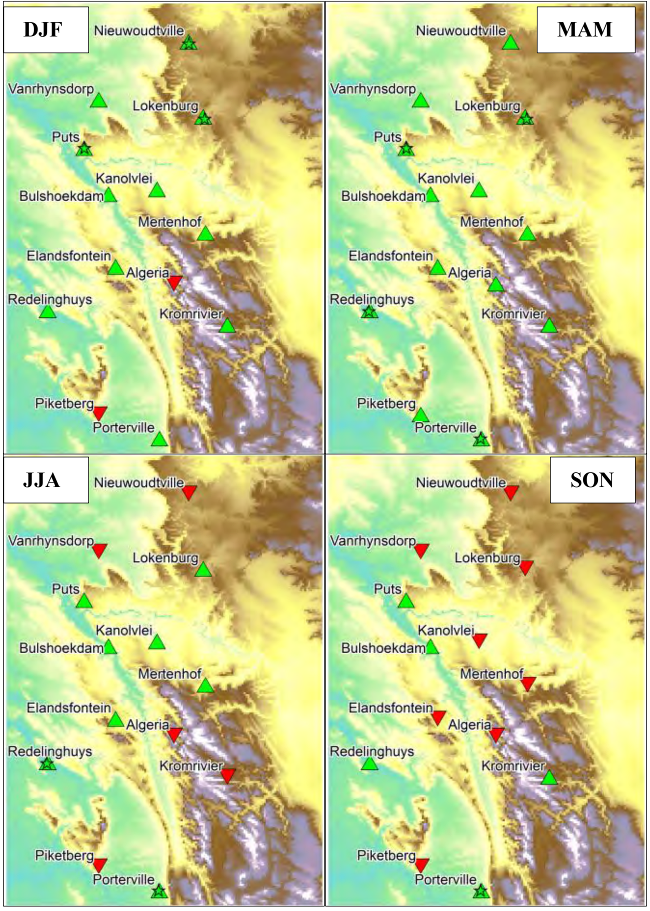
The most consistent trends in indices related to rainfall frequency were seen during the winter season (Table 2.6). All stations except Kanolvlei showed a positive trend in consecutive dry days and mean dry spell lengths. However, statistically significant trends were only registered for the consecutive dry days index over 4 stations. Of these 4 stations, Redelinghuys had the maximum positive trend of 11 consecutive dry days for June, July and August over the 50 year period. The positive trend in dry days during June, July and August supports the findings of a negative trend observed in the June, July and August seasonal totals for most stations. Redelinghuys, Elandsfontein and Puts exhibited minor negative trends in winter rainfall along with a significant increase in dry days (Fig 2.5C).

Figure 2.5 Map indicating the spatial pattern in trends of key variables (a) total seasonal rainfall, (b) simple daily intensity and (c) consecutive dry days for each climate station. The green (red) triangles indicate increasing (decreasing) trends, respectively. Trends significant at the 95% confidence interval are indicated with a star inside the symbol. Seasons are summer (December, January, February), autumn (March, April, May), winter (June, July, August) and spring (September, October and November).

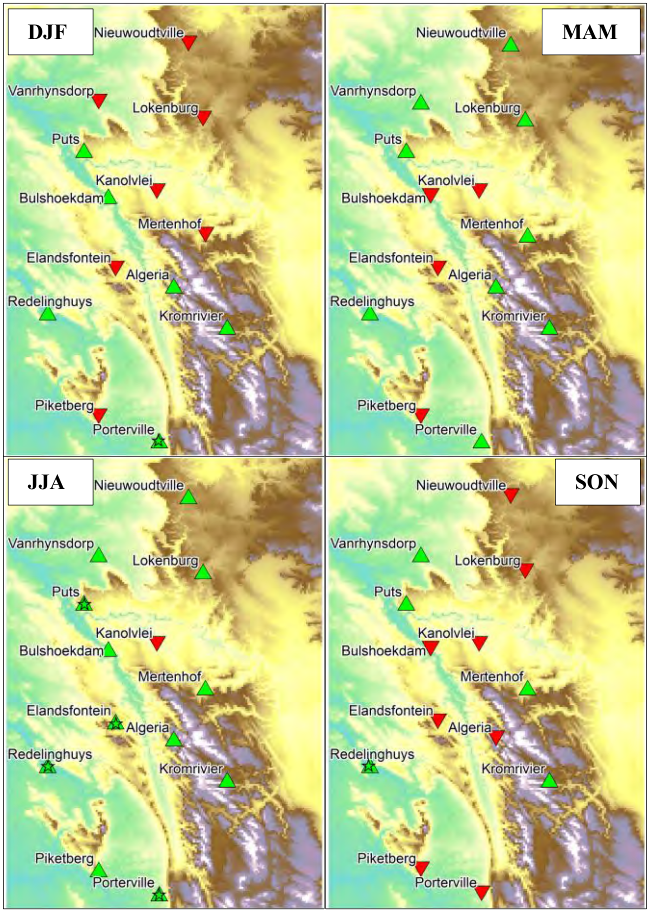
(a)



(b)



(c)



Elevation

meters

High : 1898

Low : 0

Temperature trends

Annually, the four weather stations showed a significant increase in both maximum (2.0˚C) and minimum temperatures (1.4˚C) between 1961 - 2010. Maximum positive trends were registered during summer and autumn resulting in an increase of 2.1˚C and 2.5˚C (1961-2010) respectively when averaged across stations (Table 2.7). Significant monthly maximum temperature changes were observed for all stations in January and March, with the steepest trends occurring in March. Minimum temperatures displayed positive trends across all seasons throughout the year, with the most significant increase during SON (Table 2.7). On average, the minimum temperature change was slower than maximum temperature change for the period 1961-2010. The most significant monthly minimum temperature change across stations were found in May (2.2°C) which also coincides with the significant increase in rainfall observed during the same month.

Table 2.7. Trends in monthly, seasonal and annual minimum and maximum temperatures for stations in the semi-arid west coast region of South Africa (1961-2010). Values represent the slope of the trend line for minimum and maximum temperatures (°C a^{-1}). Values marked in bold indicate trends statistically significant at the 95% confidence interval according to the Mann-Kendall test.

Station		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	DJF	MAM	JJA	SON	Annual
		°C a^{-1}												°C a^{-1}				°C a^{-1}
Graafwater	tmin	**0.038**	0.028	0.036	-0.004	**0.044**	-0.011	0.037	0.021	0.023	**0.054**	**0.047**	0.025	0.027	0.024	0.021	**0.045**	**0.031**
	tmax	**0.050**	0.040	**0.051**	0.027	0.028	0.008	0.053	0.017	0.044	**0.050**	0.016	0.030	**0.037**	**0.038**	0.023	**0.036**	**0.035**
Nortier	tmin	**0.056**	**0.066**	**0.055**	**0.052**	**0.058**	0.043	**0.039**	0.024	**0.027**	0.030	0.042	**0.043**	**0.053**	**0.057**	**0.036**	**0.030**	**0.042**
	tmax	**0.032**	**0.061**	**0.076**	**0.065**	-0.004	0.018	0.041	-0.003	0.013	0.029	**0.054**	0.034	**0.043**	**0.047**	0.023	**0.029**	**0.036**
Klawer	tmin	0.013	0.026	0.013	-0.015	0.021	-0.007	**0.026**	0.002	0.015	**0.047**	**0.032**	0.006	0.012	0.007	0.013	**0.040**	**0.014**
	tmax	**0.052**	0.053	**0.061**	0.052	0.037	0.020	**0.059**	0.036	0.050	**0.067**	**0.047**	0.038	**0.040**	**0.049**	**0.036**	**0.059**	**0.043**
Porterville	tmin	0.028	0.028	0.032	0.005	**0.054**	0.037	**0.039**	**0.029**	0.023	**0.038**	0.006	0.020	**0.022**	**0.030**	**0.031**	**0.027**	**0.027**
	tmax	**0.067**	**0.047**	**0.064**	**0.059**	**0.059**	0.019	0.047	0.020	0.030	**0.060**	0.008	**0.052**	**0.050**	**0.061**	0.031	**0.036**	**0.044**

Temperature extremes

A few core indices for extreme temperature events have been selected from 24 seasonal *Stardex* indices for further analysis (Table 2.8): the 10th and 90th percentile of minimum/maximum temperatures, heat wave duration, 90th percentile of heat wave duration, % days Tmax > 90th percentile and % days Tmin < 10th percentile. These indices have been selected since an intensification of extreme temperatures in the Mediterranean summer may significantly enhance leaf heat stress and limit growth and survival of plants due to severe restrictions on photosynthesis (Llorens *et al.,* 2004). Moreover, higher temperatures may exacerbate existing water stress for rain-fed crops during summer drought through indirect effects on evapotranspiration and soil dryness (Larcher, 2000). Annual values for the 90th percentile of maximum temperatures have significantly increased by more than 2° C over 50 years for all stations. Seasonally, the most significant increase in the 90th percentile of maximum temperatures was observed during summer and autumn. Heat wave days during MAM have increased significantly between 3 and 5 days for all but one station over the 50 year period. The finding that the most significant increases occurred during MAM was consistent for all except for percentage of days Tmin < 10th percentile. For all stations, a significant negative trend was found in the annual percentage of days Tmin < 10th percentile. The magnitude of extreme minimum temperature increase was generally less than that for maximum temperatures.

Table 2.8 Indices relating to seasonal and annual extreme temperature events for stations in the semi-arid west coast region of South Africa (1961-2010). Values represent the slope of the trend line (change per year). Values marked in bold indicate trends statistically significant at the 95% confidence interval using a Kedall-tau significance test.

Station		DJF	MAM	JJA	SON	ANN
Graafwater	Tmax 90th percentile (˚C)	**0.0452**	**0.0369**	0.0364	0.04	**0.0427**
	Tmin 10th percentile (˚C)	**0.037**	0.0223	0.0214	**0.0326**	**0.0297**
	Heat Wave Duration (d)	-0.0228	0.0185	-0.0235	-0.0435	-0.0243
	90th Percentile Heat Wave Duration (d)	0.0058	0.0254	0.0333	0.0033	0.005
	% days Tmax > 90th percentile (d)	**0.0011**	**0.0019**	0.0013	**0.0017**	**0.0016**
	% days Tmin < 10th percentile (d)	**-0.0023**	-0.0013	-0.001	**-0.0028**	**-0.0019**
Nortier	Tmax 90th percentile (˚C)	**0.077**	0.049	0.018	0.017	**0.049**
	Tmin 10th percentile (˚C)	**0.046**	**0.066**	**0.034**	**0.038**	**0.044**
	Heat Wave Duration (d)	0.000	0.055	-0.003	0.034	0.092
	90th Percentile Heat Wave Duration (d)	**0.040**	0.019	0.017	-0.009	0.028
	% days Tmax > 90th percentile (d)	0.001	**0.001**	0.001	0.001	**0.001**
	% days Tmin < 10th percentile (d)	**-0.004**	**-0.004**	**-0.003**	-0.002	**-0.003**
Klawer	Tmax 90th percentile (˚C)	**0.040**	**0.064**	0.055	**0.060**	**0.066**
	Tmin 10th percentile (˚C)	0.020	0.014	**0.015**	**0.029**	**0.023**
	Heat Wave Duration (d)	0.012	0.146	0.040	0.005	**0.280**
	90th Percentile Heat Wave Duration (d)	-0.003	**0.054**	0.034	0.008	0.024
	% days Tmax > 90th percentile (d)	0.001	**0.003**	0.002	**0.002**	**0.002**
	% days Tmin < 10th percentile (d)	-0.002	-0.001	-0.001	**-0.003**	**-0.002**
Porterville	Tmax 90th percentile (˚C)	**0.045**	**0.066**	0.024	**0.033**	**0.046**
	Tmin 10th percentile (˚C)	**0.029**	**0.035**	**0.030**	**0.035**	**0.041**
	Heat Wave Duration (d)	0.045	0.060	0.013	-0.023	**0.139**
	90th Percentile Heat Wave Duration (d)	0.028	**0.045**	0.037	0.003	0.034
	% days Tmax > 90th percentile (d)	**0.002**	**0.003**	0.001	0.001	**0.002**
	% days Tmin < 10th percentile (d)	**-0.001**	-0.001	**-0.001**	**-0.002**	**-0.001**

Wind run

Wind run (U_{total}) decreased significantly at all stations between 1961–2010 (Table 2.9). The average annual rate of decline for the 4 weahter stations over the study period was 18.77 km a^{-2} which translates into a 35.6% decline from 2145 to 1380 km a^{-1}. During JJA, all stations showed a significant decline of between 2.8 and 9 km a^{-2}.

Table 2.9 Average annual values (U_{total}) and trends in monthly, seasonal and annual wind run for stations in the semi-arid west coast region of South Africa (1961-2010). Values represent the slope of the trend line (km a^{-2}). Values marked in bold indicate trends statistically significant at the 95% confidence interval according to the Mann-Kendall test.

Station	U_{Total}	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	DJF	MAM	JJA	SON	Annual
	km a^{-1}						km a^{-2}								km a^{-2}			km a^{-2}
Graafwater	1110.9	-0.61	-0.32	-0.27	-0.41	**-1.27**	**-1.32**	-0.73	-0.37	-0.21	-0.15	-0.19	0.14	-0.09	-2.07	**-2.76**	-0.18	-5.73
Klawer	1882.1	**-2.12**	**-1.05**	**-1.64**	0.07	**-1.82**	-0.98	-0.62	**-2.35**	**-1.47**	**-1.57**	-1.15	**-1.29**	**-4.01**	-3.82	**-3.52**	**-4.38**	**-19.42**
Nortier	2467.5	**-3.04**	**-3.01**	**-2.29**	**-2.15**	**-3.74**	**-2.82**	**-3.47**	**-2.85**	**-2.97**	**-3.06**	**-2.49**	**-3.10**	**-8.81**	**-7.58**	**-8.95**	**-9.20**	**-32.69**
Porterville	1178.4	**-1.46**	**-1.33**	**-1.26**	**-0.90**	**-1.77**	**-1.49**	**-1.39**	**-1.33**	**-1.80**	**-1.34**	**-1.42**	**-1.59**	**-4.06**	**-4.15**	**-5.20**	**-4.56**	**-17.25**

2.4.2 Future projected climate scenarios

Rainfall

The results of the seasonal rainfall and rain day anomalies are given as a median value for the 6 regional climate models. Although the median value is presented here, there is an envelope (range) of future projections from the 6 models as indicated by the JJA rainfall anomalies (Fig. 2.6). During JJA the entire west coast of South Africa is projected to be drier, although slight increases are plausible over parts of the area as indicated by 2 out of 6 models. The magnitude of change is greater away from the coast and towards the south. Throughout the seasons spring to autumn the west coast is projected to become generally drier although slight increases in rainfall during DJF are also plausible (as suggested by 2 ensemble members) over the north eastern interior (Fig. 2.7).

Figure 2.6 Total rainfall anomalies (2041–2070 simulation minus 1961–1990 control; in mm month^{-1}) for June, July and August for six coupled global climate models.

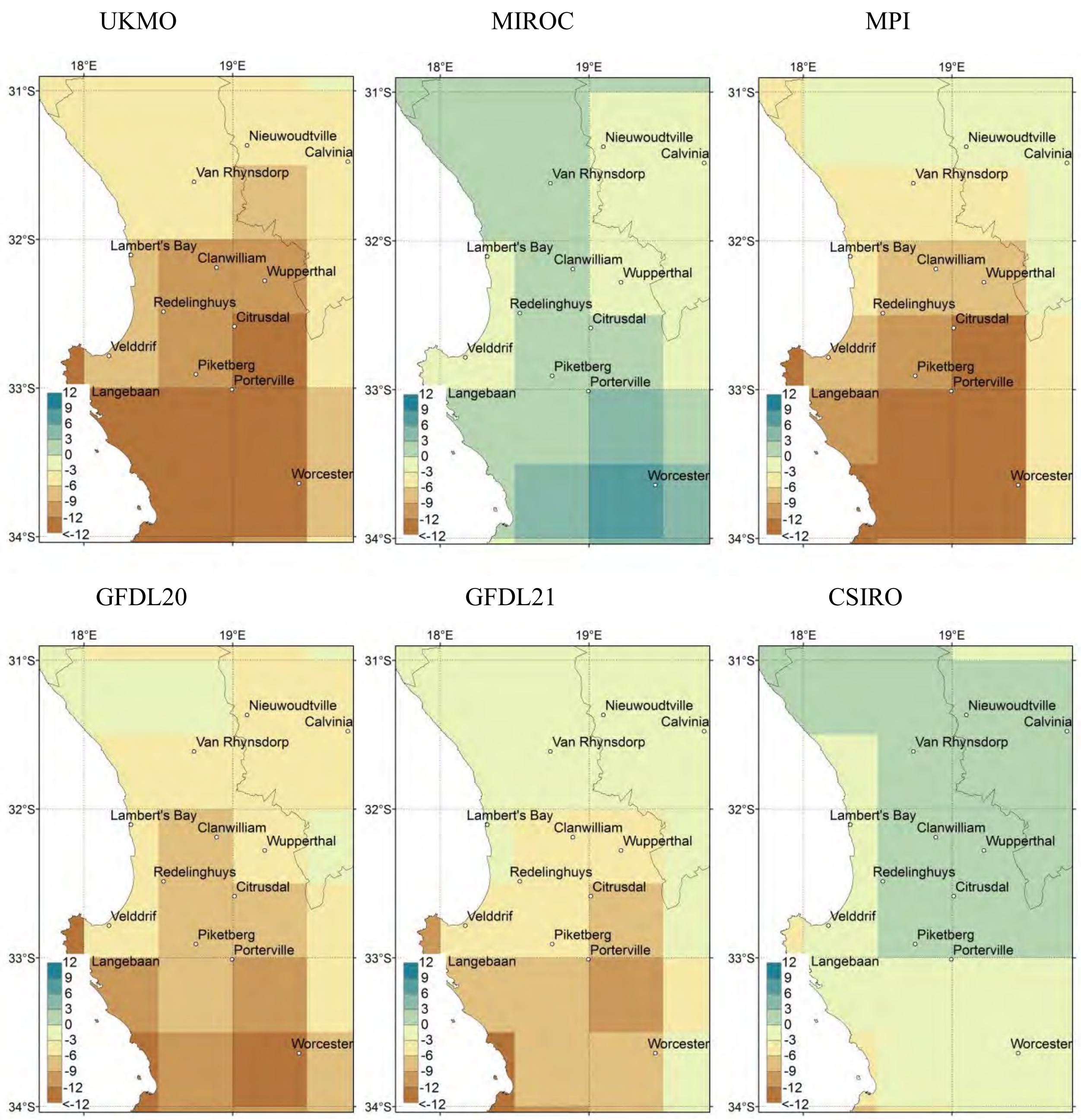
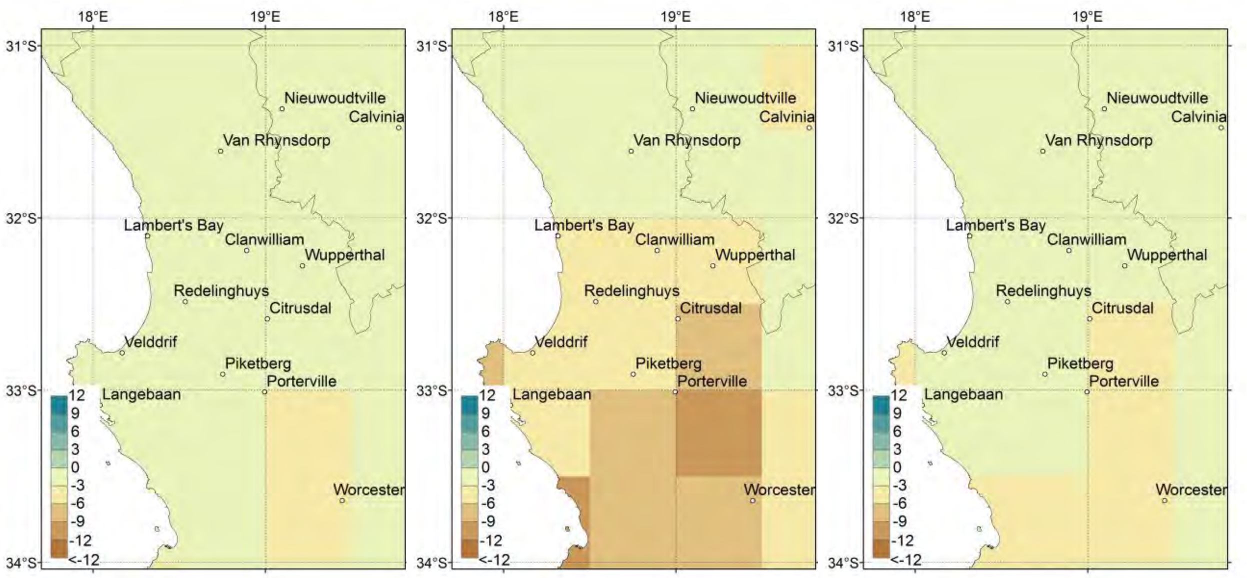


Figure 2.7 Best estimate (50th percentile of six coupled global climate models) of rainfall change for Dec, Jan and Feb (DJF), March, April and May (MAM), June, July and August (JJA), and September, October and November (SON) for the period 2041 – 2070 vs 1961 – 1990 in mm month^{-1}.



DJF MAM SON

Temperature

The results of the temperature anomalies (2041–2070 simulation minus 1961–1990 control) indicate a noticeable increase in both maximum and minimum temperatures throughout the year over the entire study area (Fig. 2.8 and Fig. 2.9). The lowest projected changes are along the coastal regions (Lambert’s Bay) while further away from the coast maximum and minimum temperature is projected to increase in excess of 2°C. For these inland areas, the warming tendency of maximum temperatures is projected to be strongest during March-May when maximum temperatures are predicted to increase up to 2.5 °C for the period 2041-2070 (Fig. 2.8). Minimum temperature trends do not vary much between seasons, and generally range between a minimum increase of 1°C at the coast and a maximum increase of 2.3°C further inland (Fig. 2.9). Both minimum and maximum temperature increases are greatest towards the north of the study region.

Figure 2.8 Best estimate (50^{th} percentile of six coupled global climate models) of maximum temperature change for December, January and February (DJF), March, April and May (MAM), June, July and August (JJA), and September, October, November (SON) for the period 2041–2070 vs 1961–1990 in ˚C.

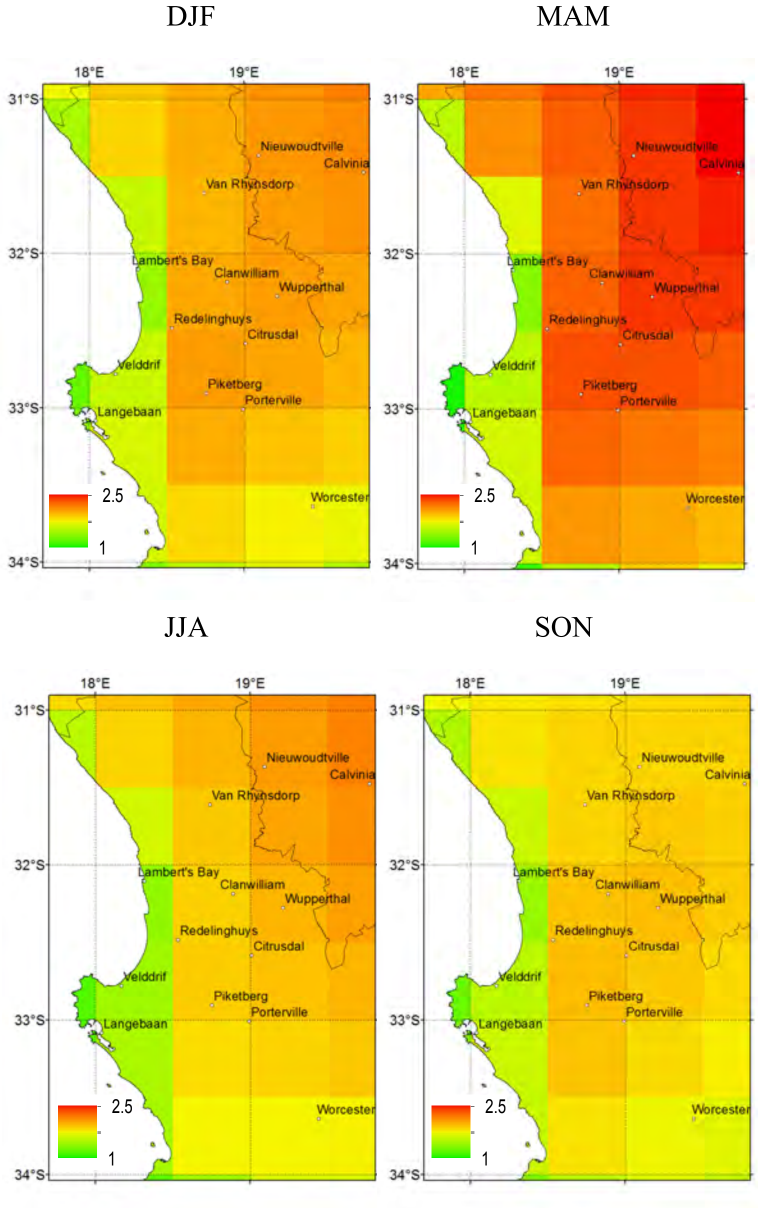
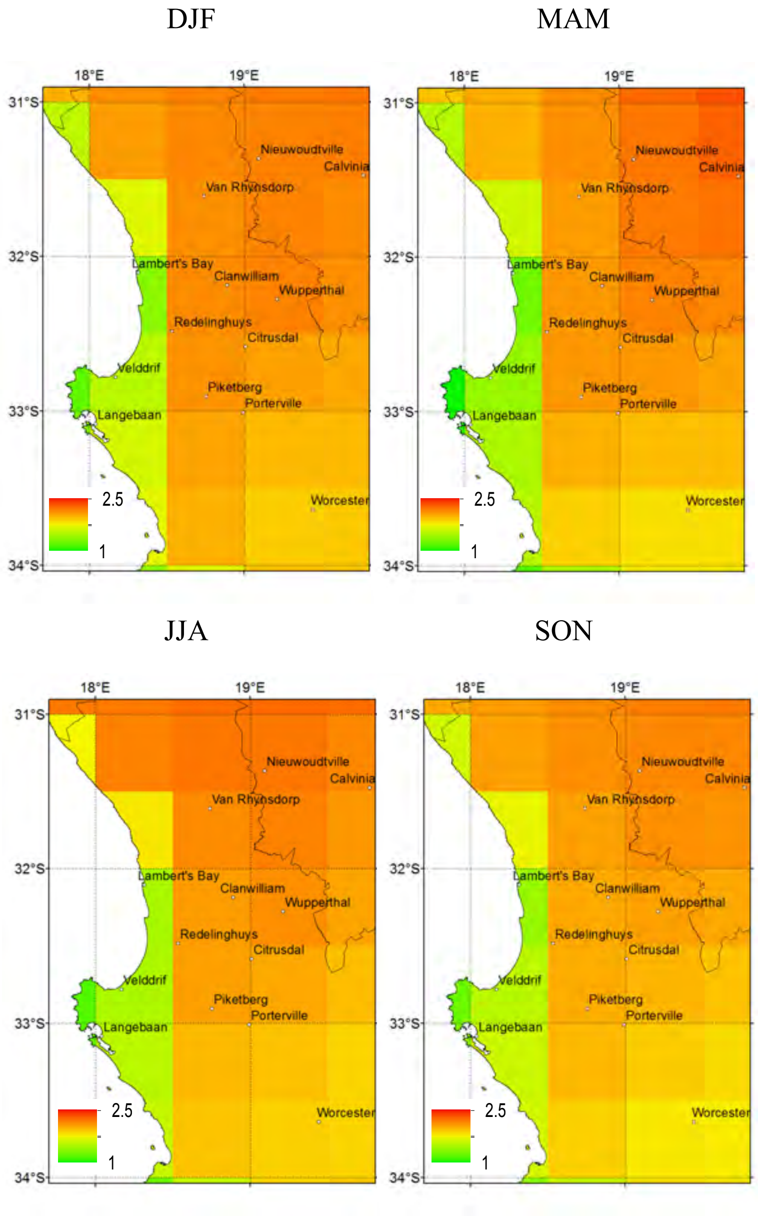


Figure 2.9 Best estimate (50^{th} percentile of six coupled global climate models) of minimum temperature change for December, January and February (DJF), March, April and May (MAM), June, July and August (JJA), and September, October and November (SON) for the period 2041–2070 vs 1961–1990 in ˚C.

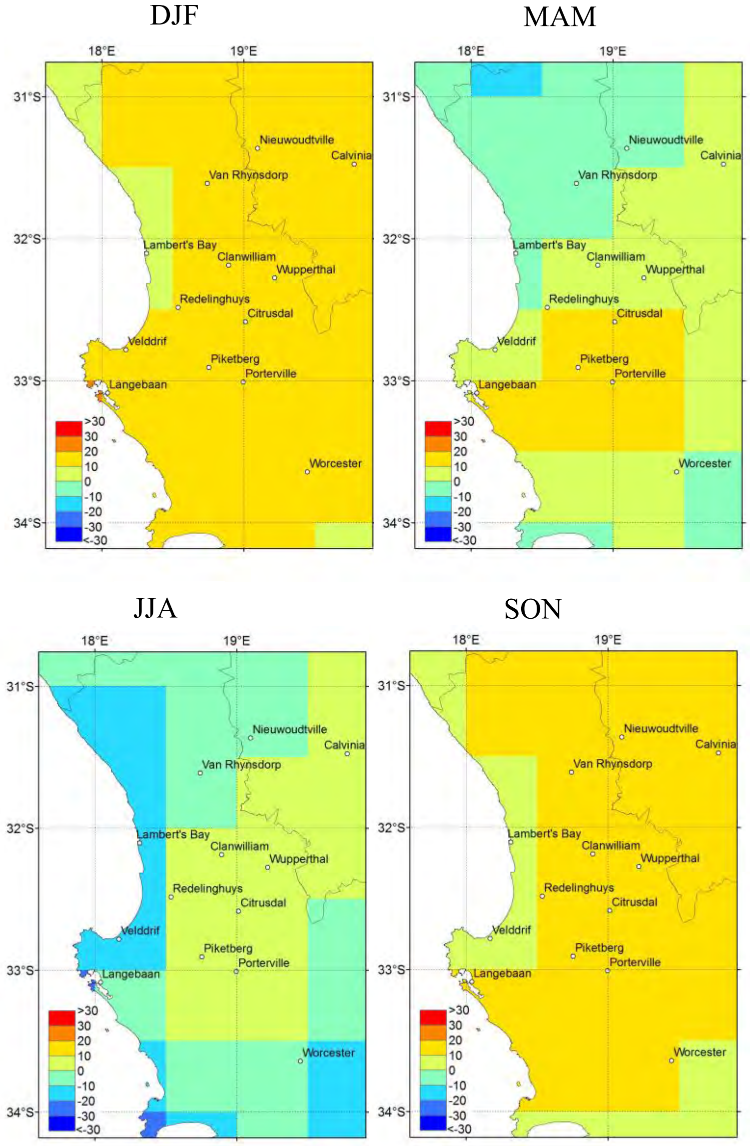


Wind run

Model to model uncertainty in average wind speed change is high, but there is a tendency for decreases in wind speed over most of the study area for the months of May-August (Fig.

2.10). For the remainder of the year, some marginal increases are projected especially over the interior parts of the study area.

Figure 2.10 Best estimate (50th percentile of six coupled global climate models) of wind speed change (10m above), for December, January and February (DJF), March, April and May (MAM), June, July and August (JJA), and September, October and November (SON) for the period 2041–2070 vs 1961–1990 in m/s month^{-1}.



2.5 Discussion

2.5.1 Rainfall

Detecting significant changes in rainfall over time and space is particularly difficult in heterogeneous landscapes with highly variable climates such as can be found in the western regions of South Africa. Nevertheless, this analysis found some marked trends in the characteristics of rainfall and their spatial coherence. Where positive trends in total annual and seasonal rainfall were detected, these were mostly attributed to an increase in heavy rainfall events as indicated by the relevant statistics of the daily rainfall. These findings concur with the review by Easterling *et al.,* (2000) which indicates a tendency for increased extreme rainfall in the south-western parts of South Africa during most of the 20th century. Moreover, the distinct pattern of increases in the intensity of large rainfall events, especially during March-May, supports Hewitson *et al.,* (2005), who also found increases in the intensity of rain events during late summer/early winter over the Western Cape. These trends may be linked to an increase in the frequency of strong low pressures centered south of South Africa between 1958 and 2001 (Midgley *et al.,* 2005).

The increase in summer rainfall on the Bokkeveld escarpment, in the northeast of the rooibos production region, suggests a possible increase in rainfall of convective origin which is mainly a feature of summer rainfall areas in South Africa (Tyson and Preston-White, 2000). According to Tadross *et al.,* (2008) increases in thermal heating and subsequent increases in atmospheric moisture may result in more convective rainfall during mid-late summer.

Negative trends in total rainfall during the main winter rainfall season (JJA), especially towards the north were accompanied by increases in the length of dry spells which indicates that rain has been falling less frequently. Hewitson *et al.,* (2005) linked drying during the winter period to atmospheric circulation changes, through a systematical southward displacement of frontal systems during JJA. However, marginal increases in JJA total and intensity of rainfall, especially towards the south concur with Mackellar *et al.,* (2014) which have also shown slight insignificant increases in winter rainfall for some stations in the Western Cape for the period 1961-2010.

The results for future rainfall (2040–2070 relative to 1960-1990) are generally in agreement with those of previous studies (Engelbrecht *et al.,* 2011; Malherbe *et al.,* 2013) which project drying across all seasons over the south-western Cape with biggest rainfall reductions occurring during JJA. These trends are consistent with the projected physical changes in the regional climate system over South Africa, which indicates a displacement of frontal rain bands towards the south, which are unfavourable for rainfall over the winter rainfall region of South Africa (Tennant and Reason, 2005). Hence both historical data and future simulations concur to some extent on the trend in mid to late winter drying over the study area. There are however, several discrepancies in the extent to which future predictions of rainfall change coincide with observed rainfall trends. The area of historical wetting during autumn (MAM), gives way to substantial drying under future climate simulations. Similarly, the marginal summer wetting in the north-east is not projected to persist in the future.

2.5.2 Temperature

Contrary to the historical rainfall analysis, the temperature data generated more significant and consistent signals. Annual temperatures have been steadily climbing for all stations, while the hottest 10 years have all occurred in the last decade of the observed period. This is consistent with the global average temperature which indicates an increasing rate of change (IPCC, 2013). The largest change in maximum temperatures coincides with the warmest and driest time of the year which is December-March. These changes in temperature are further associated with an increase in frequency of hot extremes which may contribute to the intensification of heat stress and low water availability during summer months when rainfall is limited. Similarly, Kruger (2006) reported an overall significant increase in the frequency of hot extremes and a decreased frequency of cold extremes, with the greatest changes tending to occur in the western and northern interior of the country. Consistent with previous studies (Bonnardot and Carey, 2008; Kruger and Sekele, 2013; Mackellar et al., 2014), minimum temperatures did not increase to the same extent as maximum temperatures during the latter part of the 20th century. This may be linked to the negative trend in annual rain days, which is associated with less cloud cover, increased solar radiation, resulting in warmer maximum temperatures.

While there is some disagreement between models on rainfall changes, the pattern and magnitude of projected temperature increase shows close correspondence across ensemble members. Both maximum and minimum temperatures are projected to increase by a further 1.5 - 2.5˚C for the period 2040-2070 relative to 1960-1990 which clearly coincides with magnitude of trends witnessed in the historical data. In particular, the same seasonal trend of greatest increase in maximum temperature during autumn emerges from both historical and future simulations. The slower increase in minimum temperature in the historical data was however not observed for the future projections.

2.5.3 Wind run

This analysis confirms both the trend and magnitude of wind run decline reported previously for the Western Cape (Hoffman *et al.,* 2011). It has been suggested by Midgley *et al.,* (2005) that an increase in vegetation (surface drag) or the changing temperature gradient between polar and tropical regions may be related to this phenomenon. The projected decrease in near-surface wind speeds for parts of the study area during May – August therefore coincides with the observed trends, whereas the simulated increase in wind speed for most of summer is inconsistent with historical data. Wind speed projections do however coincide with simulations for similar latitude regions in south west Australia (CSIRO, 2007).

2.6 Implications and conclusions

Seed germination and seedling emergence are critical stages in successful plant establishment (Quintana *et al.,* 2004). In most Fynbos species, germination after fire is closely tied to winter rainfall and plants are particularly vulnerable to drought stress during these phases (Kozlowski, 1968; Keeley, 1994). An important signal in terms of rainfall seasonality for rooibos germination and recruitment is, therefore, the observed decrease in winter rainfall (and corresponding increase in dry spells) which are projected to become more prominent in the future. The frequent occurrence of dry periods throughout the winter months may impair post-fire regeneration. Drought conditions may result in failure of germination, while rainfall adequate to induce germination may be followed by prolonged dry spells that would result in high seedling mortality and, therefore, a high risk of extinction of seedling populations. By contrast, in commercially propagated rooibos the early stages of germination and seedling emergence are artificially overcome by sowing seed in irrigated seed-beds. Regeneration phases of cultivated rooibos will therefore not be equally affected by early winter drought when germination is manipulated by irrigation, but rather by late winter dry spells and summer drought when supplementary watering of transplanted seedlings is not possible.

Evidence of a decrease in minimum temperature as well as a decline in near-surface wind speed during May – August may offset some of the potential negative effects of drought on rooibos by reducing the risk of frost (high altitude areas) and wind damage in young plants.

The temperature increase may also allow farmers to transplant seedlings earlier in the winter rainfall season to allow more time for establishment before the dry season commences.

In Mediterranean ecosystems most seedling mortality occurs in the first dry season of a plant's life cycle. The ability to survive the first summer drought season after initial establishment during winter is therefore critical to successful plant recruitment (Richards & Lamont, 1996). Maximum temperatures during summer months have increased by almost 2°C and the impacts of rising temperature would lead to a net drop in water availability and further intensify or prolong summer drought. Seeders and sprouters may however be affected differently by the intensity and duration of summer drought during these initial recruitment stages. Since seeder populations depend entirely on post-fire seedling recruitment for persistence and growth, a long first summer drought period would be fatal for recruited seedlings and lead to extinction of the population. Although prolonged drought periods would also hamper the establishment of resprouter seedlings, resprouter adults which have survived a fire may persist under those conditions given effective recruitment of seedlings in years when more favourable climate conditions occur (le Maitre & Midgley, 1992).

Climate change effects may not be experienced equally across the rooibos production region. Rooibos plantations occur at varying altitudes in lowland and mountain areas. Low lying areas have lower rainfall rates and are subjected to higher levels of evapotranspiration. These areas are already marginal for crop production and may experience the effects of climate change first. By contrast, in mountainous regions, especially towards the south of the study area where average rainfall is far above the minimum required for rooibos production, a decrease in rainfall and increase in temperature will be insufficient to make these areas unsuitable for production.

CHAPTER 3: ROOIBOS DISTRIBUTION UNDER CLIMATE CHANGE

Modelling the distribution of *Aspalathus linearis'* (Rooibos tea): Implications of climate change for livelihoods dependent on both cultivation and harvesting from the wild.

Abstract: *Aspalathus linearis* (Burm. f.) R. Dahlgren (rooibos) is endemic to the Fynbos biome of South Africa, which is an internationally recognised biodiversity hotspot. Rooibos is both an invaluable wild resource as well as commercially cultivated crop in suitable areas. Climate change predictions for the region indicate a significant warming scenario coupled with a decline in winter rainfall. First estimates of possible consequences for biodiversity point to species extinctions of 23% in the long term in the Fynbos biome. Bio-climatic modelling using the maximum entropy method was used to develop an estimate of the realized niche of wild rooibos and the current geographic distribution of areas suitable for commercially production. The distribution modelling provided a good match to the known distribution and production area of rooibos. An ensemble of global climate models which assume the A2 emissions scenario of high energy requirements were applied to develop possible scenarios of range/suitability shift under future climate conditions. When these were extrapolated to a future climate (2041-70) both wild and cultivated tea exhibited substantial range contraction with some range shifts south-eastwards and upslope. Most of the areas where range expansion was indicated are located in existing conservation areas or include conservation worthy vegetation. These findings will be critical in directing conservation efforts as well as developing strategies for farmers to cope with and adapt to climate change.

3.1 Introduction

There is compelling evidence of climate change induced impacts on species diversity through amongst others, species composition changes (Bertrand *et al.,* 2011; Ruiz-Labourdette *et al.,* 2013), range shifts (Bertin, 2008; Colwell *et al.,* 2008) and altered phenology (Cleland *et al.,* 2007; Prieto *et al.,* 2009; Hulme, 2011). Given the rate and magnitude of changes in the gobal and regional climate, knowledge of what determines species ranges is critical in understanding the potential consequences for agriculture, forestry and biodiversity conservation (Araùjo and Rahbek, 2006; Falk & Mellert, 2011; Bradley *et al.,* 2012). Increasing attention has, therefore, been focussed on implementing a proactive approach through developing plausible scenarios of future climate change and modelling the associated species range and ecosystem shifts.

Decision tools such as correlative spatial distribution models (SDMs) have become key in assessing biodiversity responses to climate change (Midgley *et al.,* 2003; Guisan and Thuiller, 2005; Heikkinen *et al.,* 2006; Araùjo *et al.,* 2011; Rodríguez-Castañeda *et al.,* 2012). Several SDM methods have been developed and applied to investigate species' geographic ranges and possible shifts under global climate change. These include mechanistic models, climatic envelope methods and machine learning techniques (Yates *et al.,* 2010). All of these methods estimate a species actual or potential geographic range through relating field observations of species occurrences to environmental and climatic variables. This relationship can then be used to assess species-range shifts under different climate scenarios to undertake risk assessments in specific focal areas.

In the light of the importance of accurately modelling species' responses to a changing climate, numerous papers have been devoted to exploring the relevance, application and shortcomings of these models (Guisan *et al.,* 2006; Heikkinen *et al.,* 2006; Elith and

Leathwick, 2009; Soberòn and Nakamura, 2009; Miller, 2010; Araújo and Peterson, 2012). Some cross-cutting objections against these models are 1.) They do not include biotic interactions and assume species distribution is primarily affected only by climatic variables 2.) When extrapolating to the future they make the assumption that the limiting factors and biotic interactions will remain the same 3.) The spatial and temporal resolution at which data is collected and applied raises several statistical issues 4.) While species distribution models usually deal with the mean climatic range of a species potential current and future suitability it is more often the changes in climatic variability and occurrence of extreme events that determine their distribution range. A prerequisite for distribution modelling is, therefore, a thorough understanding and interpretation of the many factors interacting within the environment where the species occur. Modelling range shifts also requires an in depth understanding and rigorous analysis of the species at hand. By acknowledging and being aware of the limitations of these methods, we can make them useful support tools exploring climate change associated range shifts.

Globally, numerous species distribution models investigating the impact of climate change on species predict that more species will experience substantial range shifts with a changing climate (Parmesan and Yohe, 2003; Thuiller, 2005; Broennimann, 2006; Chen *et al.,* 2011;). Locally, climate change related species distribution research (Midgley *et al.,* 2002; Midgley *et al.,* 2003) in the Cape Floristic Region (CFR) of South Africa suggests a reduction in the geographical ranges of endemic species, and reductions in species richness under climate change. The extent of the Cape Fynbos biome could decline by between 51% and 65% depending on the warming scenario. There is consensus between climate models that the climate in the CFR is expected to become warmer and drier, with a decline in winter rainfall, especially in the western region. This could eventually result in species extinctions of 23% in the Fynbos biome.

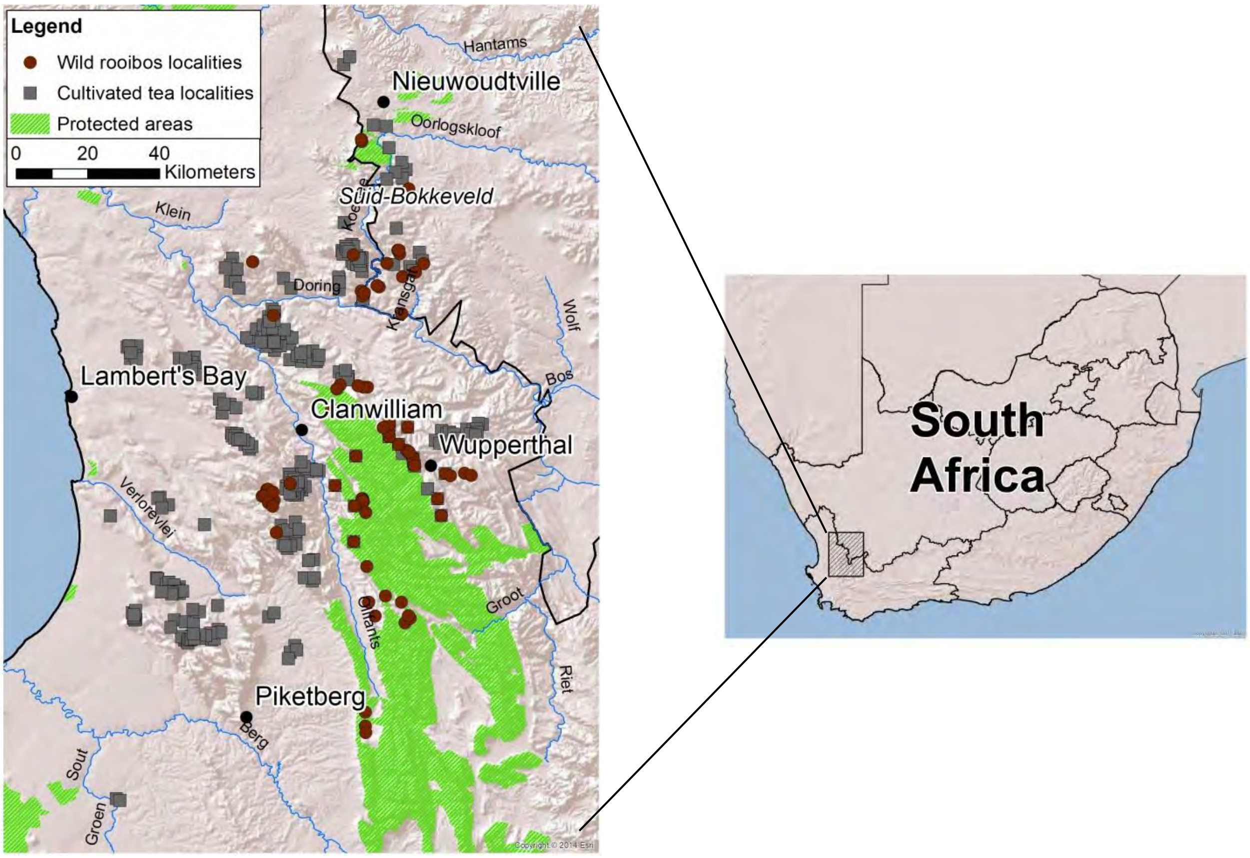
Aspalathus linearis is a leguminous shrub indigenous to the Fynbos biome of the Cape Floristic Region (Dahlgren, 1968) which has successfully made the transition from wild resource to an agriculturally important plant. Wild populations of *A. linearis* have a narrow geographic range within the Fynbos biome and are largely confined to mountain ranges of the far south western part of the Northern Cape Province and Cederberg mountains of the Western Cape. The species grows mainly in nutrient poor, highly acidic and well-drained, sandstone-derived soils (pH 3–5.3) typical of the mountainous areas in the area (Muofhe and Dakora, 2000). Its climatic distribution is dictated particularly by the combination of winter rainfall and hot dry summers with an annual rainfall of at least 300-350mm (Dahlgren, 1968). Cultivated and wild *A. linearis* differ mainly in terms of morphology, growth and flowering patterns (Malgas *et al.,* 2010). Cultivated plants are re-seeders whereas certain ecotypes of wild *A. linearis* are slower growing re-sprouters. Rooibos is ant dispersed and its fire-stimulated seeds germinate in the early winter months after the passage of the first rain bearing cold fronts. Ant dispersal provides a number of benefits for the species. Ants may move seeds many metres away from the parent plant helping it to escape from herbivores and minimizing competition with parent plants/siblings (Bond and Slingsby, 1983). In commercially propagated rooibos these critical stages of seed germination and seedling emergence are artificially overcome by sowing seed in well prepared, irrigated seedbeds after which it is then removed and established in plantations.

The species was first described circa 1768, but wild plants have been collected and utilized by local inhabitants of the Cederberg and Bokkeveld mountains (Fig. 3.1) for centuries (Morton, 1983). Based on rock art and archaeological evidence hunter-gatherers have lived in the area for 10 000-20 000 years and herders (khoi) since around 1200 AD (Barnard, 1992). Rooibos has always formed an integral part of the heritage of these people and they have a rich knowledge of managing and utilizing the plant to produce tea as well as for its medicinal and health properties. The economic value of rooibos was, however, not exploited until the 1930's when intensive research on the cultivation of the plant enabled the development of the fully-fledged industry as it stands today. The industry is one of the largest providers of permanent and seasonal employment in the rural areas of South Africa (DAFF, 2011). Recent years have seen an unprecedented growth in the rooibos industry as the demand for rooibos from international markets has steadily increased.

Alongside the well- established commercial rooibos industry, traditional small-scale farming fulfils a vital role in maintaining the economic and social stability of historically-neglected rural communities in the semi-arid Cederberg region of South Africa. Small-scale farmers are concentrated in isolated and remote rural areas around Nieuwoudtville and Wupperthal. Wild rooibos is marketed by these small-scale farmers as an organic and fair trade certified product to niche markets overseas. Many rural communities therefore depend on *A. linearis* for their livelihoods so the tea has ecological, economic and cultural significance.

In this paper bio-climatic modelling was employed to model *A. linearis*' distribution. The objectives of the study were to: 1.) to identify the environmental factors limiting or determining the natural distribution; 2) use this to develop a first estimate of the realized niche and potential geographic distribution of wild rooibos and the current geographic distribution of areas suitable for commercially production; 3) inform the location and design of field experiments to assess its ability to survive under different climatic conditions and 4.) develop possible scenarios of range/suitability shift under future climate conditions.

Figure 3.1 Map of the study area: surveyed locations of wild (red dots) and cultivated rooibos (grey squares) stretching from Nieuwoudtville in the Northern Cape, south towards Piketberg.



3.2 Data and Methods

3.2.1 Maxent

The maximum entropy method is a robust model widely applied in the field of ecology (Elith and Leathwick, 2009) and similar to Poisson point process models (Renner and Warton, 2013). The Maxent software (version 3.3.3k http://www.cs.princeton.edu/~schapire/maxent/) was used in this study to model species geographic distributions. It is a machine learning technique that uses a data matching algorithm to make predictions from incomplete information (Phillips *et al.,* 2006). Subject to known constraints, Maxent estimates a probability distribution which has the maximum entropy while matching the value of each environmental variable as closely as possible to the empirical values observed at the species' occurrence records. The output can either be raw (relative occurrence rate), cumulative or logistic (probability of presence). There is a debate about whether MaxEnt output should rather be presented in the raw format as opposed to the more widely used logistic format which relies on post-processing assumptions (Royle *et al.,* 2012; Merow *et al.,* 2013; Hastie and Fithian, 2013; Yackulic *et al.,* 2013). As this issue is still unresolved, and suitability is easier to interpret, this study presented the results as logistic output which is an estimate of the suitability (scaled from 0-1) of each grid cell within the study area as a function of the values of the climatic and environmental variables in that grid cell. Based on known occurrences of rooibos in the area that it actually occupies, MaxEnt therefore gives an approximation of the suitability for the species which approximates its realized environmental niche. It therefore fits the model in an environmental space which is a conceptual area defined by climatic and environmental variables and then projects it back to geographic space (Pearson, 2007).

3.2.2 Species occurrence data

Presence only locations for both cultivated and wild rooibos were obtained separately. Seventy-one presence location records for wild types of *A. linearis* were assembled during field work and from literature (van der Bank *et al.,* 1995; van Heerden *et al.,* 2003). Due to the limited data on localities for wild rooibos, location records of both resprouters and seeders were used as input for the model. Distribution data supplied by the South African National Biodiversity Institute's PRECIS database (Germishuizen and Meyer, 2003) indicate a natural distribution of rooibos stretching from Nieuwoudtville south towards Cape Town and even Bredasdorp in the southern Cape. The south easternmost occurrences were not included in the final dataset as they were only available at a quarter degree resolution and could therefore not be used to provide accurate information on the values of the environmental variables at the collection locality. This is further complicated by the significant variation which exists in climate and soils over short distances due to the heterogeneous and undulating character of the environment. Some locations as far east as Bredasdorp are also thought to be misidentifications (Ben-Erik van Wyk, pers comm, 2011). Only those records of wild rooibos (Fig. 3.1) that could be reliably confirmed and located were used. These locations correspond with the mountain areas of Nieuwoudtville and Wupperthal where abundant wild populations occur and most of the wild species harvesting is done by the small-scale farmers and, thus, where the study is focussed.

Intensive cultivation of rooibos occurs on the mountain slopes and on top of plateaux or plains in the mountains of the greater Cederberg region. The southernmost production area is the Piketberg and it extends northwards as far as Nieuwoudtville. One-hundred and one presence records for cultivated rooibos which represent point localities (midpoints of field boundaries) were used as input to develop the current distribution map for commercial rooibos. These were treated separately in the modelling process as they almost certainly represent an artificially modified distribution.

3.2.3 Environmental variables

A list of variables appropriate for modelling rooibos was obtained from previous studies of species distributions within the Fynbos biome (Midgley, 2002, Malgas *et al.,* 2010) as well as from farmers' knowledge of the variables limiting rooibos distribution. In her study of abiotic and biotic parameters as drivers of *A. linearis'* environmental suitability, Gerard (2010) concluded that rooibos is mainly driven by abiotic factors and indicates climate as a limiting aspect of its distribution. There is a strong correlation between elevation, temperature and rainfall in the study area and hence the following climatic variables were used as input for the MaxEnt model: total winter rainfall, total summer rainfall, average winter min temperatures and average summer maximum temperatures. In a winter rainfall climate, rooibos regeneration will require sufficient soil moisture during the winter months for seed germination and establishment while some precipitation during the summer season is necessary to enable young seedlings to survive through the dry summer months. This guided the choice of variables relating to seasonal rainfall. The wild plants' altitudinal distribution lies between 450m and 900m above sea level. Allowing for potential elevation shifts brought on by climate change, minimum and maximum temperatures were included. The climate variables were also chosen to correspond to variables which could be obtained for future climate scenarios. Baseline climate data representing interpolations of observed data for the time period 1950 – 2000 were obtained from WORLDCLIM (Hijmans *et al.,* 2005) in ESRI grid format at a resolution of 30 arc-seconds. Topography, soil depth and drainage are other important factors affecting the establishment of rooibos plantations and distribution of wild populations. Slope and land type were therefore also included as predictor variables. Slope was derived from a 90m digital terrain model (Jarvis *et al.,* 2008). Land types were obtained from the Agricultural Research Council's Institute for Soil, Climate, and Water (SIRI, 1987). Land types describe the unique combination of macroclimate, terrain form (i.e. location on a catena) and soil pattern as determined by the underlying geology and weathering patterns. A key environmental and evolutionary factor in fynbos which was not included in this study is fire. The fire regime, particularly the recurrence intervals and seasonal distribution, have shaped the traits of fynbos species and the distribution of fynbos (Kraaij and Wilgen 2014).

Although this would seem to make it an obvious factor to include in distribution modelling, there is no evidence that fire regimes vary significantly between the natural range of rooibos and adjacent fynbos areas and, thus, are a key determinant of its distribution in the fynbos.

A winter rainfall region was delineated as background for the modelling process as rooibos is known to occur in an area where winter rainfall during May – September accounts for 60% or more of the total rainfall for the year. There are no known records of rooibos occurring in a bi-modal or summer rainfall region. All of the surveyed locations are thus captured by this background. This criterion centres the focus on the region where the activities of local farmers could potentially be impacted on by climate change. The selected background extends well beyond the areas where rooibos presently occurs and includes adequate environmental space to quantify low suitability as well as allowing for possible range shifts. All the environmental variables were converted to a 30 second grid by resampling using the nearest neighbour method.

3.2.4 Model building

The selected species occurrence records and environmental variables were used to develop a model of the potential distribution of wild and cultivated rooibos under current climatic conditions. The MaxEnt algorithm was first run with presence locations of wild rooibos. The rooibos presence records were randomly assigned so that 75% of the localities were used for training data, and the remaining 25% were reserved for testing the model. The MaxEnt algorithm was run with hinge and quadratic features with several combinations of the environmental variables. The model was run with different sets of the training data where after a different fraction of the data were withheld for each run. Variables were then narrowed down to the best combination based on the contribution that each variable, as well as all variables collectively, made to the bio-climatic envelope. Response curves also gave an indication of the dependence of the predicted suitability on a specific variable as well as the range under which the variable reaches its optimum suitability. The output was projected as a map of species distribution showing the suitability on a scale from 0 (least) to 1 (most) suitable. To discriminate between truly "suitable" and "unsuitable" areas a threshold of occurrence was chosen to correspond to the lowest predicted suitability for a species occurrence record following Pearson (2007).

To assess wild rooibos species range adjustment in the face of climate change, the model for the current conditions was projected using an ensemble of scenarios under potential climate change. A distribution map for each scenario was developed and further analysed in ArcGIS to establish the level of agreement between ensemble members. A model average was then calculated from the suite of projections and compared with the existing species distribution map to quantitively assess range expansions or shifts. These maps were based on the assumption that species are allowed unrestricted dispersal to new areas that satisfy their climatic limits (Guisan *et al.,* 2006; Loarie *et al.,* 2008).

3.2.5 Model evaluation

The model needs to be assessed to determine how well the model fits the training data and predicts the current distribution of the species. As noted by Elith and Graham (2009), this is a semi-subjective process and different methods of performance can be applied. Amongst others variable importance was assessed by observing the change in gain when certain variables were excluded. All maps and functions were also visually evaluated for irregularities. Specific features have been made available in the latest version of MaxEnt to test model appropriateness and fit when projecting to a new environment. The multivariate environmental similarity surface (MESS maps) supplied with the software was used to show whether the portion of the predicted range is within the environmental space defined by the ranges of the variables in the input to the model.

3.2.6 Climate scenarios

To assess rooibos species range adjustment or shift in the face of climate change, the underlying model structure that has been developed under current conditions was applied under potential scenarios of future climate change. An ensemble of models which assume the A2 emissions scenario were used to explore rooibos suitability under future conditions. This emission scenario was selected because it reflects high energy requirements and the current actual trajectory of emissions are already ahead of the higher end emissions scenarios. Five coupled climate models, which were dynamically downscaled for Southern Africa by means of the conformal-cubic atmospheric model (CCAM) (Engelbrecht *et al.,* 2011), were obtained from the climate modelling and environmental health research group at the CSIR. These simulations were used to generate future changes in temperature and precipitation data by adding the mean differential (Hewitson, 2003) between future scenarios (2041-2070) and the baseline (1960-1990) for each climate model to the corresponding observed climate variable.

3.3 Results

3.3.1 Current climate: cultivated tea

The MaxEnt output indicates that the model was statistically significant ($p < 0.0001$) and performed relatively well in predicting suitable crop areas with an area under cover (AUC) of 0.969. The variables that made the largest contribution to explaining crop suitability, based on the “jacknife” procedure in MaxEnt, are winter precipitation (45.3%), soil types (28.2%) and summer precipitation (14%). The strong influence of land types indicates that the sandy, infertile soils derived from Table Mountain sandstone origin are required for rooibos production. The marginal response curves for cultivated tea are indicated in Figure 2. The response curve for winter rainfall was open-ended, indicating increasing suitability with increasing winter precipitation (Fig. 3.2A). The opposite is true for summer precipitation which showed decreasing suitability as rainfall increased (Fig. 3.2C). However, when summer rainfall was excluded from the model, winter precipitation displayed a bell shaped response curve, indicating reduced suitability as rainfall increases above a certain threshold value. Minimum temperature during the coldest months made a significantly greater contribution to the model than mean annual temperature. The optimum temperature (between 2°C and 6°C) corresponds to an elevation range in mountainous areas at which there is a comparatively high winter rainfall.

The districts of Citrusdal, Clanwilliam, Nieuwoudtville and Wupperthal are known to be key cultivation areas where the unique microclimate and soil combine to form a rather limited geographical area which is suitable for rooibos production. The final suitability map (Fig. 3.3A) provides a good representation of these existing core production areas. It also indicates that some small areas in the mountains to the south of the traditional production area are marginally suitable.

Figure 3.2 Marginal response curves of the most important predictor variables explaining cultivated tea suitability: (a) average winter rainfall, (b) land types, (c) average summer rainfall, (d) average winter minimum temperature, (e) slope derived from a digital terrain model, and (f) average maximum summer temperature.

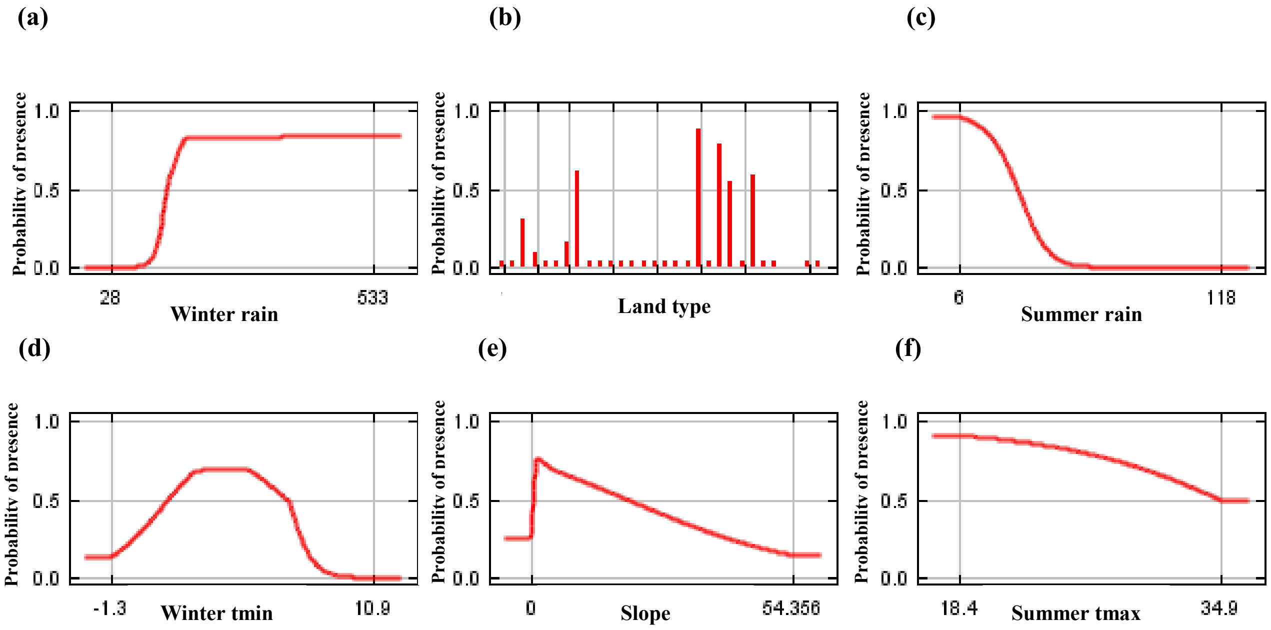
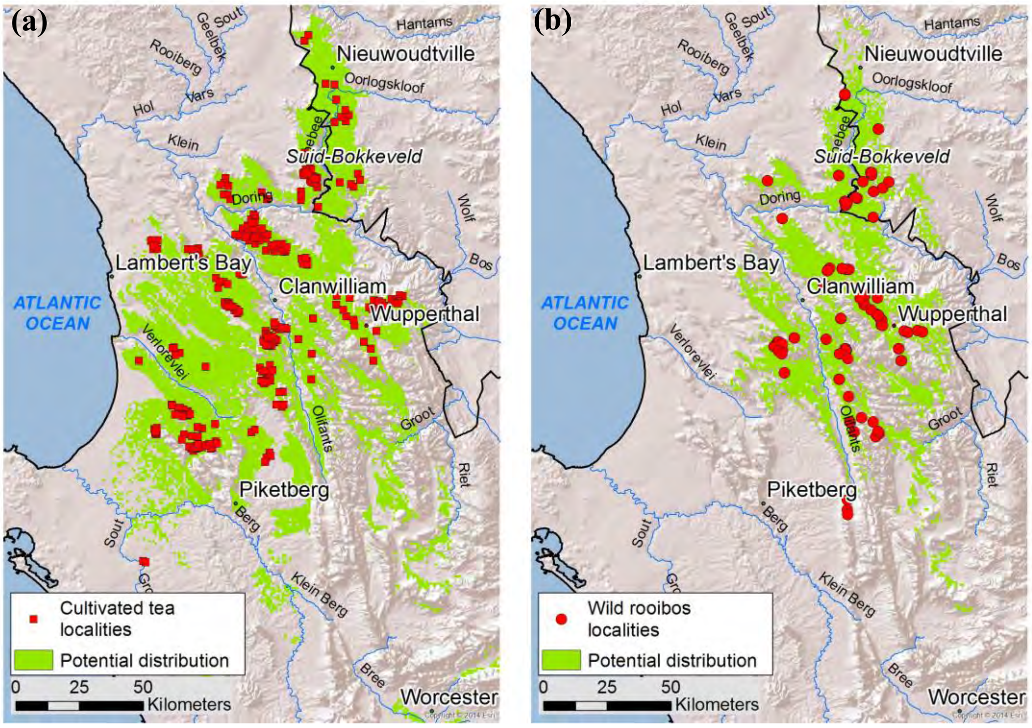


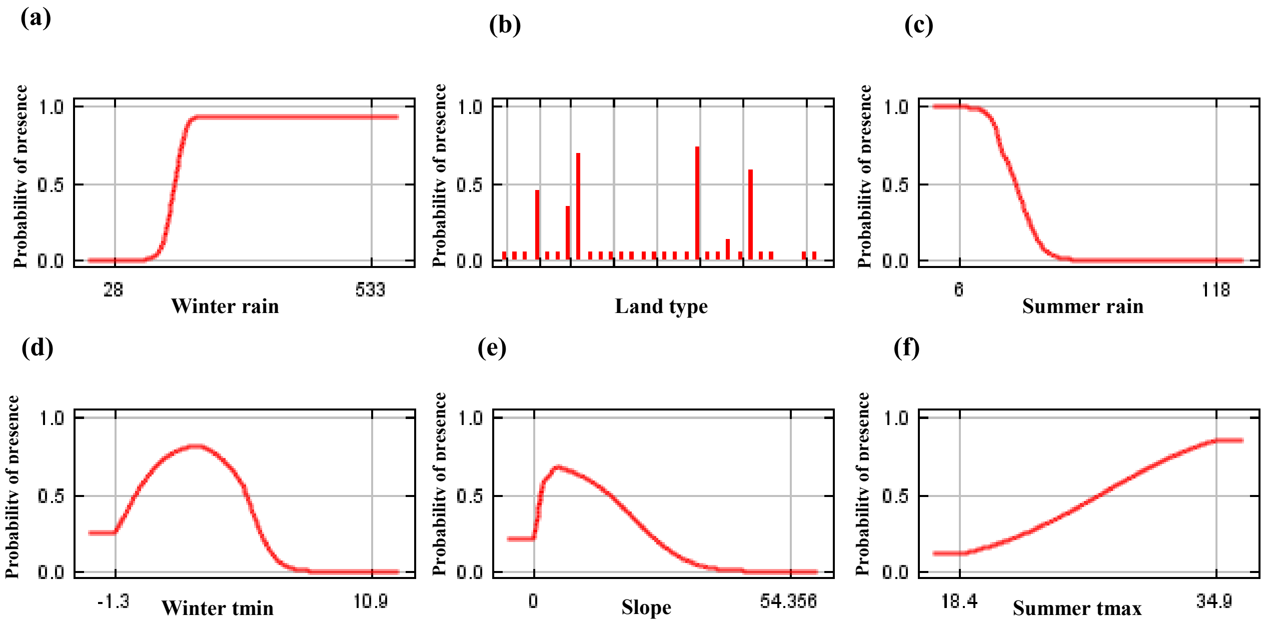
Figure 3.3 Suitability maps for (a) cultivated and (b) wild *A. linearis* for the current climate. Maps were generated with the MaxEnt algorithm using presence locations of cultivated and wild rooibos.



3.3.2 *Current climate: wild tea*

The potential distribution of *A. linearis* in geographical space (Fig. 3.3B) was predicted with reasonable success with an area under cover (AUC) of 0.989. It exhibits a good match to the known distribution of *A. linearis* in the Greater Cederberg region. The environmental variables used to define the bio-climatic envelope of *A. linearis* corresponded with the variables used in modelling cultivated tea, but differed in the contribution each variable made to define the envelope. According to the jacknife procedure winter precipitation (31.2%), min temperatures (26%) during winter and land types (18.6%) were the most important variables. Summer precipitation remained a significant variable, although to a lesser extent than for the cultivated tea. Land types of sandstone origin were still significant and also included soils with more rocky outcrops. In addition, slope increased a little in importance with an optimum between 3° and 10°. Both winter and summer precipitation displayed the same open ended response curves as cultivated tea (Fig. 3.4A and 3.4C). They displayed a similar response of decreased suitability as rainfall increases above a certain value, indicated by the response curves generated using only the corresponding variable. The potential distribution of wild tea is similar to that of the cultivated tea, although more restricted to mountainous areas. The environmental space was also somewhat different. This can be seen in the different optima of the environmental variables' and the shape of the response curves (Fig. 3.4). Minimum temperature during winter exhibits a narrower range of temperatures which corresponds to the narrower elevation range of wild compared with the cultivated tea.

Figure 3.4 Marginal response curves of the most important predictor variables explaining wild tea suitability: (a) average winter rainfall, (b) land types, (c) average summer rainfall, (d) average winter minimum temperature, (e) slope derived from a digital terrain model, and (f) average maximum summer temperature.



3.3.3 Extrapolation to future climate

A suite of five models were applied to model suitability of cultivated tea under altered climate conditions (Figs. 3.5A-F). All the climate models consistently predicted an average increase of 2.7–3.2°C in annual temperatures across the region of interest. Projections for precipitation amongst models are more variable. The UKMO and MPI models projected the most significant decreases in winter precipitation whereas the MIROC model mostly predicted increases in winter precipitation. Winter precipitation anomalies therefore ranged from decreases of 52 mm to increases of 32 mm. This resulted in a suite of envelopes of suitability changes depending on the climate model in question. The MIROC model consistently yielded the most conservative predictions because it projects less drastic temperature increases and some winter precipitation increases. There was some overlap in the predicted areas between the models (Fig. 3.5G). The model ensemble average (Fig. 3.7A) for an intermediate future period (2041-2070) indicates rooibos tea suitability will remain the same in the higher elevation areas of the traditional rooibos production area. In the western areas along the coast considerable decreases are however projected, especially in the lower lying regions. Across all scenarios the most significant increases in suitability are expected in the mountainous region towards the south of the study area indicating a general shift southwards and to higher altitudes (Fig. 3.7A).

The same suite of climate models was used to model the potential distribution of wild *A. linearis* (Figs. 3.6A-F) for an intermediate future scenario (2041-2070). Each projection is quite different and the extent of agreement is less than for the cultivated rooibos (Fig. 3.6G). The distribution map (Fig. 3.7B) shows a marked contraction in its bio-climatic range in the northern part of the study area as opposed to the cultivated tea. The Suid-Bokkeveld small scale farmer community is located in this region and is one of the most important areas where wild rooibos is currently harvested. A further significant range contraction is also visible along the western parts of the study area at lower elevations. These are the areas most vulnerable to species loss. Further south and to the eastern part of the study area, another key harvest locality is found in the region of Wupperthal. Most of this region is not expected to undergo any range shifts (Fig. 3.7B). Areas that were not predicted to undergo range shifts under future conditions are restricted to elevation ranges of between 800m and 1050m above sea level. Range expansion however, is noticeable towards the south especially along the mountain ranges. This depends on the ability of the species to colonize new sites. Overall a similar trend of range shift southwards and to higher elevations is observed for both wild and cultivated tea. From the range of climate models employed, the most conservative outcome for cultivated tea is a 49.8% reduction in the extent of the bio-climatically suitable localities, represented by the MIROC climate model (Fig. 3.8A). At worst, the UKMO climate model predict an almost 75.5% reduction by 2070 while the ensemble mean indicate a loss of 57.2% in bioclimatically suitable areas for cultivated tea. For wild tea the most favourable outcome is a 76.2% reduction, and at worst an almost 88.7% reduction in the extent of the bio-climatically suitable localities by 2070 (Fig. 3.8B). On average, 42.7% of pre-existing bioclimatically suitable areas remain suitable for cultivated tea, while only 21.2% remain suitable for wild tea by the year 2070.

Figure 3.5 Suitability of areas for cultivated *A. linearis* under 6 future climate change scenarios for the period 2041-2070: (a) CSIRO, (b) GFDL 2.0, (c) GFDL 2.1 (d) MIROC, (e) MPI, (f) CSIRO, (g) all models. Map (g) shows the measure of agreement amongst models on a scale of 1-6 where 6 indicates the strongest overlap.

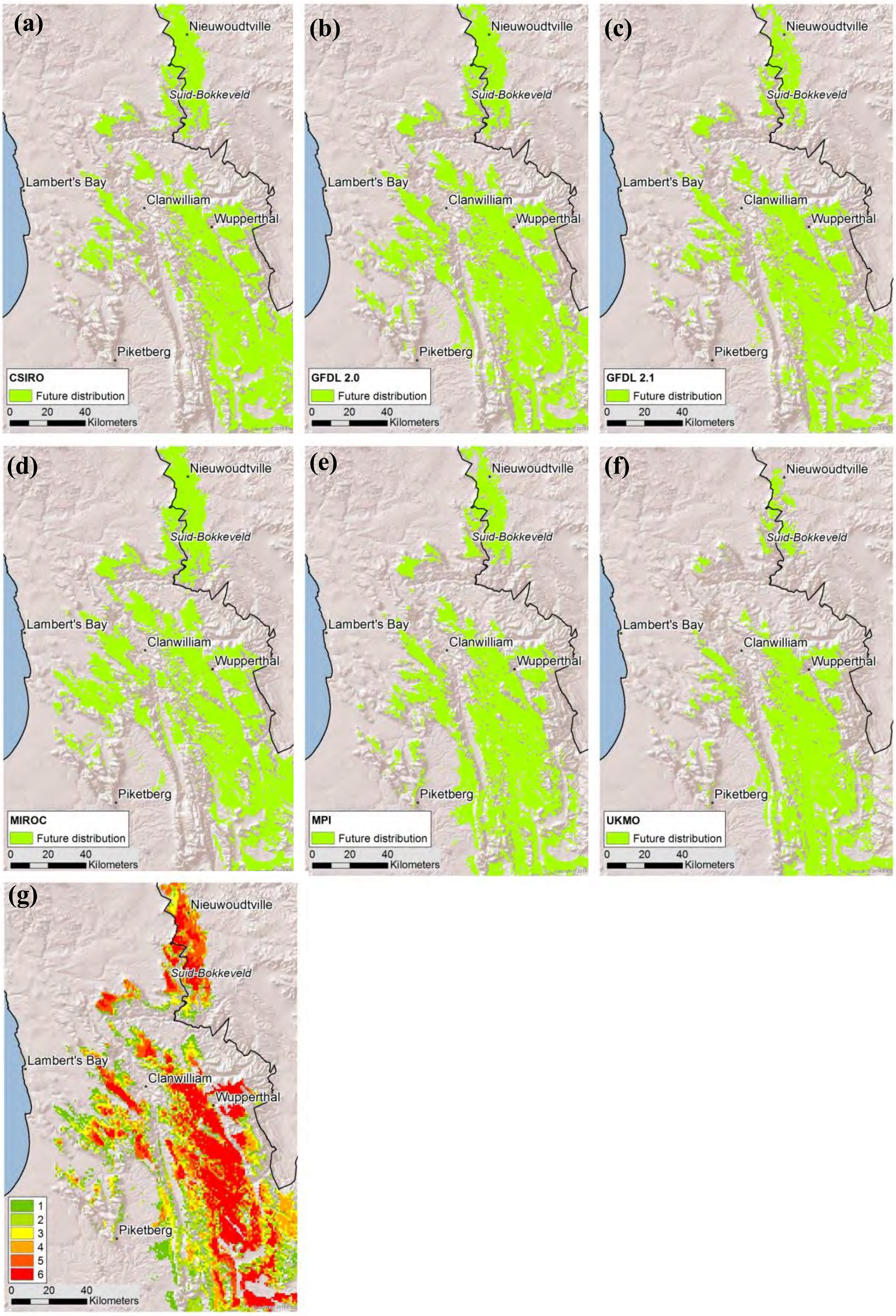


Figure 3.6 Suitability of areas for wild *A. linearis* under 6 future climate change scenarios for the period 2041-2070: (a) CSIRO, (b) GFDL 2.0, (c) GFDL 2.1 (d) MIROC, (e) MPI, (f) CSIRO, (g) all models. Map (g) shows the measure of agreement amongst models on a scale of 1-6 where 6 indicates the strongest overlap.

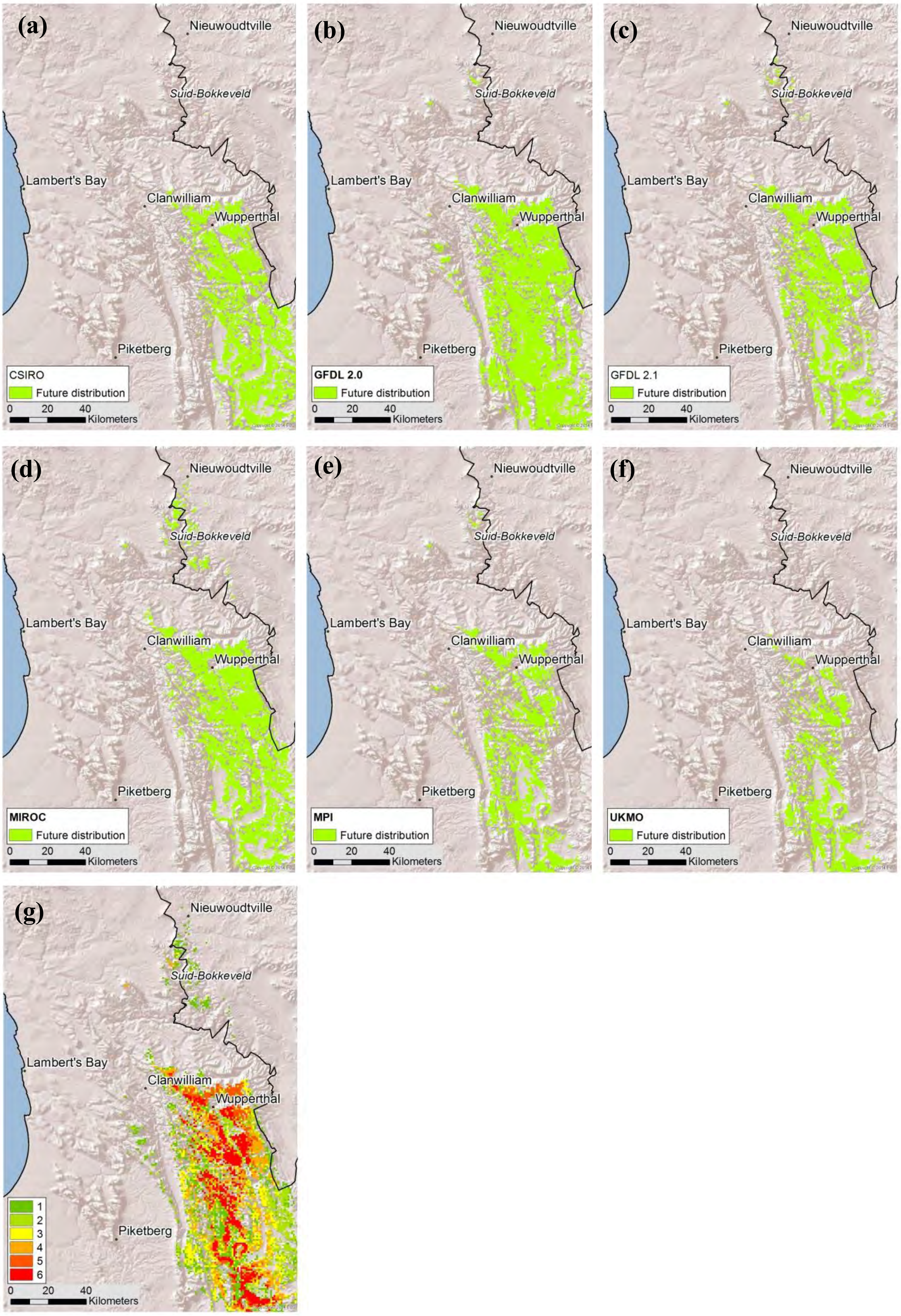


Figure 3.7 Suitability of areas for (a) cultivated and (b) wild *A. linearis* under future climate change for the period 2041-2070. Maps show areas where range contraction, no change and range expansion occur relative to the current climate (1960-1990).

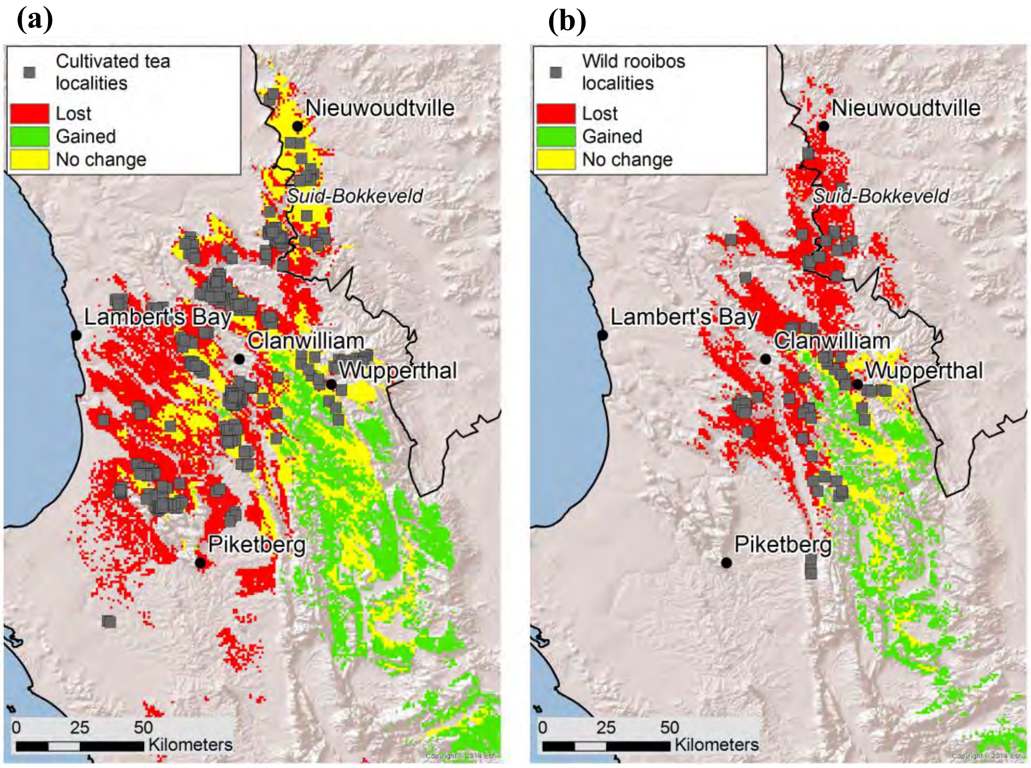
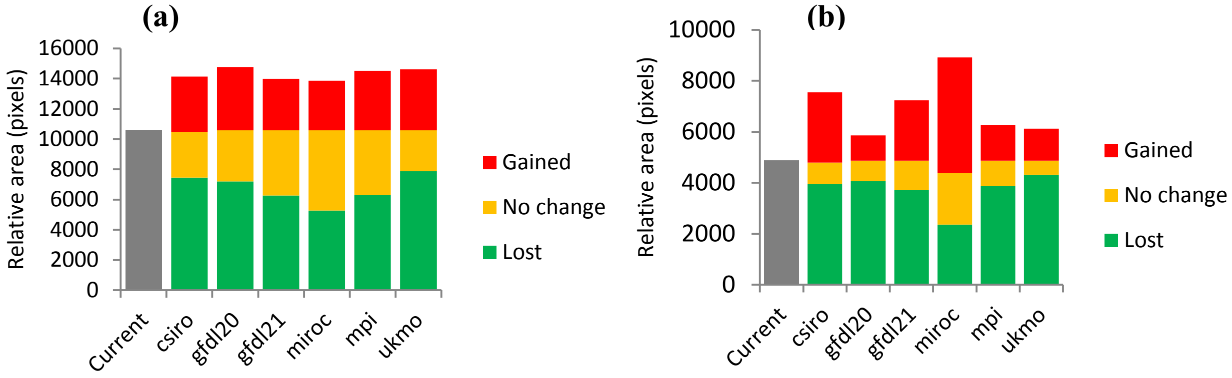


Figure 3.8. Area analysis overview. Predicted climate change outcomes for (a) cultivated and (b) wild rooibos for the year 2070. Stacked bar-charts based on area analysis maps (Figure 3.7).



3.4 Discussion

3.4.1 Model scenarios of rooibos crop and wild type distribution

Predictor variables defining rooibos' distribution in the study area were mostly similar for both wild and cultivated tea but differed in their relative contributions to the envelope. The climatic variables mainly pertained to summer precipitation, winter precipitation and minimum temperatures during winter. The findings agree with those of Hawkins *et al.,* (2010) which indicated elevation and rainfall as best predictors of rooibos species distributions. The MaxEnt response curves suggest that even limited increases in summer rainfall will negatively affect the predicted suitability of areas for rooibos. Farmers have however reported that rainfall during summer, which is mainly brought about by thunderstorms, is particularly important for rooibos persistence. The response functions further indicated that rooibos suitability increases as winter rainfall increases above 150mm. Lastly, the responses to minimum temperature during winter indicated that min temperatures exceeding 5.8°C negatively affect predicted suitability for rooibos. The MaxEnt model predicted *A. linearis* to occur mainly in mountainous areas with well-drained sandstone and quartzitic soil types and is therefore consistent with ecological knowledge of the species. This potential distribution under current conditions is comparable, (yet somewhat more restricted in some areas) to the distribution of wild rooibos developed by Malgas *et al.,* (2010) which may be due to the absence of soil information in their analysis. Although the model indicated both cultivated rooibos as well as its wild relatives tend to prefer similar habitats, the cultivated rooibos had a significant greater range.

The relative difference in distribution between the wild and cultivated types can mainly be attributed to the aid of mechanisation, soil manipulation practices, and transplanting seedlings into fields. Hence cultivated rooibos can be grown on a much larger scale and over a wider geographical range than the natural distribution, thus expanding the limits of its range. This often results in the cultivation of rooibos in marginal areas where the climatic conditions are outside the limits of its natural range. The use of seedlings rather than sowing seed to establish new plantations helps the plant through the critical stages of germination and seedling establishment, which is known to be an important determinant the distribution of populations in the landscape (Harper, 1967; Harper and White, 1974; Clark *et al.,* 2007). Commercial cultivation is thus not limited by germination and establishment requirements to the same extent as wild tea and therefore more widely distributed.

Over the western parts of southern Africa the temperatures are projected to increase at about twice the global rate while winters are projected to become drier (Engelbrecht, 2010). Increased temperatures and decreased rainfall, especially during critical stages of rooibos growth and development, may have serious implications for the sustainability of rooibos production in certain areas. The projected shifts in the distribution and suitability found in this study under future climates are comparable to those found in other studies of endemic plants (Midgley *et al.,* 2003; Lenoir *et al.,* 2008) and in crop suitability (DAPA, 2011). The direction of range shifts for both wild and cultivated tea is generally south-eastwards and upslope. This means that lowlands on the west coast will first experience climate change impacts whereas higher altitude mountain areas will experience little if any impacts for the period 2041-70. Similarly, Loarie *et al.,* (2008) projected that more species are likely to persist in mountain areas or expand their ranges to higher elevations. These novel areas might be viable for cultivation as propagation and plantation establishment is supported by human intervention. However, most of the areas where range expansion is indicated are located in existing conservation areas or include conservation worthy vegetation. The ability of wild rooibos to successfully migrate to these novel areas is uncertain. It will depend on complex interactions between abiotic and biotic variables, the species ability to disperse, population size and regeneration strategies (Midgley, 2003). This will require further investigations which fall outside the scope of this study.

3.4.2 Implications for livelihoods, conservation, and adaption planning

Emerging, small and resource poor farmers are often particularly vulnerable in that they do not have sufficient resources and access to timely information to deal with adverse effects of climate change. The bulk of wild tea is harvested by small-scale farmers located in near-pristine natural environments around Nieuwoudtville and Wupperthal. Small-scale farmers harvest proportionally less wild tea in relation to the cultivated variety, yet income per tonne from these teas is substantially higher and a valuable commodity. If species' ranges shrink or shift in the future as is predicted by the models, it is doubtful whether farmers will relocate to areas where species have colonized new sites. More pressure might be placed on harvesting the remaining populations and may contribute to the species decline. Research regarding the direction and rate of species distribution shifts is therefore important for local non-governmental organisations engaging with small scale farmers in developing strategies to cope with and adapt to climate change.

Land use change driven by the massive expansion of the rooibos industry in recent years has led to extensive habitat loss of many indigenous and endemic species including *A. linearis* (Raimondo *et al.,* 2009). The total rooibos crop footprint in this global biodiversity hotspot is currently 79 000 ha (Pretorius, 2009). Some of these locations in the lowland areas of the production area are already marginal for commercial rooibos cultivation. Existing climate variability exerts pressure on sustainable production and these areas may be the first to experience the effects of climate change. Future projections of crop suitability shifting towards mountain catchment areas are therefore a cause of concern and knowledge of future distribution patterns in the landscape will be critical in directing conservation efforts. Such information will aid the Rooibos Biodiversity Initiative which has become an important instrument in regulating land clearance and promoting sustainable land management practices.

Globally, numerous medicinally and economically useful endemic plants contribute significantly to the well-being and cultural heritage of indigenous communities. Many of these medicinal plant species are however under pressure due to unsustainable resource exploitation and degradation of habitats, while the additional challenges posed by climate change could drive some species to extinction (Cavaliere, 2009; Gairola *et al.,* 2010; Gaikwad *et al.,* 2011; Ray *et al.,* 2011). Similarly, habitat destruction and climate change pose a significant threat to rooibos' future survival. Rooibos tea is a treasured South African commodity, creating many employment opportunities and inextricably intertwined with the heritage and traditions of the local communities. Timely management intervention and adaptation to climate change is therefore of paramount importance to protect wild rooibos populations and sustain commercial production.

CHAPTER 4: PLANT PHYSIOLOGICAL RESPONSES

Physiological responses of a Fynbos legume, *Aspalathus linearis* to increasing aridity.

Abstract *Aspalathus linearis* (rooibos) is a medicinally and economically useful shrub which is endemic to the Mediterranean west coast region of South Africa. Species distribution modelling indicates potentially severe consequences in range shift for rooibos tea under changing climate conditions. However, they are based on several assumptions and simplifications which may compromise future predictions. In an effort to improve the accuracy of the species distribution model, results obtained from the modelling were used to further investigate the species' climatic limits through experimental manipulation of drought. Using seedlings of *A. linearis*, the effects of drought on plant performance was tested in a glasshouse and field setting by measuring growth, photosynthetic performance and leaf carbon and nitrogen isotope ratios ($\delta 13C$ and $\delta 15N$). In the glasshouse study, rooibos seedlings revealed several adaptation mechanisms (increasing water use efficiency and higher root: shoot ratio) to partially offset drought, albeit with negative effects for plant productivity. In the field trial, plant volume, height and basal diameter were significantly reduced amongst drought stressed seedlings. The significant decline in productivity and subsequent mortality of these seedlings indicate that the plants were not able to offset the effects of the drought through similar adaptation characteristics such as those displayed in the glasshouse trial. Hence, given a drought of sufficient intensity similar to conditions outside the plant's bioclimatic limits and corresponding to future climate scenarios, rooibos will not survive the first critical phases of seedling establishment.

4.1 Introduction

Mediterranean-type ecosystems are expected to be particularly vulnerable to climate change as they are already under immense pressure due to widespread land transformation and associated habitat fragmentation (Peñuelas *et al.,* 2004, Christensen *et al.,* 2007). The Fynbos biome is a Mediterranean-type ecosystem which is of particular concern owing to its high levels of endemic and rare species. Global climate models predict higher temperatures, lower rainfall, and greater potential evapotranspiration, as well as greater frequency of extreme weather conditions in this eco-region. Depending on the specific future climate scenario, species distribution models (SDM's) suggest that the Fynbos biome could experience a significant loss of habitat along its north-eastern distribution limits (DEA, 2013) which would impact on the bioclimatically suitable range of several species.

One such species is the endemic leguminous shrub *Aspalathus linearis* (rooibos tea) which has a particularly restricted natural distribution in the Cederberg mountains of the Western Cape in South Africa with isolated populations to the south-west. Rooibos grows in sandstone derived soils characterized by high acidity and poor nutrient availability. As medicinally and economically useful shrub, the rooibos plant contributes to the well-being and cultural heritage of local communities and is currently the most important commercially cultivated indigenous crop in South Africa (Hawkins *et al.,* 2011, Van der Bank *et al.,* 1995). Estimates of the potential impact of climate change on rooibos tea using a spatial distribution model (MaxEnt) predict that wild rooibos tea may lose up to 88.7% and cultivated tea 75.5% of their climatically suitable habitat range by 2070 (Lötter *et al.,* 2014). The rooibos bioclimatic model predicted the climatic range of cultivated rooibos tea to be particularly limited by total winter rainfall (below 115 mm), while winter temperature (below 2˚C and above 7˚C) and summer temperature (above 32˚C) also constrains the climatically suitable area for crop production.

Bio-climatic models give an approximation of the suitability for the species as a function of the values of the climatic and environmental variables where it is known to occur. This yields the climatic range limits of the species' distribution and allows the model to forecast geographic range shifts due to climate change. However, the bio-climatic modelling is based on several assumptions and simplifications which may compromise future predictions (Heikkinen *et al.,* 2006). Plants may make certain physiological adjustments in response to water limitation through a decrease in transpiration and increase in water use efficiency to compensate the effects of drier conditions. Therefore these models are useful as first-cut assessments of species distribution, but require further testing to provide more robust predictions.

Combining modelling with manipulative field or glasshouse studies could greatly improve confidence in assessing SDM predictions under climate change and provide more robust predictions for management. However, very few studies have applied this combined approach to evaluate predictions from species distribution studies. Pattison & Mack (2008) validated their predictions from a CLIMEX model with field trials, testing seed germination and plant growth rates within and beyond the current range of *Triadica sebifera*. West *et al.,* (2012) performed a multi-year experimental drought manipulation in a mountain Fynbos shrubland to improve the categorization of plant functional responses to drought to make more accurate predictions regarding plant response to future climate change. Both of these papers highlight the need to provide eco-physiological and experimental confirmation of species distribution models.

To date, little is known about the growth, nutritional and photosynthetic response of *Aspalathus linearis* to increased aridity. Several plant productivity and physiological responses of other legumes to drought and or heat stress have been studied, although different species have different traits and adaptation mechanisms to cope with adverse environmental conditions (Karatassiou *et al.,* 2009). In this study, field and glasshouse trials were carried out to further explore prospects of rooibos tea under climate change. The specific aim of this study was to investigate the nutritional and photosynthetic performance of *A. linearis* during drought and heat stress and to establish if survival and growth of *A. linearis* correspond to limiting factors predicted by SDM's. These results will enable us to make better predictions of potential consequences for plant growth and productivity under climate change and improve the accuracy of correlative distribution models.

4.2 Materials and methods

4.2.1 Field study

Site and plant material description

The study was carried out on farmland (Skimmelberg) in one of the main rooibos growing areas of the greater Cederberg region between Clanwilliam and Citrusdal (32.35˚S, 18.79˚E, 515 m.a.s.l.). The site is located on organically certified tea lands which have previously been prepared for cultivation. The soil is typical quartzitic sandstones of the Table Mountain Group which are well leached nutrient-poor and acidic soils. The long term mean annual precipitation is 512 mm, of which 60.5 % occurs from June to September. The temperature range is typical of a Mediterranean climate with a mean maximum temperature of 35˚C in February, and a mean minimum temperature of 3.5˚C in July. Five months old healthy *A.linearis* seedlings of uniform size according to plant height, basal stem diameter, and root length were obtained from seedbeds on the local farm and transplanted into the experimental tea land. The transplanted seedlings were allowed sufficient time to become established before the treatment commenced during July 2012.

Experimental design

In the study site, ten 4m × 4m plots were delimited on a prepared rooibos tea field and enclosed by a fence to prevent vertebrate browsing. Five plots which were randomly selected received the drought treatment, while the remaining five plots did not receive any treatment and were considered control plots. Each plot consisted of nine seedlings which were regularly spaced at 50cm from each other. Fixed-location rainout shelters according to the design of Yahdjian and Sala (2002) were erected over each of the experimental plots. To avoid the effect of micro-climatic and other disturbance-related factors, 0.5 m around the perimeter of the plot was considered to represent edge effects and excluded. The shelter consisted of a metal frame with ten clear polycarbonate roof panels at a height of 0.6–1.2 m above the soil. These bands were spaced at a distance of 20cm from each other to intercept 55% of incoming precipitation. This simulates a precipitation regime at the lower limit of the species predicted suitability. A gutter on the lower side of the roof channelled the intercepted water into a pipe which carried the water off site. The rain exclusion treatment started in July 2012 and lasted until March 2013. Gravimetric soil water content was measured each month throughout the experiment at a depth of 20cm and 40cm. After collection, soil samples were immediately weighed. Soil samples were then dried at 60°C for 48 h and reweighed. Gravimetric soil moisture content was calculated as (fresh weight – dry weight)/dry weight. Meteorological data was obtained from the nearest weather station as well as from records kept by the local land owner.

Seedling monitoring

Seedling survival and growth were monitored from July 2012 until March 2013. Canopy spread (plant height, length and breadth) and basal diameters were recorded four times: on July 20 2012, October 15 2012, December 15 2012 and Feb 15, 2013, respectively to monitor plant growth and changes in plant volume. Volume was calculated as for an ellipsoid spheroid (Phillips and McMahon, 1981):

$$V = \frac{\pi a^2 \times b}{6} \tag{1}$$

Where:

V = volume

a = the average of length and breadth, or height, whichever is shorter

b = the average of length and breadth, or height, whichever is the longer

Basal diameters were measured with a digital calliper to the nearest 0.01mm. To allow re-measuring a line was painted on the stem in order to measure the exact point on the stem later.

At the end of the experiment, some of the surviving seedlings were collected and then separated into root, stem and leaves in the laboratory, where all parts were oven-dried at 80˚C for 48 h and weighed.

Isotope and elemental analysis

For both the field and glasshouse trials the C and N stable isotope analyses were carried out at the Archaeology Department, University of Cape Town. In the field trial five *A. linearis* plants per plot were sampled at the end of the experiment in 2013 by using a 20cm portion of disease-free and undamaged branch cut off the top of each shrub and placed in paper bags. Plant material was clearly marked and dried in an oven at 60˚C for 72h. After drying, the plant material was finely ground for C and N stable isotope analysis. Values were expressed relative to the Pee-Dee Belemnite (PDB) standard for δ^{13}C and relative to atmospheric N for δ^{15}N, as (%), according to the following equation:

$$\delta Z = (R_{sample}/R_{standard} - 1) \text{ x } 1000 \qquad (2)$$

where Z is the heavy isotope of either N or C, and R is the ratio of heavier to lighter isotope for the sample and standard (^{13}C/^{12}C or ^{15}N/^{14}N).

The oven-dried plant components were milled in a Wiley mill where after samples were combusted in a Flash 2000 organic elemental analyzer. Three in-house standards (Merck Gel, Lentil, Acacia saligna) were used and calibrated against International Atomic Energy Agency (IAEA) standards. N is expressed in terms of its value relative to atmospheric N, while C is expressed in terms of its value relative to Pee-Dee Belemnite.

Integrated water use efficiency (WUEi) was derived from the δ^{13}C values and describes leaf WUE (based on stomatal behaviour) over the whole growing season and can be estimated using C isotope discrimination, whereby plants discriminate against ^{13}C during photosynthesis.

Effect size

After West *et al.,* (2012) the significance of differences between treatment and control measurements (for soil moisture, growth and mortality), data were plotted as effect size (ES):

$$\mathrm{ES} = \frac{M_{\mathrm{T}} - M_{\mathrm{C}}}{\sqrt{\varepsilon_{\mathrm{T}}^{2} + \varepsilon_{\mathrm{C}}^{2}}} \qquad (3)$$

(M_T and M_C represent the mean values for treatment and control plots, respectively, and εT and εC are the standard errors of the mean for treatment and control plots, respectively.) Thus, ES is in units of standard errors. We considered times where $-2 > ES > 2$ to represent significant differences between treatment and control.

For leaf stable isotopes and biomass partitioning, the significance of differences between treatment and control measurements were tested with an analysis of variance (ANOVA, using Kaleidagraph for Macintosh). The means (4-5) were subsequently separated using the post-hoc t-test ($P \leq 0.05$).

4.2.2 Glasshouse study

Plant growth conditions

One hundred *Aspalathus linearis* (Burm. f.) R. Dahlgren (rooibos) seeds were treated with Sulphuric Acid (H_2SO_4, 95%-99% concentration) for 15 minutes and rinsed in distilled water to increase the permeability of the seed coat. The seeds were left overnight in smoke primer before being germinated in seedling trays containing soils collected from the root regions of *A. linearis* plants growing naturally in the Cederberg Mountain region. Germination took place during December and January in an east-facing glasshouse at the University of Stellenbosch, South Africa. The range of midday irradiances was between 630 and 680 mol m^{-2} s^{-1} and the average day/night temperature and humidity were 23/15°C and 35/75%, respectively. Seedling trays were watered daily with distilled water until seeds germinated. Upon germination seedlings were watered once every 2 d for 3–4 weeks, until seedlings became infected with the indigenous rhizobia and nodule formation had occurred. Once nodule formation was established, the seedlings were transferred to 20 cm diameter pots containing 3 kg of quartz sand under the same glasshouse conditions. All the rooibos plants received 100 ml once a week of a low nutrient solution (25% strength Long Ashton Nutrient Solution, modified to contain 200 μM N and 150 μM P). The rooibos plants were grown for 16 weeks following transplantation, after which a six week period of drought stress was induced. Prior to commencing the drought treatment, the soil in all the pots was flushed with distilled water. Subsequently, drought was induced by withholding water from the plants, while the control plants received 100 ml of distilled water once per week. This was done to ensure that the nutrient supply remained the same, and that only the H_2O supply was varied during the drought period.

Photosynthesis

Five replicates of each treatment were used for the photosynthetic determinations. Groups of leaves of each plant was used to determine the photosynthetic rate, stomatal conductance and transpiration rate at midday, using a portable infrared gas analyser (LiCor, Lambda Instruments Corporation, USA). Light-response curves were derived using the facility on the infrared gas analyser, by manually adjusting the irradiance level in the leaf chamber. Photosynthetic readings were taken at 12 irradiance intervals (PAR 0– 2000 μmol $m^{-2}s^{-1}$) with 5-20 min at each irradiance level before gas exchange was recorded. To assess heat effects, the leave temperature of drought stressed and control plants were increased to 32˚C by using the internal heater of the photosynthesis system. All measurements were the average of 10 logs.

The leaves that were used for photosynthetic measurements were removed and oven dried to calculate their respective weights. All photosynthetic values were adjusted and expressed on a leaf dry mass (g) basis. The light response curves were used to derive the light-saturated rate of photosynthesis (Pmax), quantum yield (φ), light compensation point (LCP) and dark respiration. Photosynthetic water-use efficiency (PWUE) was calculated from measurements of Pmax and transpiration rate.

Plant harvest

The plants were divided into root, leaf and stem components upon harvest. The harvested material was then placed in a drying oven at 60˚C for 72h and dry weights were recorded. The dry weights of the respective components were expressed in terms of plant biomass gain over the experimental period. The relative growth rate (whole plant), shoot growth rate, root growth rate and root:shoot ratio of the control and drought stressed plants were determined from the baseline plants.

Statistical analysis

The effects of drought and temperature on the plant physiological functions were tested with an analysis of variance (ANOVA, using Kaleidagraph for Macintosh). The means (4-5) were subsequently separated using the post-hoc t-test ($P \leq 0.05$). Different letters indicate significant differences between treatments.

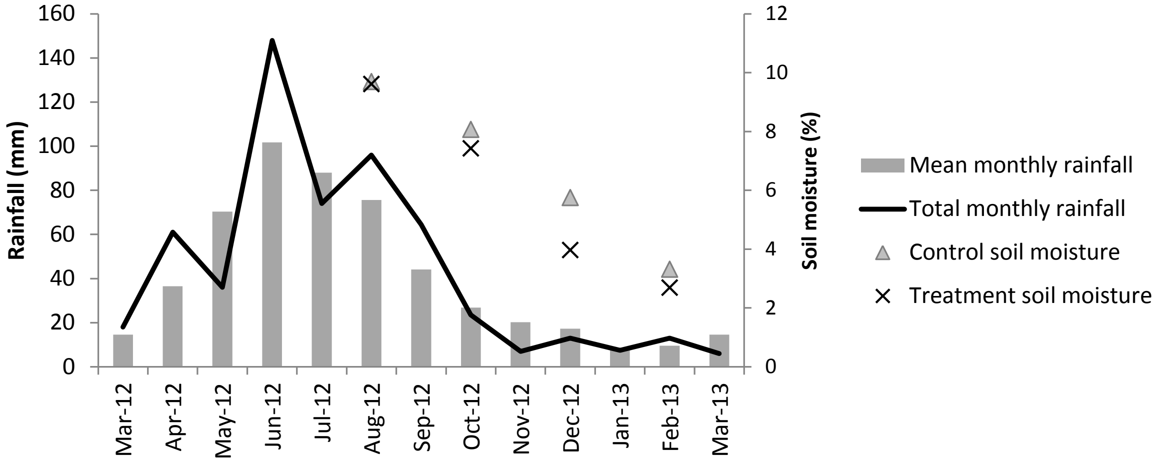
4.3 Results

4.3.1 Field study

Environmental conditions

The seasonal rainfall totals during the study period were slightly above the long term average values for the study area. Winter rainfall (June to August) of 318 mm was followed by 95 mm during spring (September to November) and 33.5 mm through summer (December to February) (Fig. 4.1). Temperatures were typical of the region with summer temperatures occasionally greater than 40 ˚C. Soil moisture content for both sites (treatment and control) started to decrease in October, and was further reduced as the drought period progressed/intensified during summer, reaching values in February of 2.68% at 20 cm (Fig. 4.1). The rain exclusion treatment induced significant differences in soil moisture content between sites from October onwards.

Figure 4.1 Rainfall and soil moisture dynamics during the course of the rain exclusion treatment (excluding 55% of incoming precipitation).

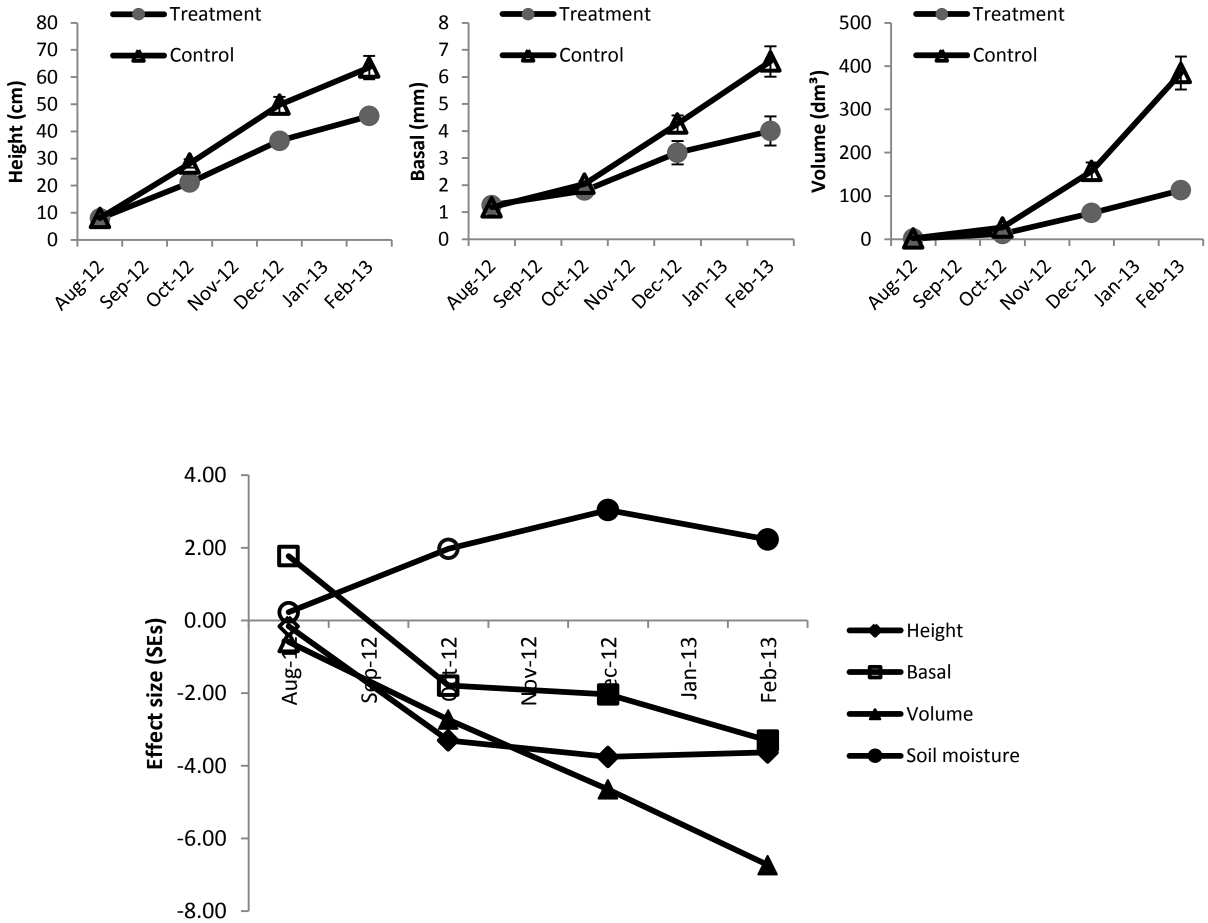


Physical growth parameters and mortality

Differences in mean plant height, volume and basal diameter were not statistically significant at the start of the study between the control and rain exclusion treatment. The individual heights of cultivated plants ranged between 5.5 cm and 13.5 cm, while volume was on average 1.68 dm^3 and basal diameter 1.26 cm respectively (Fig. 4.2). However, from October onwards, basal diameter, height and volume showed significant responses to the rain exclusion treatment. The more the drought stress increased, the slower the three parameters would increase. After 6 months, the volume of plants in the rain exclusion treatment was 3.4

times less than that of the plants in the control site. Plant height was significantly influenced by the drought treatment being 1.4 times smaller compared to the control plants. The rain exclusion treatment reduced basal diameter in seedlings by 2.57 dm^3. In addition, the experimental drought had a considerable impact on mortality. During the treatment, mortality amongst the droughted *A. linearis* seedlings was 25, while little mortality (9 plants) occurred amongst the control plants.

Figure 4.2 Plant growth responses to experimental drought (top panel) and treatment effect (bottom panel) on rooibos seedlings over the course of the study in the greater Cederberg region, South Africa. Differences < ± 2 SEs from 0 are regarded as insignificant (open symbols). Differences > ± 2 SEs from 0 are regarded as significant (closed symbols).

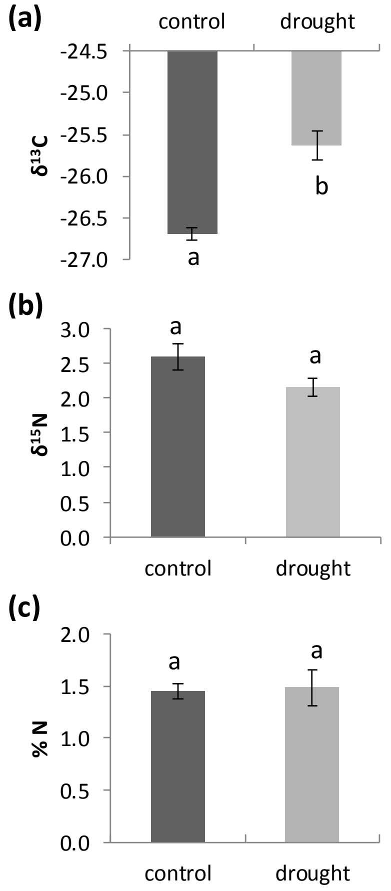


There were substantial differences in $\delta^{13}C$ values between treatment and control plants. On average drought treated tea plants had 1.04 times lower $\delta^{13}C$ values than control plants (Fig. 4.3A). There was however no difference in the ability of control plants and plants receiving the rain out treatment to fix atmospheric N via biological N_2 fixation (BNF) (Fig. 4.3B). Average foliar $\delta^{15}N$ values for the control plants were 2.15 as opposed to the 2.60 for the plants receiving the treatment. There was a significant inhibitory effect of drought on overall plant growth (Table 4.1). During the six month period of drought stress both above ground and below ground biomass showed a significant decline compared to the control plants. There was however no significant difference in the root:shoot ratio between treatment and control plants.

Table 4.1 Biomass (g) of A. linearis after 6 months in control and drought plots. Values represent the means (n=5) with standard error bars. The different letters next to the means, indicate significant differences among the treatments, ($P \leq 0.05$).

Parameters	**Control**		**Drought stressed**	
Biomass				
Shoot (g)	78.9 a	± 7.6	29.2 b	± 2.1
Nodulated roots (g)	32.3 a	± 4.9	15.8 b	± 0.9
Plant (g)	111.1 a	± 3.6	45.0 b	± 2.7
Root/shoot ratio	0.41 a	± 0.1	0.54 a	± 0
Roots from total (%)	29.3 a	± 5.1	35.2 a	± 1.5
Shoots from total (%)	70.7 a	± 5.1	64.8 a	± 1.5

Figure 4.3 Isotopic ratio of $\delta^{13}C$ **(a)**, isotopic ratio of $\delta^{15}N$ **(b)**, and C:N ratio **(c)**. Error bars represent the standard error of the mean (n = 5). Different letters indicate significant differences among the treatments, ($P \leq 0.05$).



4.3.2 Glasshouse study

Photosynthesis

A. linearis showed a significant decrease in the maximum photosynthetic rate due to drought imposition, while increasing temperature produced an increase in Pmax (Fig. 4.4A). The combined factors (temperature and water stress) produced a decrease of almost 84% with respect to the control treatment. Changes in light saturated photosynthesis rates were mostly coupled with a concomitant increase or decline in stomatal conductance except for the drought treatment where there was a disproportionate decline in Gs (53%) compared to the 35% decline in Pmax (Fig. 4.4B). The drought treatment alone and in combination with elevated temperatures produced significant lower transpiration rates (Fig. 4.4C). By contrast, as the temperature increased (32˚ C) the transpiration rates increased considerably due to greater stomatal opening with a simultaneous increase in water loss. Increased temperature therefore, produced a decline in WUE, while the combination of drought and temperature also had a significant inhibitory effect on WUE. Conversely, drought stressed plants exhibited an increase of 37.4% in photosynthetic water-use efficiency compared to the control (Fig. 4.4D). Furthermore drought and the combination of factors (drought and increasing temperature) affected the mechanism of photosynthesis by decreasing the quantum yield (Fig. 4.5A). The light compensation point of drought stressed plants was somewhat lower than the values for control plants, although not statistical significant (Fig. 4.5B). At high temperatures, water stress also caused a displacement of the light compensation point to a higher value (Fig. 4.5B). The efficiency of energy storage was however less affected by temperature alone. Opposite changes in leaf dark respiration rates were observed for the different treatments. Respiration rates exhibited a decrease (less negative net CO_2 exchange) under drought stress while it increased with both temperature and the combination thereof with drought (Fig. 4.5C).

Figure 4.4 Maximum photosynthetic rate (Pmax) **(a)**, stomatal conductance (g_S) **(b)**, transpiration rate (E) **(c)**, and photosynthetic water-use efficiency (WUE) **(d)** of 6 month old *Aspalathus linearis* plants, cultivated under glasshouse conditions. Plants were grown under well-watered conditions for 4 months, and thereafter the drought treatment was induced for 6 weeks. Temperature effects were measured alone and in combination with drought. Values represent the means (n=5) with standard error bars. The different letters on top of the means, indicate significant differences among the treatments, ($P \leq 0.05$).

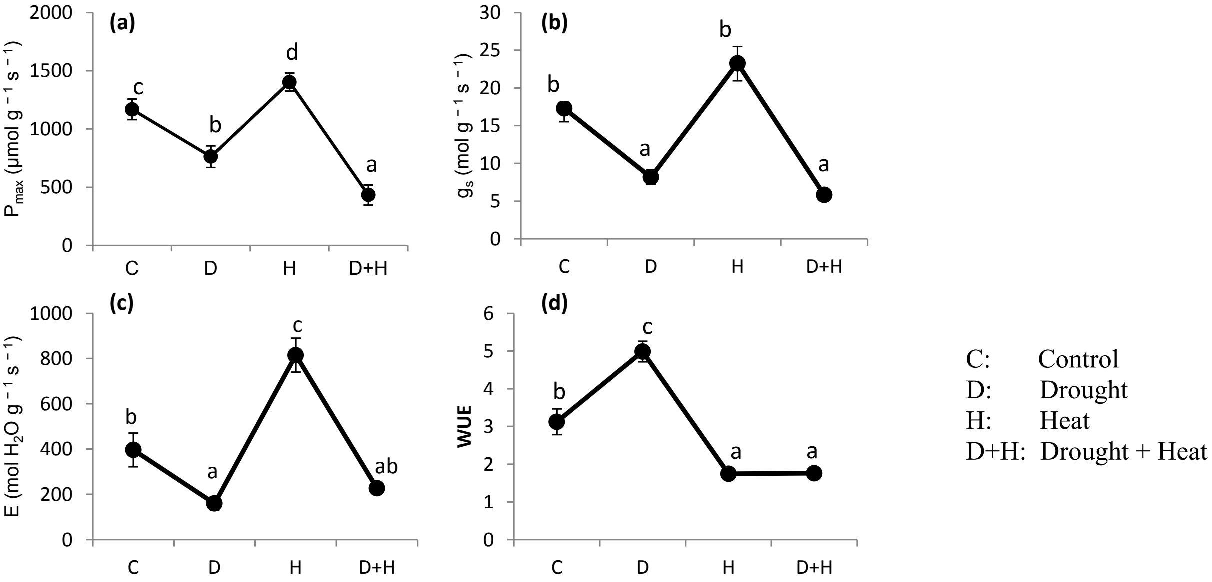
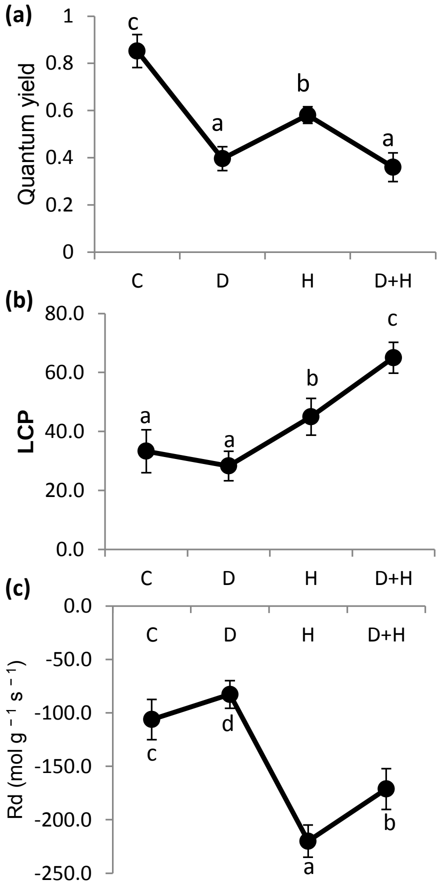


Figure 4.5 The quantum yield **(a)**, light compentation point (LCP) **(b)**, and dark respiration rate (Rd) **(c)**, of 6 month old *Aspalathus linearis* plants, cultivated under glasshouse conditions. Plants were grown under well-watered conditions for 4 months, and thereafter the drought treatment was induced for 6 weeks. Temperature effects were measured alone and in combination with drought. Values represent the means (n=5) with standard error bars. The different letters on top of the means, indicate significant differences among the treatments, (P ≤ 0.05).



C: Control
D: Drought
H: Heat
D+H: Drought + Heat

Biomass

There was a significant inhibitory effect of drought on overall plant growth (Table 4.2). During the six week period of drought stress the relative growth rate declined by 50 %. However, an adaptation of the drought period was the changes of biomass partition in plant organs. While root growth was not significantly affected by drought, there was a major decline in shoot production as seen in the higher root:shoot ratio.

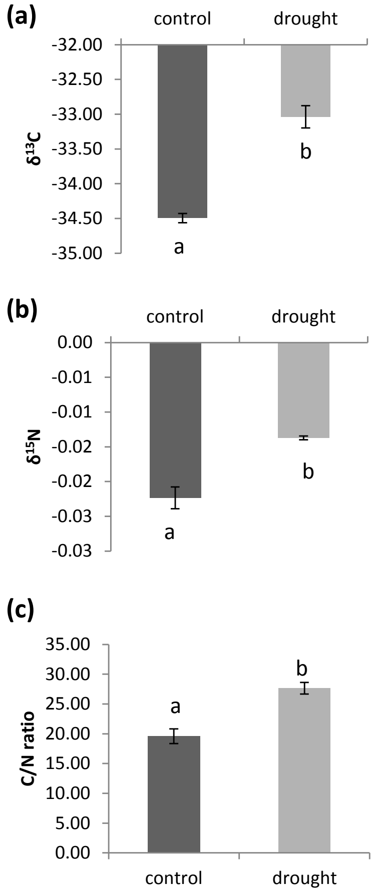
Table 4.2 Biomass of 6 month old Aspalathus linearis plants, cultivated under glasshouse conditions. Plants were grown under well-watered conditions for 4 months, and thereafter the drought treatment was induced for 6 weeks. Values represent the means (n=5) with standard error bars. The different letters next to the means, indicate significant differences among the treatments, ($P \leq 0.05$).

Parameters	**Control**		**Drought stressed**	
Biomass				
Shoot (g)	0.069 a	± 0.002	0.046 a	± 0.003
Nodulated roots (g)	0.033 a	± 0.002	0.035 b	± 0.004
Plant (g)	0.102 a	± 0.004	0.081 b	± 0.007
Root/shoot ratio	0.474 a	± 0.017	0.753 b	± 0.049
Roots from total (%)	32.12 a	± 0.772	42.85 b	± 1.65
Shoots from total (%)	67.88 a	± 0.772	57.15 b	± 1.65
Relative growth rate (mg/g/day)				
Plant	32.65 a	± 2.99	16.50 b	± 2.31
Root	12.15 a	± 1.482	13.67 a	± 2.14
Shoot	20.50 a	± 1.575	2.83 b	± 0.16

Isotopes

The $\delta^{13}C$ isotope discrimination indicates that the drought treatment had a significant effect on stomatal closure (Fig. 4.6A). These less negative values suggest that the plants were experiencing water stress with accompanying stomatal closure (Brugnoli and Lauteri, 1991). Further effects of the drought on nutrition, is the decrease in N nutrition via BNF, compared to the control (Fig. 4.6B). In addition, because of the sharp increases in C concentration and slight changes in N concentration, the C/N ratio in the drought treatment increased significantly (Fig. 4.6C).

Figure 4.6 Isotopic ratio of δ^{13}C **(a)**, isotopic ratio of δ^{15}N **(b)**, and C:N ratio **(c)**. Error bars represent the standard error of the mean (n = 5). Different letters indicate significant differences among the treatments, ($P \leq 0.05$).



4.4 Discussion

4.4.1 What is the effect of increased aridity on nutritional and photosynthetic performance of A. linearis?

The drought-induced reduction in maximum photosynthetic rate, stomatal conductance and transpiration is congruent with previous findings that drought-induced closure of stomata can lead to a reduction in photosynthesis (Cornic, 2000, Flexas and Medrano, 2002, Benešová *et al.,* 2012). When plants are drought stressed stomata close progressively, decreasing stomatal conductance and hence resulting in reduced rates of photosynthesis and transpiration. However, there may have been an imbalance in the relative percentage of reductions in stomatal conductance (61% decline) and photosynthesis (40% decline), which may have caused the increased water use efficiency (WUE) under drought stress. The adaptive value of increasing WUE is that more CO_2 can be gained per unit of water lost via transpiration. The comparatively larger reduction in photosynthesis compared to leaf respiration under water stress concurs with Ribas-Carbo *et al.,* (2005) where changes in respiration were less pronounced than those in photosynthesis, resulting in a decreased photosynthesis-to-respiration ratio. The benefit of a reduction in leaf respiration may be to reduce the energy burden on drought stressed plants as photosynthesis provides the substrate for respiration (Cannell and Thornley, 2000). It is known that plant C balance for plant growth is greatly influenced by respiratory losses of photosynthetically fixed CO_2 (Poorter *et al.,* 1990, Atkin *et al.,* 1996, Loveys *et al.,* 2002) and that most of the CO_2 respiration occurs in plant leaf material (Atkin *et al.,* 2007). Therefore, the ability to reduce respiratory C-costs during drought stress confers a distinct advantage to plants for improved growth and production. Moreover, Grubb (1998) suggested that the decline in CO_2 respiration rates could be an adaptive strategy typical of sclerophylls in arid regions, in order to cope with and survive through dry periods.

Furthermore, the effects of drought on the mechanisms of photosynthesis, as evidenced by the reduction in quantum yield, indicates that there was a decrease in the photosynthetic efficiency of the light reaction, because fewer CO_2 molecules were fixed per unit photon of light (Cornic and Massacci, 1996). This decline may be associated with less CO_2 being assimilated under drought stress, as evidenced by the 61% lower stomatal conductance in drought stressed plants. This is further supported by the $\delta^{13}C$ findings of smaller stomatal apertures following imposition of water stress.

While the drought treatment induced several inhibitory effects on plant performance, the increase in temperature under well watered conditions had a synergistic effect on plant performance. More specifically, elevated temperature (32 ˚C) stimulated carbon fixation and associated stomatal conductance and transpiration. The increase of transpirational activity caused an increase in water loss relative to the photosynthetic carbon gain, thereby reducing water use efficiency. However, the combined effects of drought and temperature were additive and resulted in the suppression of photosynthesis well below the values for drought alone. Water deficit caused stomatal closure which in turn, limits transpirational cooling and intensifies the effects of already high temperature.

The significant decline in total plant biomass during drought stress was underpinned by reduced shoot growth in favour of roots. This is consistent with observations in previous studies of plant responses to drought stress where above-ground biomass was compromised by water stress conditions to maintain root growth and increase the R/S ratio (Liu and Stützel, 2004, Vicente, *et al.,* 2012, Yang *et al.,* 2012). This may be a morphological adaptive response to drought (Lei *et al.,* 2006) whereby the proportional increase in water absorbing biomass facilitates the acquisition of water and nutrients in deeper more humid soil layers.

The increase in $\delta^{13}C$ (higher ^{13}C /^{12}C ratio = less negative) in seedlings of water stressed rooibos indicates that water stress altered the source of carbon fixation. The altered ^{13}C to ^{12}C ratio could be due to stomatal closure under drought, resulting in an increase of ^{13}C fixation, leading to less ^{13}C discrimination (Fotelli *et al.,* 2003, Kume *et al.,* 2003). As reported in the literature higher δ^{13}C values are associated with higher intrinsic WUEs (Farquhar *et al.,* 1989) when water availability decreases. This is in agreement with a study by Bellaloui (2011) where water stress caused significant changes in the ^{13}C to ^{12}C ratio of soybeans as an N_2-fixing plant. Drought-induced stomatal closure, evidenced by δ^{13}C values indicates more long-term stress and concurs with the short-term reduction in stomatal conductance (Gs) during drought stress. The relatively greater decrease in stomatal conductance than in photosynthetic rates produces an increase in instantaneous WUE (Peñuelas *et al.,* 2000).

The reduction in N nutrition due to lower BNF during drought indicates a reliance of the N2-fixing bacteria on soil water and healthy plant water relations (Arrese-Igor *et al.,* 2011). Both the infection of legumes by rhizobia and nodule functioning have been found to be highly sensitive to soil water deficiency (Sprent *et al.,* 1988; Zahran, 1999; Vicente *et al.,* 2012). However, since nodule initiation and development in the experimental plants was already well established before the drought treatment commenced, the negative effect of drought on BNF could rather have been related to direct effects on nodule activity and functioning. The drought-induced increase in the C:N ratio is a known phenomenon in nutrient-poor ecosystems during water stress (Groom and Lamont, 1999) where sclerophyly usually increases when the environment evolves towards drier conditions (Sardans *et al.,* 2008). According to Chaves *et al.,* (2002) sclerophylly is a known protective measure for an evergreen plant in a Mediterranean environment to survive extreme climate conditions.

4.4.2 Does survival and growth correspond with predictions from species distribution models?

Field study results largely support SDM projections for rooibos tea. Reduced rainfall similar to that experienced outside the species' predicted range changed seedling performance and biomass accumulation, indicated by decreased plant height and volume. In contrast to what was observed in the glasshouse experiment, both root and shoot biomass decreased significantly. This may be ascribed to the duration and intensity of the drought that the plants experienced since they were not able to improve water use efficiency by increasing the proportion of water-absorbing root biomass relative to aboveground biomass. Based on the $\delta^{13}C$ values, field grown plants were significantly more water stressed than plants propagated in the glasshouse. The $\delta^{13}C$ values coincide with values obtained from cultivated adult rooibos plants during the dry summer season when drought is most intense (Lötter *et al.,* 2014). Contrary to the inhibitory effect of drought on BNF in the glasshouse, the absence of a significant difference in N fixation for the field plants may be attributed to a higher availability of mineral soil N. Since legume plants have the ability to obtain their N from BNF and from available N sources in the soil, symbiotic N_2 fixation takes place against a changing background of mineral N availability (Zahran, 1999). Hence both control and drought stressed plants were less dependent on biological N fixation for their nutritional requirements.

In conclusion, a mild short term drought (glasshouse trial) inhibited plant performance in *A. linearis,* most notably through significant decreases in net photosynthesis, which resulted in a decline in particularly above ground biomass accumulation and nutrient assimilation. Since the leaves and twigs of the plant comprise the harvestable component, a greater investment in the root system is therefore expected to reduce productivity of commercially cultivated tea, especially in areas where the crop is at or near its climatic limits. However, by increasing

WUE, developing a higher level of sclerophylly and altering the allocation of photoassimilates the plants were able to partially offset the effects of drier conditions. This ability of the species to adapt to the negative effects of water deficit stress may enable seedlings to survive and persist, albeit with reduced biomass. Yet, given a drought of sufficient intensity similar to conditions where the crop is outside its climatic limits as indicated by the rooibos bioclimatic model, *A. linearis* species are likely to suffer hydraulic failure and mortality.

CHAPTER 5: DIFFERENCES BETWEEN ROOIBOS TYPES

Seasonal variation in the N nutrition and C assimilation in wild and cultivated *Aspalathus linearis* (Rooibos tea).

Abstract: The Fynbos biome of Southern Africa is a Mediterranean-climate ecosystem with highly infertile soil. It is home to the endemic leguminous shrub *Aspalathus linearis* (rooibos tea) which is both an invaluable wild resource and commercially cultivated plant. Wild rooibos has a narrow geographic range and is confined to mountain ranges of the Cederberg Region. Under projected climate change, warmer and more arid conditions may place additional pressure on these range restricted plants to survive in an already resource limited environment. To understand the adaptive strategies that may allow rooibos to persist in its habitat under future climate change this study evaluated changes in the photosynthetic activity and nutrient cycling of wild and cultivated rooibos, at the temperature and rainfall extremes of summer and winter. Ten mature shrubs (20 shrubs in total) of similar age and size were randomly selected from two sites where wild and cultivated rooibos populations occurred separately. Plants were assessed for leaf gas exchange rates, nutrient cycling and natural leaf abundances of δ15N and δ13C during the late summer months (dry season) as well as during the late winter months (wet season). Wild and cultivated rooibos tea revealed significant differences (between the two types and within seasons) in water use efficiency, biological nitrogen fixation and N reabsorption. In particular, the wild plants were better able to tolerate summer drought by increased water use efficiency and maintaining higher levels of biological nitrogen fixation compared to the cultivated tea.

5.1 Introduction

Aspalathus linearis (Burm. F) Dahlg., Fabaceae is an evergreen leguminous shrub indigenous to the Fynbos biome of the Cape Floristic Region (Dahlgren 1968) and one of South Africa's most successful commercially propagated endemic crops. The Fynbos biome is one of five Mediterranean climate regions of the world which are all recognized as biodiversity hotspots (Cowling *et al.,* 1996). There is a particularly remarkable ecological convergence between two of the five, namely the Cape Floristic Region and the kwongan of Southwest Australia (Cowling *et al.,* 1996; Wisheu *et al.,* 2000). Both Fynbos and kwongan occur on severely nutrient-impoverished soils, display similar fire regimes, a remarkable convergence in growth forms and species diversity at different spatial scales (Cowling *et al.,* 1996; Wisheu *et al.,* 2000). Both areas are particularly species rich with high levels of rarity and endemism where 68–75% of plants are endemic as opposed to 23-50% in other Mediterranean climate regions (Cowling *et al.,* 1996). In addition several of the Fynbos and kwongan species are restricted to extremely small distribution ranges and face similar threats in terms of habitat fragmentation and climate change (Bomhard *et al.,* 2005; Miller *et al.,* 2007; Yates *et al.,* 2010).

The Mediterranean climate is characterized by a strong seasonal pattern of rainfall, with marked aridity during summer months and most of the annual rainfall falling predominantly during the winter period. The water deficit conditions during summer months are generally considered to be the main environmental constraint to plant growth and productivity in Mediterranean type vegetation (Larcher 2000). Low availability of soil nutrients is a further limiting factor in Mediterranean ecosystems (Kruger *et al.,* 1983; Sardans *et al.,* 2006). Fynbos soils are sandstone-derived and characterised by high acidity and poor nutrient availability and are especially deficient in N, P and Ca (Kruger *et al.,* 1983; Moufhe and

Dakora 2000). N and P are known to be limiting nutrients for plant productivity in Mediterranean type ecosystems, while the concentrations thereof in the ecosystem may also change in response to warming and drought (Lloret *et al.,* 1999; Sardans *et al.,* 2008). Plant growth and fitness in such an environment depend largely on a species ability to optimally utilize available resources. Many species possess certain eco-physiological traits which enable them to adapt to the existing environmental constraints. One such important trait is biological nitrogen fixation. Being a legume, rooibos has a proven ability to fix N_2 and is known to be able to fix well over 100 $kgNha^{-1}$ annually (Sprent *et al.,* 2009). According to Lamont (1983) and Mitchell *et al.,* (1986) several plants growing in Mediterranean ecosystems have developed specialised nutrient-uptake and internal nutrient cycling strategies. An important mechanism by which critical nutrients are maintained is via nutrient reabsorption, in which nutrients are mobilized from senescing leaves and transported to other plant tissues (Killingbeck 1996). Another functional strategy to deal with restrictive environments is sclerophylly, which is associated with a high ratio of carbon to nitrogen in a plant's leaves (Rundell 1988). Sclerophylly is a typical characteristic of Fynbos vegetation and it has been suggested to be an adaptation to specific stresses such as seasonal water deficits (Bussotti *et al.,* 1998) and low-nutrient soils (Gutshick 1999). Apart from nutrition, plants may also adapt via photosynthetic and water relations adjustments (Chaves *et al.,* 2002, 2003; Vitale *et al.,* 2012). It has been observed in field grown plants that as summer drought progress, leaf photosynthesis is increasingly limited due to a decline in stomatal conductance (Chaves *et al.,* 2002). This leads to a reduction in water loss and an increase in water use efficiency (the ratio of water loss to biomass gained). Water use efficiency (WUE), defined as the ratio between biomass (usually shoot) production and transpiration (Farquhar *et al.,* 1989) is one measure of the ability of a plant to perform well under incipient drought (Thomas, 1997). Instantaneous water use efficiency (WUEi) through gas exchange measurements relates two physiological parameters: the photosynthetic rate and the transpiration rate (Bacon, 2004). Long term water-use efficiency however describe WUE over the whole growing season and can be estimated using carbon isotope discrimination, whereby plants discriminate against ^{13}C during photosynthesis (Nilsen and Sharifi, 1997). An increase (less negative) in carbon isotope composition ($\delta^{13}C$) correlates with an increase in long-term tissue WUE.

Climate change which is predicted to cause an increase in aridity for the west coast of South Africa by the end of the century (Hewitson and Crane 2006; IPCC 2007; Engelbrecht *et al.,* 2009) may place additional pressure on plants to survive in an already resource limited environment. Previous research (Archer *et al.,* 2008) has suggested that these changing climate conditions might impact severely on rooibos tea. It is therefore essential to understand the eco-physiological behaviour of these plants in relation to current climatic and soil constraints. However, not much is known about the nutritional and water relations physiology of *A. linearis* under drought stress in its natural habitat. Local knowledge suggests that wild rooibos shrubs may have higher drought resistance than the cultivated type (Louw 2006). *A. linearis* is one of the relatively few economically important Fynbos species which has successfully made the transition from wild resource to an agriculturally important plant in the 20th Century. Wild rooibos was historically used by local inhabitants of the Cederberg region to produce tea as well as for its medicinal and health properties (Dahlgren 1968). Based on growth form, several wild ecotypes of *A. linearis* have previously been identified (Malgas *et al.,* 2007). Preliminary genetic testing suggests a high level of genetic diversity within *A. linearis* (Van der Bank *et al.,* 1995, 1999; Malgas *et al.,* 2009). The cultivated type is thought to have originated from a wild type found in the Pakhuis area of the southern Cederberg during the 1930's (Cheney and Scholz 1963; Morton 1983). The entire commercial industry therefore hinge on one *A. linearis* selection made ca. 80 years ago. This selection was based on qualities such as growth rate, seed production and especially taste.

A useful approach to gain information on plant physiological performance is to study seasonal patterns in nutrient cycling and gas exchange (Llorens 2003). Hence, the aim of this study was to determine how the wet and dry seasons affect the photosynthetic gas exchange, nutrient cycling and water use efficiencies of wild and cultivated *A. linearis*. This will allow us to better understand the ability of *A. linearis* to persist in its habitat under future climate change.

5.2 Materials and Methods

5.2.1 Site and plant selection

The study was conducted on communal land outside Heuningvlei (32.2˚S, 19.13˚E, 858 m a.s.l.), which is an outpost of the Moravian church village of Wupperthal in the Cederberg mountains, Western Cape, South Africa (Fig.5.1). The soils in the area comprise mainly quartzitic sandstones of the Table Mountain Group which are well leached nutrient-poor and acidic soils. Cederberg sandstone Fynbos vegetation predominates in this area and contains numerous endemic plant species of which rooibos is one. The study site included a plantation of cultivated tea and a nearby site with wild rooibos (resprouters) growing amongst other Fynbos plants. Wild populations of *A. linearis* have a narrow geographic range within the Fynbos biome and are confined to mountain ranges of the Cedarberg region (Dahlgren 1968). The wild plants occur naturally between 450m and 1050m above sea level, while cultivated rooibos is grown over a wider geographical range than the natural distribution. Cultivated tea is propagated in plantations, although it does not receive any irrigation or chemical fertilizers.

In both sites, there were no fire events during the last 10 years, and plants were not subjected to a harvesting cycle during the two years of this study.

The study site was therefore chosen based on three criteria, 1.) an area where cultivated and a wild rooibos co-occurred closely, 2.) accessibility and 3.) known fire history and harvesting practices. Precipitation and air temperatures during the sampling period were obtained from the nearest weather station Mertenhof (32.14° S, 1919° E) which is 10 km from the study site and meteorological data is reported in Fig. 5.2. Climate conditions were typical for a Mediterranean–type climate: hot and dry summers, relatively cold winters, and rainfall periods concentrated in May until Sept. During the experimental period, summer drought occurred from November to March, when monthly rainfall ranged between 1 and 10 mm while maximum daily temperatures averaged over 30 ºC. Total rainfall during winter (JJA) was 145 mm and minimum temperatures ranged between 3.5 and 4.4 ˚C.

Figure 5.1 Map of the Cederberg region, showing the natural distribution of rooibos and location of the study site. The area is located in the north western part of the Western Cape, South Africa.

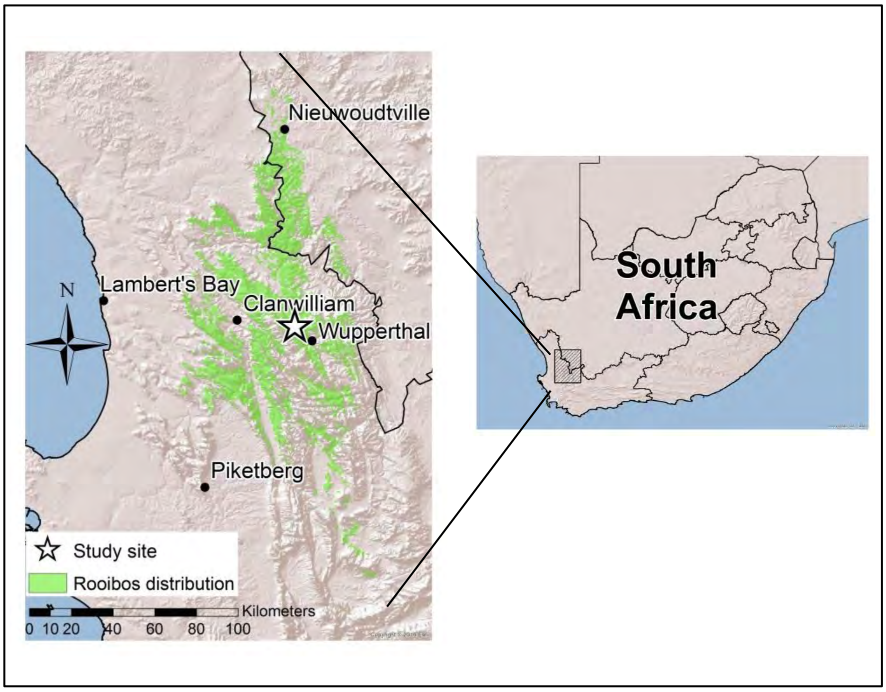
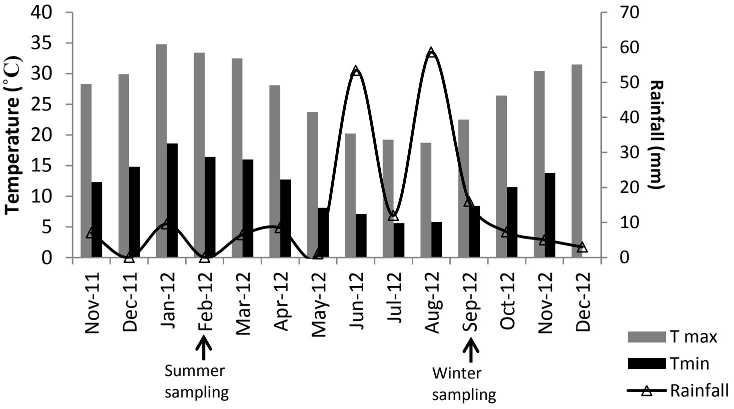


Figure 5.2 Monthly mean maximum and minimum temperatures and monthly precipitation from November 2011 until December 2012 in the study area. Arrows indicate the sampling dates.



5.2.2 Plant and soil sampling

Data were collected from two sites where wild and cultivated *A. linearis* populations occurred separately. The first site included cultivated rooibos tea, where plants were grown in rows, set 1.5 m apart on an area of approximately 50 x 60m. The second site featured wild resprouter rooibos plants growing amongst other Fynbos species, and was located approximately 100 metres from the first site.

Ten mature shrubs of similar age and size were randomly selected in both sites, thus 20 shrubs in total. Sampling was conducted during late summer months (dry season) as well as during the late winter months (wet season). The shrubs were numbered and the same shrubs were sampled each season.

For the plant samples, a 20cm portion of disease-free and undamaged branch was cut off the top of each shrub and placed in paper bags. Recent leaf litter was collected at the base of each selected plant (litter deposited most recently according to the local farmers was taken). Plant material was clearly marked and dried in an oven at 60˚C for 72h. After drying, the plant material was finely ground for C and N stable isotope analysis. For the soil samples, three samples (each 10 cm deep) were taken from underneath each shrub, equally spaced from each other. These three samples were pooled to form one bulk sample for each shrub. Another three soil samples (each 10 cm deep) was taken 1m away from each shrub, equally spaced from each other. The samples were once again combined to form a single sample for each plant. Soil samples were kept at 15°C until analysed.

5.2.3 Calculations of N-reabsorption efficiency, $\delta\,^{15}N$ and $\delta\,^{13}C$

N-reabsorption efficiency was calculated as the difference between maximum N pools in green leaves and N pools in senescent leaves as described by Killingbeck (1996).

$$\frac{X_{Gr} - X_{sen}}{X_{Gr}} \times 100 \tag{1}$$

(X_{Gr}, N concentration of green foliage; X_{Sen}, N concentration of senesced leaves). Calculations were made separately for each plant prior to statistical analysis.

The stable isotope analyses were carried out at the Archaeology Department, University of Cape Town. Values were expressed relative to the Pee-Dee Belemnite (PDB) standard for $\delta^{13}C$ and relative to atmospheric N for $\delta^{15}N$, as (%), according to the following equation:

$$\delta Z=(R_{sample}/R_{standard} - 1) \times 1000 \tag{2}$$

where Z is the heavy isotope of either N or C, and R is the ratio of heavier to lighter isotope for the sample and standard ($^{13}C/^{12}C$ or $^{15}N/^{14}N$).

The oven-dried plant components were milled in a Wiley mill where after samples were combusted in a Flash 2000 organic elemental analyzer. Three in-house standards (Merck Gel, Lentil, Acacia saligna) were used and calibrated against International Atomic Energy Agency

(IAEA) standards. N is expressed in terms of its value relative to atmospheric N, while C is expressed in terms of its value relative to Pee-Dee Belemnite.

5.2.4 Gas-exchange measurements

Maximum photosynthetic rates (Pn), stomatal conductance (Gs) and transpiration rates (E) were measured using a using a LI-6400 portable infrared gas analyzer (Li-Cor, Lincoln, Nebraska, USA). The reference CO_2 concentration was maintained at 400 ppm, flow rate was 500 µmol s^{-1}, while light intensity inside the chamber was set at 1500 µmol photons.m^{-2}.s^{-1} (light-saturated value derived from light saturation curves). Measurements were made during mid-morning on clear, sunny days. The measured leave material was removed, oven dried and weighed to express gas exchange rates on a leaf dry mass (g) basis.

5.2.5 Instantaneous and integrated water-use efficiency

Instantaneous (photosynthetic) water use efficiency (WUE_i) was calculated from gas exchange in order to assess the efficiency of water usage during photosynthesis. Instantaneous water-use efficiency (WUEi) was estimated by dividing the photosynthetic rate (Pn) by the transpiration rate (E), at the same light level and is expressed as net photosynthetic rate/transpiration rate. Integrated water use efficiency describes leaf WUE (based on stomatal behaviour) over the whole growing season and can be estimated using C isotope discrimination, whereby plants discriminate against ^{13}C during photosynthesis.

5.2.6 Statistical analysis

A two-way analysis of variance (ANOVA) with season and type (wild and cultivated rooibos) as fixed factor was performed on gas exchange, stable isotopes composition and N reabsorption to reveal differences in responses within seasons and between types. The normality of data and homogeneity of variance were tested by the Shapiro-Wilk and Levene tests, respectively before ANOVA to ensure the assumptions of ANOVA were met. The different variables and their interactions were tested to detect significant factors (Kaleidagraph, Synergy Software, USA). Where the ANOVA revealed significant differences between treatments, the means ($n = 10$) were separated using a post hoc Tukey's HSD, multiple-range test based on a significance level of $p \leq 0.05$. Different letters indicate significant differences between treatments.

5.3 Results

5.3.1 Plant nutrient and isotope analysis

Leaf N concentrations displayed a marked difference between wild and cultivated rooibos, being significantly lower for wild tea. These differences were observed in both summer and winter. Both wild and cultivated rooibos types displayed the highest leaf N concentrations during winter, with values ranging between 0.91% - 1.53% (Fig. 5.3A).

The nutrient reabsorption of N followed a similar seasonal trend to the foliar N concentration. Absorption of N from senescing material was highest during winter when 32% of cultivated and 23% of wild plant N was reabsorbed (Fig. 5.3B). Cultivated tea displayed a significant higher N reabsorption compared to wild tea in both seasons. The greatest difference between wild and cultivated rooibos was observed during the summer period.

In contrast to the N concentration, the leaf C values were higher in wild tea compared to the cultivated tea, though this was only significant during the winter period (Fig. 5.3C). Seasonally, leaf C content was significant lower during winter compared to summer in cultivated tea. The C to N ratio (C/N ratio) was significantly higher in the wild plants than in cultivated plants. During summer, wild plants showed a comparatively higher (10%) C/N ratio (Fig. 5.3D). The C/N ratio followed a similar trend for both wild and cultivated plants, with the highest values during summer and lowest in winter.

There was a distinct difference in the ability of wild and cultivated rooibos to fix atmospheric N via biological N_2 fixation (BNF). On average, BNF in wild rooibos plants was 2.3 times higher than in the cultivated plants (Fig. 5.4A). Differences between wild and cultivated rooibos were most prominent during summer where BNF in wild tea was approximately nine times higher. However, the lower BNF rates in the cultivated plants were not accompanied by similar decreases in foliar N concentrations. Both types displayed the same seasonal pattern with significantly higher BNF during winter.

Wild and cultivated rooibos revealed substantial differences in $\delta^{13}C$ values, with wild tea being significantly lower. On average wild tea had 1.04 times lower $\delta^{13}C$ values than cultivated tea with the most significant difference observed during summer (Fig. 5.4B). Both wild and cultivated plants displayed the same pattern with the lowest $\delta^{13}C$ values occurring during winter and the maximum in summer.

Figure 5.3 Differences in N concentrations (a), N reabsorption rates (b), C concentrations (c) and C/N ratio (d) in leaves and stems of cultivated and wild *A. linearis* during late summer and winter (mean ±SE). Different letters above bars indicate significant statistical differences between treatments (P<0.05, *post hoc* Tukey's HSD, multiple-range test, ANOVA) (n=10 means).

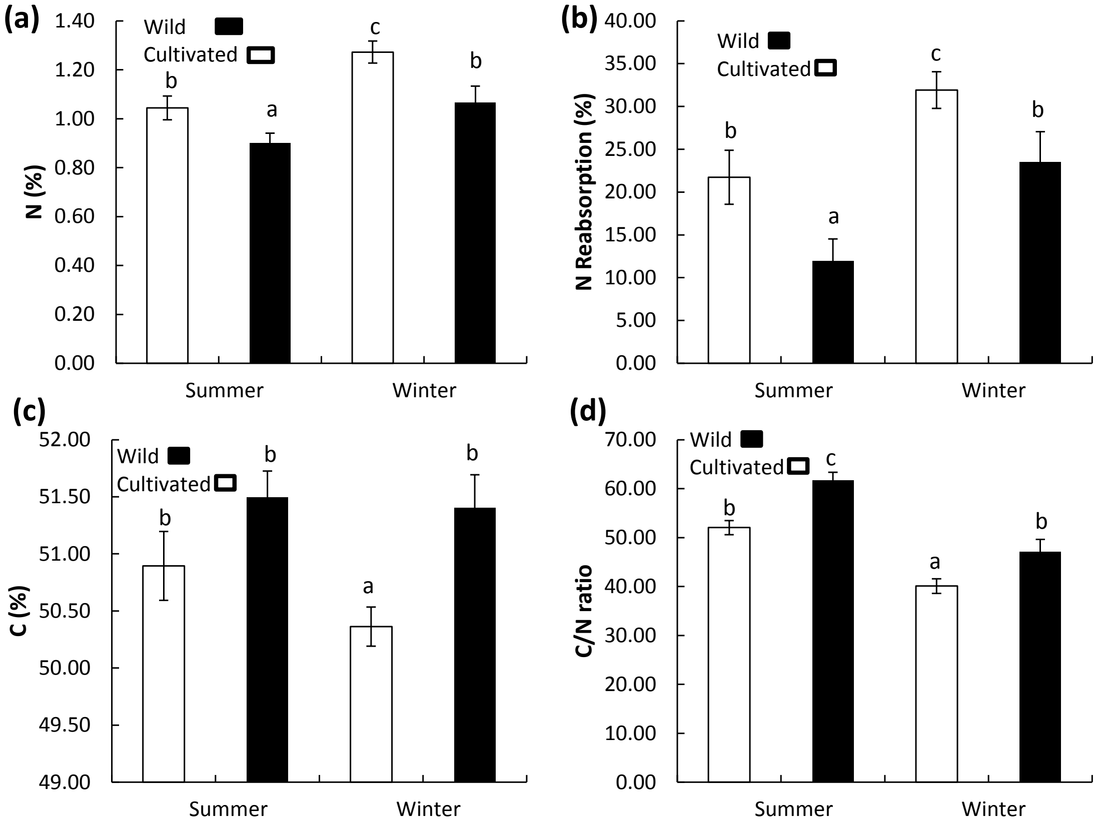
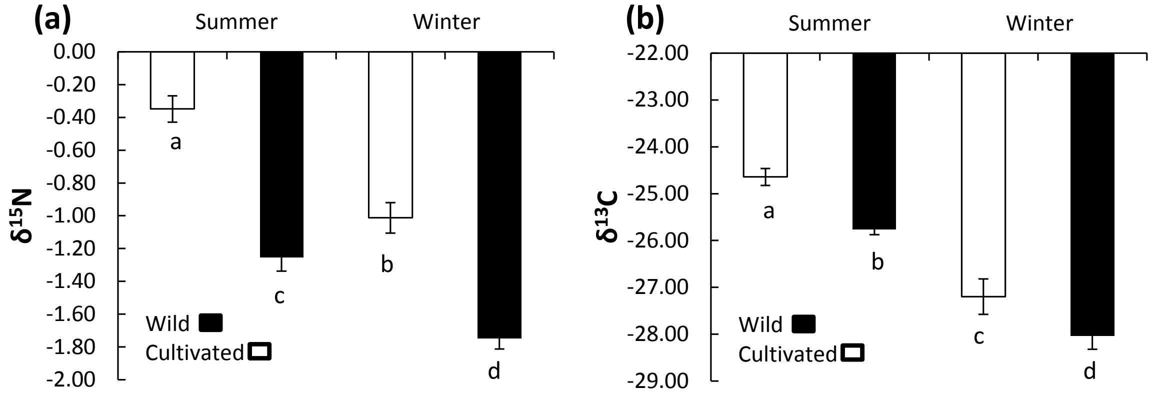


Figure 5.4. Seasonal variation in stable C and N isotopic signatures for cultivated and wild *A. linearis*: (a) $\delta^{15}N$ values and (b) $\delta^{13}C$ during late summer and winter (mean ±SE). Different letters above bars indicate significant statistical differences between treatments (P<0.05, *post hoc* Tukey's HSD, multiple-range test, ANOVA) (n=10 means).



5.3.2 Photosynthesis and water relations

There was a significant difference in photosynthetic rates between seasons and across types (wild and cultivated *A. linearis*). The lowest photosynthetic assimilation rates were recorded during the summer period for both wild and cultivated plants (Fig. 5.5). The wild type however had a higher photosynthetic rate than the cultivated type during the summer period, while this was not the case during the winter. Cultivated tea had higher photosynthetic and transpiration rates in winter. In both wild and cultivated rooibos, transpiration rates were higher in winter compared to summer. Increases in photosynthetic rates were mostly accompanied by concomitantly higher stomatal conductances.

Both wild and cultivated rooibos exhibited lower WUE_i during the wet season (Fig. 5.5D). This coincides with the $\delta^{13}C$ based integrated WUE variation between winter and summer.

There was however no difference in instantaneous WUE between wild and cultivated rooibos during winter, although the integrated WUE did show significantly higher WUE in cultivated plants. During the dry summer period the wild plants revealed significant higher instantaneous WUE.

Soil N data revealed a significant difference in soil N concentration between wild and cultivated sites during winter. Although the cultivated site had higher soil N compared to the wild site, seasonal differences were not significant (Fig. 5.6). Soil N displayed a similar seasonal pattern when compared to plant N, being highest during late winter. Soil P was significantly higher at the cultivated site. Soil C however, was significantly higher at the wild site compared to the cultivated site during both seasons. There were no significant differences between seasons for either soil C or P for the respective sites.

Figure 5.5 Differences in photosynthetic rate (Pmax) (a), transpiration (E) (b), stomatal conductance (Gs) (c) and water use efficiency (WUE) (d) in cultivated and wild *A. linearis* during late summer and winter (mean ±SE). Different letters above bars indicate significant statistical differences between treatments ($P<0.05$, *post hoc* Tukey's HSD, multiple-range test, ANOVA) (n=6 means).

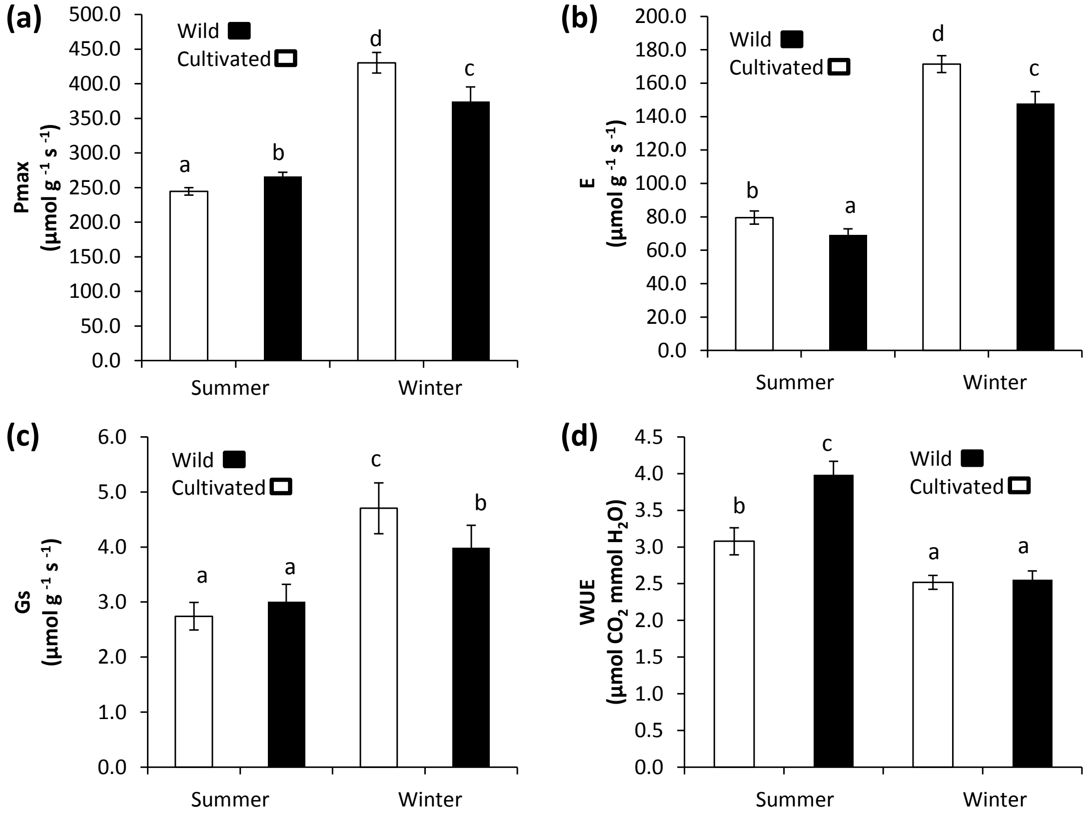
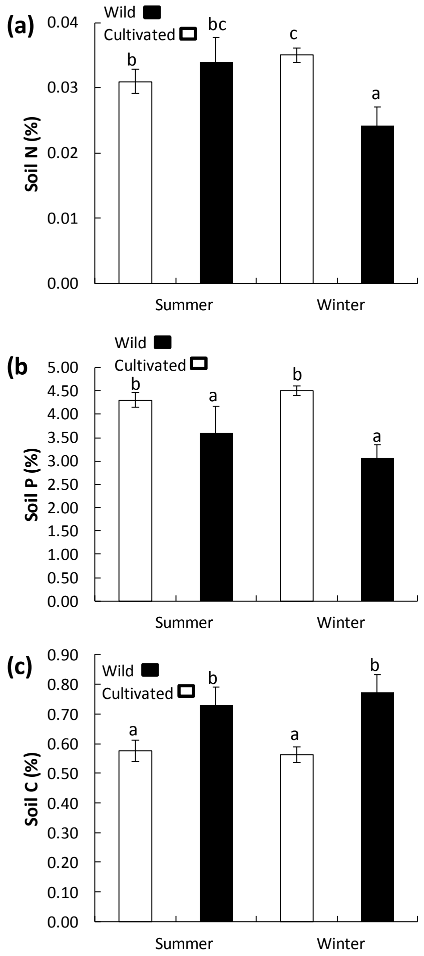


Figure 5.6 Differences in soil N (a), soil P (b) and soil C concentrations between the cultivated and wild site during late summer and winter (mean ±SE). Different letters above bars indicate significant statistical differences between treatments (P<0.05, *post hoc* Tukey's HSD, multiple-range test, ANOVA) (n=10 means).



5.4 Discussion

Dry and wet seasonal variations in N cycling and gas exchange of wild and cultivated *A. linearis* legumes were investigated. It was found that the two types of rooibos tea had different methods of adapting to N nutrition and C assimilation during wet and dry seasons.

The $\delta^{13}C$ comparison between the wet and dry season indicates that both wild and cultivated rooibos experienced water stress during the dry period of the year. Increasing aridity may cause stomatal closure which can result in less discrimination against ^{13}C during photosynthesis with associated elevated $\delta^{13}C$ values (Ehleringer 1995; Swap *et al.,* 2004). However, based on the significant lower $\delta^{13}C$ values of the wild tea type compared to the cultivated type during the dry period, it appears that the wild type was less water stressed. This improved resistance of the wild type to drought, may be related to the increased C/N ratio, as an indication of developing schlerophylly. Schlerophylly is often associated with dry climates as an adaptation to low water levels, but also to low nutrient availability (Groom and Lamont, 1999; Gutschick, 1999; Sardans *et al.,* 2008). Bussotti *et al.,* (1998, 2000) have found that the level of sclerophylly increases during the summer months in response to adverse environmental field conditions. In this regard, both types had lower C/N during winter. Seasonal changes may also cause differences in nutrient availability, and this may be enhanced in nutrient poor ecosystems such as the Fynbos.

This effect is evident in the leaf N nutrition of both plants during the dry and wet seasons. The highest N concentrations were found in cultivated tea during winter (1.27%), whilst the wild tea during the arid summer had the lowest N concentrations (0.85%). These values are well within the range of other evergreen sclerophyllous mountain Fynbos plants (Herppich *et al.,* 2002). It has been shown that plants in Mediterranean regions take up N during the winter rainy season, prior to any above ground growth, and that these minerals are stored in the old evergreen leaf tissue before being translocated to new growth in spring (Mooney and Rundel 1979). Similar seasonal patterns have been reported for other endemic Fynbos species (Stock *et al.,* 1987; Witkowski *et al.,* 1990; Herppich *et al.,* 2002). Maier et al., (1995) suggested that the mature leaves may function as sink organs during times of high nutrient availability and eventually reallocate this shared N to the newly formed sinks during spring growth.

As cultivated tea plants were less reliant on BNF, they may have been able to conserve more N from their leaves through reabsorption, prior to abscission. In contrast the wild plants relied more heavily on BNF and resorbed less N from leaves. Reabsorption from senescing material is primarily a nitrogen conservation strategy (Eckstein *et al.,* 1999; Aerts and Chapin, 2000) and essential to assist in alleviating nutrient scarcity. Although the values reported for N reabsorption in other Fynbos species (35-70%) (Stock *et al.,* 1987; Witkowski 1991) are somewhat higher, the reabsorption of N, coupled with its ability to fix N is an important mechanism to overcome nutrient deficiency in this nutrient constrained environment.

The effect of the dry season on symbiotic N_2 fixation was more evident in the cultivated than the wild plants, evidenced by the greater percentage decline in BNF during summer relative to winter, in the cultivated plants. Since these plants did not show a proportional increase in the reabsorption of leaf N, the remaining N had to be acquired from soil uptake via the roots. This increased reliance on soil N in the cultivated plants during summer, may be the result of the lower energy costs associated with soil mineral uptake of N. N_2-fixing plants incur additional C costs for the synthesis of roots and nodules and maintenance of mycorrhizal fungi (Vance and Heichel, 1991). Nodule initiation, growth, and activity are also more sensitive to moisture stress than general root and shoot metabolism (Albrecht *et al.,* 1994). During the summer stress, this ability to switch N sources may be a distinct advantage.

The seasonal pattern in net assimilation rates, observed in *A. linearis,* coincides with the results obtained in other studies, investigating schlerophyllous shrubs, from Mediterranean climate regions (van der Heyden, 1989; Flexas *et al.,* 2001). These studies revealed lowest rates of photosynthesis during summer when photosynthesis is reduced by stomatal limitation, triggered by low water availability. According to Louw (2006) drought limits biomass production in *A. linearis*, as was confirmed in our study, by the lower photosynthetic rates of both wild and cultivated rooibos during the late summer months when aridity is most pronounced. However, during late winter, under favourable soil moisture conditions, cultivated tea had elevated gas exchange rates compared to the wild tea. Conversely, during the dry summer months the wild tea exhibited an increased ability to adapt to the limited soil moisture conditions by sustaining higher photosynthetic rates. This was also accompanied by their ability to maintain higher WUE's, facilitated by effective control of transpirational water loss. This enhanced ability to adapt to dry conditions may be related to their higher sclerophylly index which Turner (1994) argues to be an important protective mechanism to resist extreme climatic events. Bussotti *et al.,* (2002) have demonstrated that leaves with a higher level of sclerophylly are able to maintain their internal moisture status, even in extreme drought conditions.

The results of our study suggest that, under the present climate conditions the wild plants are better able to tolerate summer drought with greater water economy, and more reliance on BNF for N nutrition. These wild plants may therefore be better adapted to tolerate the warmer and drier conditions predicted for the next decades which will underpin the survival of this legume species under current models of climate change.

CHAPTER 6: SUMMARY AND SYNTHESIS

This work combined different methodological approaches (climate trend and modelling analyses, ecological modelling, field and glasshouse trials) to investigate distribution patterns, drought tolerance levels and adaptive traits of cultivated and wild *A. linearis* types and gain insights into the prospects for *A. linearis* to persist and sustain commercial production in the future. Rooibos-specific climate indicators were used to guide historical trend and analyses of future climate projections and inform the development of a bioclimatic species distribution model. Results from the species distribution model and information derived from interviews with growers were used to design experimental trials to test the predictions of the bioclimatic modelling. Gas exchange measurements, isotope ratios ($\delta\ ^{15}N$ and $\delta\ ^{13}C$) and growth analysis were used to investigate drought tolerance and establish whether there is any difference in the ability of wild and cultivated *A. linearis* plants to adapt to adverse environmental conditions. This is the first research on rooibos' plant physiological functioning in the presence of climatic and environmental constraints which help anticipate responses to climate change and develop adaptation strategies to minimize the effects of such changes on wild and cultivated rooibos.

6.1 Climate trends and simulations

Characterisation and understanding of trends in historical climate variables, in conjunction with viable multi-model projections of future climates, help to identify specific climate-related risk factors and regions which may already be experiencing the effects of climate change. This was one of the primary objectives of the thesis (objective number 1 in chapter 1). Climate trend analyses found that there is strong evidence of accelerated temperature increase and spatially localized changes in rainfall characteristics during the recent past over the greater Cederberg region.

The temperature increases, and decreases in rainfall, projected for the region as simulated by the conformal-cubic atmospheric model (CCAM) are consistent with and expected to exacerbate observed trends, leading to prolonged periods of drought in summer and reducing the amount of peak water available in autumn and winter. Specific periods and indices where observed trends are in line with future simulations, relate to a drying trend and an associated decrease in frequency of rainfall events during June, August and October. For temperature indices, there is strong agreement on the average temperature increase as well as for extremes, especially during Dec-March, which is the warmest and driest period of the year. This would especially affect early rooibos plant life stages and survival success during the post-fire regeneration stage (late winter and summer) when seedlings are considered most vulnerable to environmental alterations (Howard and Goldberg, 2001; Fay and Schultz, 2009). This would also affect rooibos populations that are already growing under high drought stress more than populations growing under less drought stressed conditions, reflecting the differences found in future species suitability between the lowland areas and the mountain sites, and also between the north-western and southern range of the production area (related to objective 2 in chapter 3). On the contrary, the demonstrated minimum temperature increase and decline in wind speed, especially during May – Aug may produce positive effects such as reduced frost and wind damage in rooibos seedlings, but may be limited to specific areas.

6.2 Ecophysiological traits and distribution patterns

An important aim (objective 2) of the study was to quantitatively and spatially assess how these climate changes would affect the distribution and suitability of rooibos. When the species distribution models are projected using future climates they show a profound decrease in the area suitable for rooibos tea, largely related to the significant winter warming and decrease in annual precipitation simulated by the climate models. From the ensemble of climate models, the most favourable outcome for cultivated tea is a c. 49.8% reduction in the extent of the bio-climatically suitable localities, and at worst an almost 75.5% reduction, by 2070. For wild tea the most favourable outcome is a 76.2.5% reduction in the extent of the bio-climatically suitable localities, and at worst an almost 88.7% reduction, by 2070. Suitable areas for cultivation and species survival are likely to persist in mountain areas, while possible range expansion is indicated in a south-eastwards direction. The rooibos bioclimatic model predicted the optimal cultivated range of rooibos tea to be limited by winter rainfall (115-256mm) corresponding to an elevation range of between 94-955 m.a.s.l., while winter mean temperature (7.1-13.5˚C) and summer max temperature (above 32˚C) also constrains the climatically suitable area for crop production. Regions producing rooibos at the warmer and arid margins of these climate limits, will find it increasingly difficult to maintain high quality levels of production in conditions of further climate change. On the contrary, higher lying or south-easterly locations which are at the moment beyond the favoured areas for tea production will probably turn into areas more favourable to high quality production. This will be subject to suitable soil types and other edaphic constraints, since the mountain areas contain many rocky outcrops and steeper slopes which make it difficult for farmers to cultivate.

Consistent with objective 3 of the study to refine the results of the distribution modelling, additional methodological approaches (glasshouse and field trials) were used to assess functional constraints of *A. linearis*. Eco-physiological trials corroborated the high importance that the SDM's placed on rainfall-related variables and thresholds in explaining the presence of *A. linearis*. These trials have also revealed that specific plant adaptive strategies may compensate for some of the projected changes in climate, and that future productivity and the ability of the species to persist may not be equally affected by climate change in all areas.

In Chapter 4 it is shown that rooibos plants employ several adaptive traits to cope with stressful conditions depending on the severity of the drought. With mild water limitation, suppressed photosynthetic performance leads to a decline in overall plant growth and a reduction in aboveground biomass accumulation in favour of root growth and a subsequent increase in the R/S ratio. Additionally, plants are also able to partially offset the effects of drier conditions by developing sclerophylly and increasing water use efficiency (WUE), as indicated by both instantaneous measurements and long term $\delta^{13}C$ data. These findings illustrate the important trade-off between successful production of cultivated tea, measured in terms of yield and beverage quality, and persistence and survival of the species. The observed drought associated adaptation strategy of decreasing above ground biomass in favour of root growth will significantly compromise annual productivity, since the leaves and twigs of the plant comprise the harvestable component. Additionally, in field grown rooibos plants, drought and elevated temperatures during winter induce leaf abscission, further impacting on crop yield. For wild plants however, such morphological adaptive responses could be advantageous and may enable plants to survive and persist, albeit with reduced biomass.

This dichotomy between production success and survival may also not be captured by the distribution modelling since certain suitable locations may not coincide with the specific climatic optima required for high yield and quality. High quality tea is associated with higher altitudes and lower temperatures (typically corresponding to the natural distribution of wild tea between 450-1050 m.a.s.l) (Cheney and Scholtz, 1963; Hansen, 2006; Nel *et al.,* 2007). Many areas indicated as suitable by the distribution modelling, therefore, fall beyond this optimum production limit. Moreover, the flavonoid *aspalathin*, is an important quality marker for the rooibos industry as it contributes to the characteristic red-brown colour of processed tea and constitutes the basis of its medicinal properties (Joubert and Schulz, 2006). It has been shown in other medicinal plants that temperature and drought stress can inhibit biosynthesis and cause degradation of flavonoids, reducing their medicinal quality (Mori *et al.,* 2007; Wahid *et al.,* 2007; Yuan *et al.,* 2012; Odjegba and Alokolaro, 2013; Rienth *et al.,* 2014). Sub-optimal climate conditions (particularly higher temperatures) may, therefore, affect production success but may not significantly influence mortality, at least in the short term.

Longer and more severe droughts may, however, result in outright mortality of seedlings and lead to local extinction of seeder populations. This has been illustrated in chapter 4 where seedlings subjected to severe water limitation under field conditions (late winter and summer) were not able to reduce mortality through either a trade-off between root and shoot growth, developing sclerophylly or increased WUE. These findings emphasise the increase in the risk that more severe droughts and increased temperatures, such as expected under future climate change, may pose to tea production and species persistence.

There may however be significant differences in the extent to which cultivated and wild plants will be able to persist in a more arid climate. This addresses the last objective of the study to investigate functional traits of wild and cultivated rooibos. Seasonal patterns of nutrient cycling and gas exchange showed that wild resprouter plants had an increased ability to adapt to the limited soil moisture conditions during the warm and dry summer months. This seems to be due, at least partially, to increased water use efficiency and more reliance on biological nitrogen fixation for N nutrition. Increasing sclerophylly, which is associated with a high ratio of carbon to nitrogen in a plant's leaves (Rundell, 1988), was observed as a functional strategy to adapt to specific stresses such as seasonal water deficits. These traits would allow adult resprouter plants to persist in the landscape for considerable periods of time as the climate dries, although prolonged drought periods would also hamper the establishment of resprouter seedlings. Moreover, such differences in tolerance to environmental stress support suggestions that further selection through wild types may indeed improve the resilience of cultivated tea to drought and heat stress.

6.3 This study in context

Translation and application in conservation and adaptation planning for commercial and small scale rooibos farming.

Unlike wild tea, the distribution of cultivated tea is assisted by several management interventions which make it possible to expand or shift its indigenous range. This leads to cultivation in sub-optimal locations and production in these marginal areas is likely to decrease rapidly under the influence of accelerated global climate change. These represent the most vulnerable areas, and include lowlands along the west coast and the northern periphery of the production area. In these areas, the occurrence of optimum cultivation conditions will become erratic as the suitable areas tend to expand south-eastwards in the future. While adaptation measures such as improving soil fertility, retaining natural vegetation and minimum tillage could mitigate climate change effects, farmers in marginal areas may progressively opt to convert to crops more suited to the new conditions.

Subject to edaphic constraints, farming enterprises located in the optimal bioclimatic space (inland plateaus and mountains at elevations between 480 and 950m.a.s.l.) might be able to tolerate climate change until at least 2070. These are the areas of collective agreement amongst climate models where current cultivated areas remain suitable in a future scenario. Many of the traditional growing areas, including the Suid-Bokkeveld and Wupperthal small-scale farmer communities, correspond with these requirements. Yet, even in these core localities it may become increasingly difficult to attain optimal yield and quality, leading to increased and intensified management (e.g. the use of irrigation). Anecdotal evidence from farmers however, suggests that using irrigation as mitigation measure to supplement inadequate rainfall during adult life stages of rooibos has persistently resulted in die-back and total crop failure. Monitoring of rooibos stands, from seedling stage through to seed reproduction (by sowing prepared seeds and tracking survival annually in suitable or marginal field sites) would be an important mechanism to detect climate change effects, especially in high risk areas.

Profound range contraction of wild tea from its northern range raises concerns for the Suid-Bokkeveld small scale farmers, since the largest proportion of wild tea is sourced from this area. While most harvested tea exhibits resprouter traits, which may enable it to resist climate change for longer, these populations should be closely monitored. It will be very important to maintain intact natural habitats wherever possible along potential migration routes. The modelled range in the future tends to move south-eastwards and upslope on the mountains over time, indicating the importance of retaining strips of natural habitat both horizontally (along contours) and vertically (up and down a slope). Since rooibos is an ant dispersed species, the likelihood of *A. linearis* to successfully migrate and establish in these novel areas depends highly on dispersal rates. According to Mittermeier *et al.,* (1998) the nearly 3,000 ant-dispersed species in the CFR have low dispersal and migration capabilities, and such species may not migrate fast enough to track the new climate. Re-colonization will be limited and localized especially with increasing distance from the parent population. Other important factors such as landscape configuration and soil characteristics will further dictate the success or failure of wild rooibos types in specific localities on the landscape.

Optimal localities for persistence of wild tea correspond to the areas surrounding Wupperthal and the mountains between Clanwilliam and Citrusdal. The documented genetic diversity of *A. linearis* types found around Wupperthal (Malgas *et al.,* 2010), and the presence of established protected areas bordering the area, should make this a priority area for long-term *in situ* conservation of wild genetic material. While there is good control of the harvesting techniques in the Suid-Bokkeveld, there is little control at Wupperthal (Hansen, 2006). Wild populations should therefore be closely monitored and increased caution will be necessary to ensure that the frequency and intensity of harvesting is sustainable. Their adaptability and resilience to stresses can provide farmers with the coping strategies needed to confront climate changes.

Though managed relocation of wild tea to more suitable habitats is mostly impractical, expansion of cultivated tea to novel areas is a plausible scenario. Commercial producers could therefore potentially offset some of the consequences of climate change by moving the species to suitable areas. In future, more pressure might also be placed on exploiting remaining natural areas in existing growing areas or by increasing the proportion of wild tea being harvested. This may, however, bring farmers into conflict with the biodiversity conservation sector since these areas are almost exclusively located in existing conservation areas or include conservation worthy vegetation. Any future expansion or relocation of plantations should be subject to stringent environmental impact assessments. Moreover, the ability to cultivate tea in areas where it did not occur naturally may threaten genetic integrity of different wild types and cause homogenization of the species (Van der Bank, 1995).

6.4 Limitations and future research

Several challenges of identifying and modelling long-term trends in rainfall exist over the study area. The topographical diversity of the area contributes to high local scale variability and complexity of rainfall patterns and therefore makes rainfall surfaces subject to greater errors. Since a station represents only a single point, it may not be adequately representative of the surrounding region, especially in the presence of complex environmental gradients. Moreover, the spatial density of weather stations with consistent long term daily rainfall data may be insufficient to identify significant trends and results in larger sampling errors in trend estimates. The use of gridded data sets produced from existing station records may prove useful to better identify temporal and spatial trends. Observed rainfall changes should also be considered in the context of inter-annual variability, since naturally occurring variations in climate at yearly and decadal timescales can also greatly affect the calculation of trends (DEA, 2013).

Apart from detecting trends in annual, seasonal or monthly rainfall, future research can also focus on identifying changes in the onset and cessation of the rainy season, which is essential for timely preparation of farmland and making operational decisions regarding sowing and transplanting of seedlings. For wild rooibos seeder populations, rainfall reliability quantified as interannual variation in monthly and seasonal rainfall, and as the frequency of individual events in terms of their size, duration and intensity may be an important factor in determining post-fire regeneration success. It has been suggested that the high incidence of fire-killed shrubs in the south-western Cape are linked to the reliable rainfall regime of the region (Cowling *et al.,* 2005). Climate change related research on rooibos would therefore benefit from an analysis of rainfall reliability, onset and duration.

While the distribution modelling made a distinction between wild and cultivated types, it treated wild types as a homogenous group. This was due, in part, to the limited data on localities for wild rooibos and, the lack of data on whether wild populations were indeed resprouters or seeders or mixed. The existing model can be further refined by incorporating more environmental variables and locality data to discriminate between seeder and resprouter distributions, while some assumption about the species' dispersal ability can be made to improve the models prediction accuracy. Additionally, an important caveat related to future predictions is that they are dependent on the particular climate models chosen for the analysis. Predictions should be tested with other climate models (e.g. CORDEX regional climate models and CMIP5 GCMs) to ensure that a wide range of possible future climate conditions are considered.

Though testing the distribution model through *in situ* experiments proved helpful in corroborating the findings, much value could be added by testing the model at sites and/or areas that differed both in climate and suitability (Pattison & Mack, 2008). By using simple regression models one can then examine the relationship between growth and mortality at the various sites and their predicted suitability. Ideally, the time of monitoring for field experiments should be extended to include two or more summers/winters as longer periods of observation would give a more realistic in situ response of the species.

Since this study has shown that rainfall events will become more sporadic appearing at more irregular intervals in time during the winter period (corresponding to the germination and seedling establishment phase), understanding how plants respond to episodic drought and watering events will be helpful to implement crop management practices in a changing climate. This would involve applying intervals of drought and/or rewetting events to test drought tolerance and the recuperative potential of plants. Moreover, drought and heat factors should be integrated when testing plant responses as these stress factors occur simultaneously under natural conditions.

Crop failure due to irrigation may be ascribed to the timing, frequency and amount of water application, as it has been shown in rooibos nurseries that a humid microclimate contributes to disease development (Bahramisharif *et al.,* 2013a). In particular, overhead irrigation provides excess water on leaves rather than around the root zone which is conducive to the outbreak of *Botrytis cinerea,* the most important foliar disease of rooibos seedlings (Wessels *et al.,* 2013). Yet, supplementing soil moisture during critical stages of seedling establishment may significantly increase the productivity and survival rate of *A. linearis* during droughts. Future research can assess viability of irrigation for improving seedling establishment by applying different irrigation regimes while monitoring plants for quality, yield and diseases.

The distribution of wild tea and to a lesser extent its cultivated counterpart, is controlled by several other factors such as natural disturbance regimes (frequency and timing of fire) and biotic interactions (e.g. pests and pollinators), which are also expected to be influenced by climate change. The most important insect pest associated with reduced productivity and mortality in rooibos plants is the "Molopopterus theae". They are especially active during the summer months when plants are subjected to severe moisture stress and appear to have a preference for warmer temperatures (Prof. Justin Hattingh, personal comm. 2010, ARC). More research should therefore go into other components or determinants of the species range.

6.5 Conclusion

This study has established a baseline for assessing the consequences of climate change for cultivated and wild rooibos tea. Previous climate related research on rooibos tea was conducted within the participatory action research (PAR) framework and relied primarily on observations from farmers. In contrast, this work applied climate trend analyses, climate model scenarios of the future, ecological modelling and eco-physiological methods (gas exchange, nutrition, growth) to provide insight into *A. linearis'* response to new climatic conditions predicted for the future. Combining these approaches proved valuable in capturing many subtleties of the species physiological constraints and adaptive capacities.

By using an ensemble of six coupled global climate models (and observed climate trends), the study has sought to determine how climate change will manifest itself over the study region. In combination with ecological modelling this provided insight into the climatic limits and future distribution of rooibos tea. This has led to the identification of production areas most at risk in conditions of future climate change while also identifying vulnerable areas to be targeted for conservation actions. Spatially, the effects of climate change will be most pronounced in presently marginal areas, while the optimal localities at higher elevations on the eastern part of the study area could have the potential to withstand climate change until at least 2070 (25.5% - 50% of pre-existing growing areas remain suitable). Coupling ecological models with experimental trials have revealed functional traits of *A. linearis* which may enable the plant to offset some of the negative effects of climate change. Field trials have also shown that climate change may not equally affect wild and cultivated rooibos types, which would subsequently require different adaptive strategies. The findings of the study provide added perspective in guiding geographically specific actions for conservation planning, monitoring and future research and developing adaption strategies for sustainable rooibos production.

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