

# Effects of increased heat and drought stress approximating climate warming on the reproduction, photosynthesis and growth of Proteaceae species in a southern African Mediterranean climate ecosystem

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## DEDICATION

I dedicate this work to God, the Almighty and my creator. Jehovah Jireh, the supplier of all my needs! I also dedicate this work to my husband, David Arnolds, who encouraged me all the way through this project to give my all. To my children David, Fletcher and Chelsey who had to endure a lot! Thank you! I love you all.

*“I thank God for the mountains,  
And I thank Him for the valleys,  
I thank Him for the storms  
He brought me through;  
For if I'd never had a problem  
I wouldn't know that He could solve them,  
I'd never know what faith in God could do”.*

*(Andrae Crouch - 1942-2015)*

## ABSTRACT

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In this research project, the effects of warming and drought on three Proteaceae species (*Protea repens*, *Protea laurifolia*, *Leucadendron laeolium*) were investigated through various monitoring and experimental studies on an altitudinal moisture gradient. This study tested the hypothesis that reproduction, photosynthetic capacity and growth of selected Proteaceae species from warm, arid sites at low elevations along the gradient will be more resilient to heat and drought stress accompanying climate warming than those from cool humid sites at high elevations along the same gradient.

Experimental approaches were applied to obtain numerical data on diurnal stem diameter variations, sap flow rates, vapour pressure and photosynthetic capacity of *Protea repens* growing at five different climate stations along an altitudinal moisture gradient. Enforced seed dormancy was examined by calculating seed germination in 11 Proteaceae species in experimental mesocosms. Germination responses (% germination) of three Proteaceae species growing along a moisture gradient were tested and also in a greenhouse to increase the understanding of the impact of changing environmental conditions on germination. Drought resilience was tested on one-year-old seedlings of 16 Proteaceae species in a greenhouse.

The results of this study have indicated that the lowest elevation exhibited lower dewfall over the entire experimental period compared to the dewfall at the highest elevation. *Protea repens* indicated significantly negatively correlations in total daily amplitudes in sap flow vs station maximum diurnal temperature during four seasons. Proteaceae germination indicated that measured reductions in seedling recruitment were closely associated with increases in diurnal soil temperature minima and maxima. Measured transpiration rates declined linearly with increasing duration of drought and decreasing soil volumetric content in all 16 Proteaceae species. The test species proved to be highly tolerant of water stress/drought and are well adapted to Mediterranean climatic conditions.

This study emphasizes the importance of the non-rainfall precipitation as an insignificant, continuous supply of water to dry and semi dry areas, and also it provides an important supply to the areas water equilibrium, particularly during low rainfall episodes. Findings indicate that Proteaceae seedlings are tolerant of summer dry periods over a large part of the South African Cape Floristic Region, and that enforced seed dormancy, is induced by elevated night-time temperatures. However, the post-fire stage among Proteaceae is most

sensitive to climate change. Germination in the greenhouse took place 74 days after germination while germination in the field needed a longer period. The findings indicated that germination of Proteaceae is highly dependent on temperature.

It was concluded that Proteaceae reproductive stages, growth and survival are sensitive to drought, higher temperatures and water availability. Long term monitoring, more test species and more climate change experiments is needed to get more results to fully explain the effects of temperature and drought on Proteaceae. This study has contributed to our understanding and knowledge of climate change effects on Proteaceae.

*Key words: Climate change, drought, germination, growth, Proteaceae species, temperature, water*

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## OPSOMMING

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In hierdie navorsingsprojek is die invloed van verwarming en droogte ondersoek op drie Proteaceae spesies (*Protea repens*, *Protea laurifolia*, *Leucadendron laureolum*) deur middel van verskeie moniterings- en eksperimentele studies langs 'n hoogte- en voggradiënt. Met hierdie studie is die hipotese getoets dat voortplanting, fotosintese en groei van geselekteerde Proteaceae spesies in warm, droë, laagliggende proefpersele langs 'n hoogtegradiënt, meer weerstandig sal wees teen hitte- en droogtestremming wat gepaard gaan met klimaatsverwarming as dié op koel, vogtige hoërliggende persele oor dieselfde gradiënt.

Eksperimentele benaderings is toegepas om numeriese data oor die daaglikse stingeldeursnee-variasie, sapvloeitempo, dampdruk en fotosintetiese kapasiteit van *Protea repens* in te win wat groei by vyf verskillende klimaatstasies langs 'n hoogte- en voggradiënt. Gedwonge saaddormansie is bestudeer deur die saadontkiemingspersentasie van 11 Proteaceae-spesies, wat in mikrokosmosse gekweek is, te bereken. Om lig te werp op die invloed van die veranderende omgewing op saadontkieming, is die kiemingspersentasie van die drie Proteaceae-spesies, in situ oor die voggradiënt asook in die glashuis, getoets. Die droogtegehardheid van 16 jaaroud saailinge van Proteaceae-spesies is onder glashuistoestande bepaal.

Die resultate het aangetoon dat die laagste proefperseel het laer douval oor die hele eksperimentele periode getoon in vergelyking met die douval van die hoogste geleë proefperseel. *Protea repens* het 'n betekenisvolle negatiewe korrelasie aangedui tussen daaglikse sapvloeitempo en maksimum temperatuur oor vier seisoene. Proteaceae ontkiemingsdata het getoon dat die berekende afname in saailingopkoms nou ooreenstem met die toename in die daaglikse maksima en -minima in grondtemperatuur. Die transpirasietempo van al die spesies het lineêr afgeneem met toenemende duur van droogte en met afname in volumetriese grondwaterinhoud van al 16 Proteaceae spesies. Die proefspesies het geblyk hoogs bestand te wees teen droogte en dat hulle goed aangepas is by Mediterreense klimaatstoestande.

Die resultate van hierdie studie het beklemtoon die belangrikheid van nie-reën presipitasie, wat hoewel gering, 'n konstante bron van water vir sommige ariede en semi-ariëde ekosistels is en ook nog 'n betekenisvolle bydra lewer tot die waterbalans tydens droogte.

Hierdie resultate het ook aangetoon dat Proteaceae-saailinge weerstandig is teen periodes van somerdroogte oor 'n groot deel van die Suid-Afrikaanse Kaapse Floristiese Streek.

Verder het dit ook aangetoon dat gedwonge saaddormansie geïnduseer word deur hoër nagtemperature en dat die na-veldbrandstadium van Proteaceae die gevoeligste is vir klimaatsverandering. In die glashuis het ontkieming 74 dae na planting plaasgevind, terwyl dit langer geneem het in situ. Hierdie bevinding dui op streng temperatuurafhanklikheid van Proteaceae.

Die slotsom is dat die reprodktiewe fase, groei en oorlewing van die Proteaceae gevoelig is vir droogte, hoër temperature en die beskikbaarheid van water. Langtermynmonitering, meer toetsspesies en meer klimaatveranderingeksperimente is egter nodig om die effek van temperatuur en droogte op die Proteaceae ten volle te verstaan. Hierdie studie lewer 'n bydra tot die kennis en begrip van klimaatsverandering op Proteaceae.

*Sleutelwoorde: Droogte, groei, klimaatsverandering, ontkieming, Proteaceae-spesies, temperatuur, water*

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# CHAPTER 1: GENERAL INTRODUCTION

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## 1.1. Introduction

A change in condition of the climate, recognized (e.g. using statistical analysis) by the differences in the mean and/or the variations in its properties, which continues for a lengthy period (usually decades or longer), is called climate change. This describes any variation in climate over time, whether it is caused by natural variability or anthropogenic activity (IPCC, 2007). Climate change also mean changes such as more extreme weather events because of the increased thermal energy in the atmosphere. Greenhouse gases (CH<sub>4</sub> and NO<sub>2</sub>) and carbon dioxide (CO<sub>2</sub>) have accumulated in the atmosphere before industrialization has previously impacted on the earth's temperature and is expected to promote the increase of temperature during this century (IPCC, 2013).

Gases that contribute to the greenhouse effect are able to trap heat in the atmosphere through the absorption of infrared radiation that is reflected by the earth's surface (IPCC, 2001). High levels of infrared radiation that reaches the earth are caused by greenhouse gases. During the period from 1765 to 1995 it was established that the extra atmospheric problem of CO<sub>2</sub> (61%) and CH<sub>4</sub> (23%) have been accountable for the greenhouse effect (Bridgham & Johnson, 1995).

Extraordinary biotic reactions to the earth's surface temperature are projected (Thomas *et al.*, 2004) and also the outputs through which ecological responses create extra climate influences by altering transfer ratios of energy, water and trace greenhouse gases at the planetary surface (IPCC, 2014). In the IPCC 5th Assessment Report of 27 September 2013, Dr Rajendra Pachauri, chairman of the IPPC, announced in Stockholm that with 97% certainty, humankind is the biggest factor in climate change. Species responses assessed through numerous statistical meta-analyses have shown that the influence of human activity on global warming has already altered the earth's living organisms (Parmesan, 2006). A study has found that 59% of 1 598 species that ranged over different ecosystems, taxonomic and functional groups, presently show considerable differences in their phenologies and distributions in reaction to current, reasonably moderate warming of 0.6 °C (Parmesan & Yohe, 2003). It is presumed that maximum effect of global warming will be experienced by terrestrial ecosystems in elevated mountain regions and the arctic tundra (Grabherr *et al.*, 1994; Oechel & Vourlitis, 1994).

Reduced layers of mosses and lichens and increased cover and heights of deciduous shrubs and graminoids were the results of regulated heating trials at 11 study sites within the tundra biological areas (biomes), these findings confirming that newly detected rises in bush cover in several tundra zones are indeed a reaction to climatic temperature increases (Walker *et al.*, 2006). Contrary to other thorough studies carried out in the sub-arctic and arctic tundra, there are few data on other biomes. Meta-analyses of plant yield responses to experimental heating in 62% of the worlds locations (representative of Forest, Grassland, high and low latitude/altitude Tundra biological areas) showed reduced relative yield responses to heating with a rise in the average yearly temperature of the site (Rustad *et al.*, 2001) suggesting that plant yield is anticipated to decrease further with a decrease in altitude, subtropical and tropical areas. Foden *et al.* (2007) found that *Aloe dichotoma* populations were in decline, with potential range retraction in Namibia, and with increasing population growth in Namaqualand. The mechanisms were poorly understood.

Mediterranean-type ecosystems have been rated as especially sensitive to variations in the diversity of plant and animal life prompted by the five key drivers of biodiversity at worldwide scale (Musil *et al.*, 2005). Climate change is one of the main drivers (second to land use) that affect biodiversity when the entire ecosystems are averaged (Sala *et al.*, 2000). Various simulation models have been designed to facilitate comparisons among ecosystems and predict species responses to climate change (Kramer *et al.* 2000; Linkosalo *et al.*, 2000). The effects of climatic change on vegetation in the South African Mediterranean-climate Cape Floristic Region (CFR) are of special concern as this region is registered along with 35 worldwide biodiversity regions (Myers *et al.*, 2000) due to its extremely high species richness (8 504 species in an area of 87 892 km<sup>2</sup>) and high portion (68.2%) of indigenous species (Cowling *et al.*, 1989, 1998; Linder, 2003). Current bioclimatic models predict substantial loss of species diversity and shifts in species distributions with an anticipated up to 5°C increase in mean annual temperature for the entire region and 30% reduction in mean annual precipitation in this region near the end of a hundred years (Hulme *et al.*, 2001, De Wit & Stankiewicz, 2006; Hewitson & Crane, 2006; Field *et al.* 2014). Several bioclimatic modelling studies have indicated that climate change could result in a reduction in the fynbos biological area climate cover. This includes the main south-western areas (particularly the steep regions) staying inside the cover, then with considerable rising movement, to colder areas (DEA, 2013 b, Louw *et al.*, 2015). The most prominent changes are predicted for the dominant Proteaceae on inner coastal plains (Hannah *et al.* 2002; Midgley *et al.*, 2002; 2003).

Empirical data are needed to corroborate and improve bioclimatic model projections of vegetation responses to global climate change. Several studies from southern African

biomes have recorded shifts in community composition in response to recent severe drought episodes (Midgley *et al.*, 2005; Foden *et al.*, 2007; Hoffman, 2009). A study by Agenbag (2006) found that fynbos growth is very sensitive to temperature changes. The growth of fynbos species depends on their specific optimum temperatures in combination with regular rainfall, rather than rainfall totals. Fynbos in the CFR is relatively tolerant to short term summer drought, but the effects of a total reduction in annual rainfall could have severe implications (Agenbag, 2006).

Acclimation of temperature can follow in reaction together with periodic temperature fluctuations and long-term variations to growth temperatures. Vegetation in the arid and semi-arid areas such as the climate of Mediterranean areas present elevated temperature adjustment capacity and have indicated to be physiologically flexible (Berry & Björkman, 1980; Vitale & Manes, 2005; Louw *et al.*, 2015). According to Louw *et al.* (2015) Proteaceae are recognized by producers to offer adequate toughness and hardness under warmer conditions. Irrigated commercial Proteaceae have already spread to hotter regions in the Cape Floristic Region (CFR) and into warmer parts outside the area (Louw *et al.*, 2015). The hypothesis that Proteaceae will survive under warmer and drier climatic conditions have not been investigated yet. It however was shown that Proteaceae exhibit the ability to modify their traits to their developmental temperatures (Yamori *et al.*, 2014; Louw *et al.*, 2015).

Fynbos populations are highly species rich, because of elevated soil-related beta diversity (Linder, 1985, Cowling, 1990; Mustart, 1994). The CFR is described by a range of geological patterns and soil forms. In a study by Mustart (1994), it appeared that fynbos Proteaceae have evolved reproductive traits sustainable by their different soil types, however, not related to them but through size-related effects. As a disturbance, fire is the driving force of environmental courses such as redevelopment, succession and vegetation changing aspects (Esler *et al.*, 2015) in the Fynbos biological area (Bond & van Wilgen, 1996). According to Gill (1981), Lamont *et al.* (1991) and Mustart *et al.* (1994), the recurring fires to which these require resprouting fynbos types are subjected to, probably play a key part in establishing reproductive features which enhance the after fire spread of every type of fynbos plant.

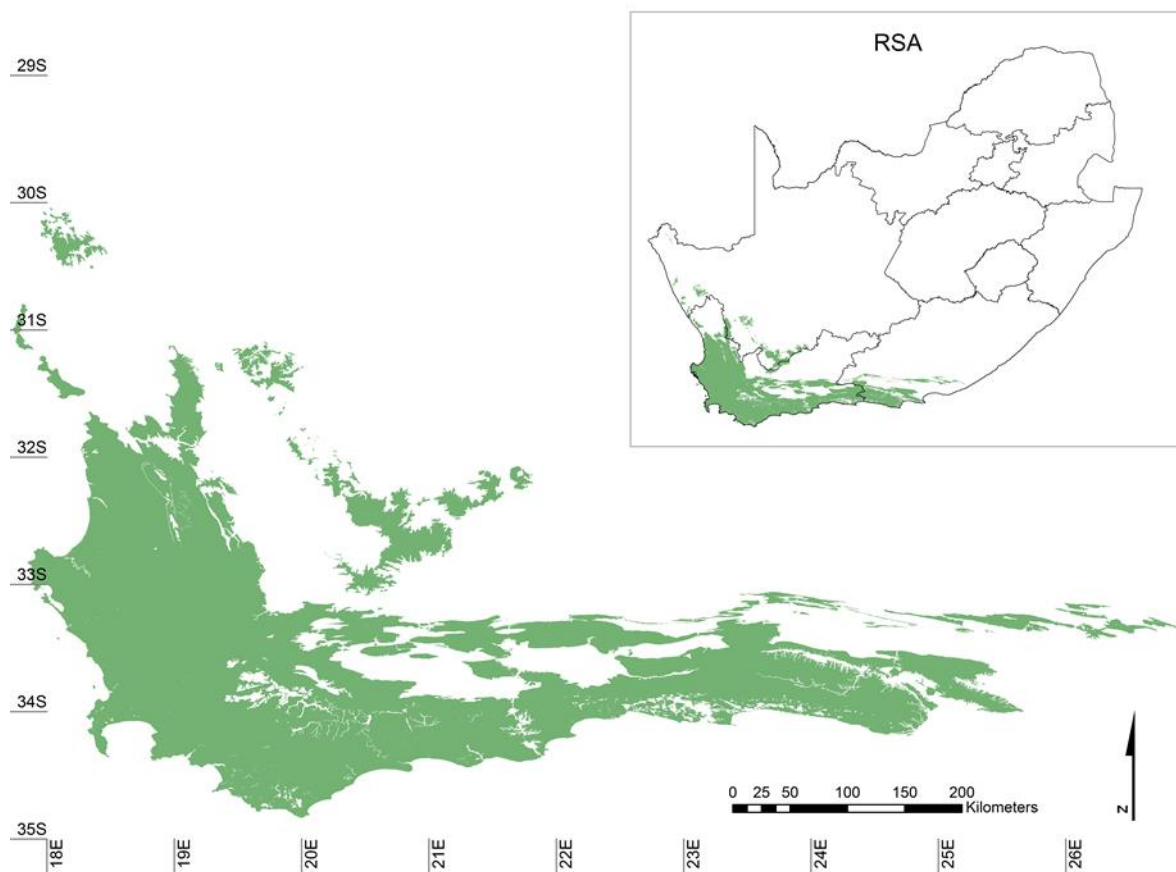


Figure 1.1. Map indicating the size of the Cape Floristic Region (CFR) (green area) on the extreme south-western corner of South Africa (insert).

Climate controlled differences in seed yield, seedling recruitment and persistence are considered the main cause causing the distribution and size of plant communities as well as their taxonomy and purpose (e.g. Cornelius *et al.*, 1991; Bowman & Panton, 1993; Hanson, 2000). Indeed, germination and seedling survival are crucial for plant species continuation, during common disturbance (Klinkhamer & de Jong, 1988; Clark *et al.*, 1998; Peters, 2000).

Numerous processes are linked to recruitment, germination and seedling formation. These processes have received little attention compared to seed yield, spreading and competition for limiting reserves (Woodward 1993; Landhausser & Wyn, 1994). To understand the outcomes of climatic differences on redevelopment need test/trial data on physiological reactions by seeds and seedlings to micro-environmental settings (Wigley *et al.*, 1984; Peters, 2000).

Plant reproductive processes are sensitive to warmer temperatures than vegetative development, and might be more susceptible to brief periods of heat stress before and through the early flowering stage (Reddy & Kakani, 2007). Hedhly *et al.* (2003) indicated that pollen tube development is affected by temperature, although little information is available on the consequences on the female side and on flower receptivity. Poor pollen viability, decreased production and poor pollen tube growth cause decreased fruit-set at warmer temperature and lead to poor fertilization of flowers (Prasad *et al.*, 2003). Floral abortion is a result of decreased seed set per plant in soybean (Thuzar *et al.*, 2010) and decreased seed production in yields such as *Brassica napus* (Angadi *et al.*, 2000), *Brapa* sp (Morrison & Stewart, 2002) and *B. juncea* (Gan *et al.*, 2004). During flowering pollen growth, fertilization, and asynchrony of stamen and gynoecium's development are sensitive to temperatures (Prasad *et al.*, 1999; Croser *et al.*, 2003; Boote *et al.*, 2005). The main reason for low seed numbers produced in some legumes such as chickpea and cowpea could be the results of the loss of pollen or stigma viability under enhanced temperature stress (Srinivasan *et al.*, 1998; Davies *et al.*, 1999; Hall, 2004). Significant negative correlations were found in groundnut between pollen yield and temperature (Prasad *et al.*, 1999). Reduced seed production at warmer temperature under ambient and higher CO<sub>2</sub> conditions were found as a result of decreased pollen viability in groundnut and bean (Prasad *et al.*, 2002, 2003). Pollen yield and pollen sterility during elevated temperatures could be associated with early deterioration of the tapetal coating of pollen (Porch & Jahn, 2001). The details of pollen viability loss are not clear and need further research (Sailaja *et al.*, 2005). Increases in temperature that increases vegetative growth and development could also reduce reproductive development that leads to seed production. Thomas *et al.* (2003) showed that the start of flowering in soybean is reduced by temperature > 32 °C and seed formation is delayed at 30-40 °C. Soybean plants exposed to a temperature of 35 °C for 10h during the day, yield seed reductions of about 27% (Gibson & Mullen, 1996) indicating the need to shield plant reproductive processes from elevated and more recurring periods of extremely higher temperature both in current and future climates (Salem *et al.*, 2007). Similarly, Gan *et al.* (2004) found that the seed yield of canola decrease by 15% when high temperature stress was applied before flowering, whereas the yield reduction was 58% when the stress was delayed to the period of flowering, and further to 77% when the stress was delayed to the pod developmental stage. Physiologically, high temperature stress during reproductive development may affect flower abortion and later pod abscission resulting in a decreased number of seeds set per plant (Duthion & Pigeaire, 1991), as well as cell growth.

Plant reproduction is also influenced by elevated night temperatures compared to higher daytime temperature (Warrag & Hall, 1984; Ahmed, 1993). Floral bud development can be

suppressed and the abortion of floral buds can be induced by premature heat stress (Patel & Hall 1990), while heat stress at later developmental stages adversely affect anther development, causing pollen sterility and flower abscission (Mutters & Hall 1992; Hall & DeMarson 1992; Ahmed, 1993). Limitations in carbohydrate supplies have been implicated as a factor responsible for reproductive failure under heat stress (Ahmed, 1993). Supportive evidence for this hypothesis has been obtained in studies with *Gossypium hirsutum*, (Guinn, 1974) tomato (Dinar & Rudich, 1985) and pepper (Aloni *et al.*, 1991), where abscission of the reproductive structures was correlated with decreased carbohydrate supplies. Decreased carbohydrate supplies have been attributed to a requirement for sink rather than to the assimilate supplied by leaves in tomato (Dinar & Rudich, 1985; Ahmed, 1993), though in *Vigna unguiculata* the reduction in carbohydrate content is related to decreases in the rate of photosynthesis (Ahmed, Hall & Madore, 1993).

With respect to seedling recruitment, there are several cues in fire prone Mediterranean ecosystems for seed germination. These include fire seed release in serotinous taxa with canopy stored seeds (Lamont *et al.*, 1991), heat-induced fracture of water and gas impermeable seed coats (Jeffrey, Holmes & Rebelo, 1988; Brits, Calitz, Brown & Manning, 1993), biochemical stimulation of seed embryos (Blommaert, 1972, van de Venter & Esterhuizen, 1988, Musil & De Witt, 1991) by smoke and gases (e.g. ethylene and ammonia) released during fires (Van de Venter & Esterhuizen, 1988; Baskin *et al.*, 1998), and increased diurnal soil temperature amplitudes in immediate post-fire environments (Brits, 1986). The latter, especially relevant where larger increases in diurnal temperature minima than maxima are anticipated with climate warming, the consequent reduced temperature amplitudes accompanying warmer night-time temperatures having potentially negative consequences for seedling recruitment and ecosystem recovery following fire. Post-fire seed germination can take place from soil seeds banks such as legumes, Cistaceae (Hanley & Fenner, 1998; Thanos, 2000; Jaleel *et al.*, 2009) or Conifers that germinate from cover seed banks (Lamont, 1991).

Species growing in fire-prone areas, display serotiny or canopy seed storage which is a common occurrence (Cowling *et al.*, 1987; Lamont, 1991; Whelan, 1995; Jaleel *et al.*, 2009). Fire resilience is increased through the storing of seeds in the canopy thus protecting them from heat and postponing distribution through the strategy of serotiny (Jaleel *et al.*, 2009; Salvatore *et al.*, 2010). Serotinous cones or fruits release the stored seeds after fire and seeds might benefit from post-fire conditions for germination and seedling formation (Ne'eman *et al.*, 1993; Henig-Sever *et al.*, 2000).

A crucial stage following seed germination and seedling establishment in Mediterranean-climate ecosystems is seedling persistence, especially over the hot dry summer period following wintertime seedling recruitment (Esler *et al.*, 2015). Consequently, an understanding of the resistance of seedlings of different taxa and life forms to drought and heat stress is required. Stomatal closure and limitation of gas exchange is a result of drought stress. Dehydration or desiccation can possibly lead to a major disturbance in metabolism and cell structure and finally lead to the termination of enzyme catalyzed reactions (Smirnoff, 1993; Jaleel *et al.*, 2007d). Severe drought stress can stop photosynthesis, disrupt metabolism and could cause the plant to die (Jaleel *et al.*, 2008c). Drought stress adversely affects plant development through interference of the physiological and biochemical activities, such as photosynthetic pigments (Anjum *et al.*, 2003b; Farooq *et al.*, 2009; Jaleel *et al.*, 2009), photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism and growth promoters (Jaleel *et al.*, 2008a-e; Farooq *et al.*, 2008; Jaleel *et al.*, 2009).

Plants respond to water stress differently and significantly at several structural levels depending upon the amount and length of the drought stress and on the species level and stage of development (Chaves *et al.*, 2002; Jaleel *et al.*, 2008b). Plants cope with drought stress through mechanisms of tolerance and avoidance (Kramer & Boyer, 1995; Neumann, 1995; Mahdi *et al.*, 2014). The relationship between plant morphological and physiological acclimations to drought stress is less well understood. Several studies have observed increased ratios in the shoots or roots (e.g. Bachelard 1986; Li & Wang, 2003; Susiluoto & Berninger, 2007). These increases affected plant nutrition which in turn affects the photosynthetic system. The question remains whether drought stress limits photosynthesis through stomatal closure or through metabolic impairment (Lawson *et al.*, 2003; Anjum *et al.*, 2003b). Both stomatal and non-stomatal (biochemical) limitations are generally accepted to be the main determinants of reduced photosynthesis under drought stress (Farooq *et al.*, 2009; Jaleel *et al.*, 2009). Several studies have found that stomatal and non-stomatal properties of photosynthesis regularly decrease together in response to drought although the major part of this decrease on photosynthesis can be attributed to stomatal effects (Collatz *et al.*, 1976; Susiluoto *et al.*, 2007). Leaves close their stomata during periods of drought, resulting in decreased the intercellular CO<sub>2</sub> concentration. The diffusion of CO<sub>2</sub> into the leaves is limited (Chaves, 1991; Susiluoto *et al.*, 2007) causing in a decrease in net photosynthesis tempo. The rate of dark reactions of photosynthesis decline, when intercellular CO<sub>2</sub> concentrations decline and the supply of CO<sub>2</sub> to the Calvin cycle is reduced. The light reactions continue uninhibited and as a result plants turn the excess energy from the photosystems into adenosine triphosphate (ATP). Cessation of photosystem

II reaction centers and the change of light energy into heat are essential for protection of the photosynthetic apparatus (Maxwell & Johnson, 2000; Susiluoto *et al.*, 2007).

Given the above issues, including the fact that Proteaceae are perennial species, it is clear that an approach that combines both controlled experimental and structured field-based observations would provide useful insights into the species' responses to climate change effects such drought and temperature. Controlled environments is good for determining specific responses to a range of environmental impacts on seed and seedling stages, while field observation methods are good for established juvenile and adult plants.

## **1.2. Objectives and thesis structure**

The objectives are mainly focused on the experimental and field-based study of drought and warming effects on seed germination and ecophysiology of seedlings, established juvenile and adult Proteaceae species. This study is a contribution to acquiring empirical data to validate and refine bioclimatic model predictions of Proteaceae vegetation responses to global climate change. It also assists us to better understand the physiological responses of Proteaceae to predicted warmer and drier climatic conditions.

The specific hypothesis tested in this thesis was that reproduction, photosynthetic capacity and growth of selected Proteaceae species from warm, arid sites at low elevations along the gradient will be more resilient to heat and drought stress accompanying climate warming than those from cool humid sites at high elevations along the same gradient. The presupposition were that Proteaceae will not be resilient to warmer and drier climatic conditions and that these changing conditions will be negatively affect reproductive growth and development. Drought will negatively affect photosynthetic performance, stem diameter and water relations within the plants

### **The specific objectives were:**

- To compare the germination of seed of different Proteaceae genera and ecotypes to elevated temperatures and diminished precipitation frequencies approximating climate warming.
- To compare the resilience of juvenile stages of different Proteaceae genera and ecotypes to drought with respect to photosynthetic capacity, water relations and stem diameter.

- To examine the effects of high temperatures and diminished precipitation frequencies on the growth, reproductive development and ecophysiological performance of different serotinous genera of Proteaceae species.
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## CHAPTER 2

**Diurnal and seasonal stem diameter variations and sap flow of a Mediterranean shrub, *Protea repens* (L) L., along a moisture gradient**



## ABSTRACT

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The ecological distribution of vegetation types are regulated by climatic conditions and precipitation is one of the most significant components driving the occurrence and diversity of species. Long-term monitoring and understanding plant responses to rapid changing environmental conditions are crucial for exploring community dynamics and evaluating the exposure of species to changes in the climate. In an attempt to assess the resilience of the Mediterranean woody shrub, *P. repens* to drought and temperature, the result of changes in temperature and moisture regime on the photosynthetic capacity and transpiration dynamics (hydraulic response) were determined by monitoring daily and periodic variations in stem diameter, chlorophyll fluorescence and diurnal monthly sap flow rates for 8 consecutive months.

Non-destructive measurements of plants were conducted in situ over a climate gradient at different times of the year at Jonaskop, Western Cape. Temperature, vapour pressure, RH and soil moisture were monitored concomitantly. Variations in stem diameter were continuously recorded at hourly intervals using linear variable differential transducers, known as stem diameter variation sensors, at five stations along a moisture gradient. Seasonal data collected over changing climatic conditions showed that *Protea repens* (L.) underwent seasonal fluctuations in stem diameter with the lowest values recorded at station one (ST1) during spring and summer, and the highest recorded values were during winter and autumn at ST1. At station five (ST5) the stem diameter fluctuations remained constant during the four seasons. The highest non-rainfall precipitation was recorded at station one during autumn (167 mm) and the lowest were recorded at station five during autumn (9.5 mm). Non-rainfall precipitation affected diurnal stem diameter amplitudes in semiarid and arid environments.

Changes in stem sap flow rates were measured at hourly intervals with relative rate sap flow sensors interfaced with loggers installed on the same terminal branches of *P. repens* at each site along the climate gradient. The sensors were mounted on woody stems ranging from 1-5 mm in diameter. A portable modulated fluorimeter calculated the effective quantum yields of PSII ( $\Delta F/F_m'$ ) in 30 minute dark-adapted *P. repens* leaves.

*P. repens* L. displayed significantly negative correlations between their total daily amplitudes in sap flow and station maximum daily temperature both in winter, spring, summer and autumn. The leaves of *P. repens* L. displayed significantly negative correlations between

their total daily amplitudes in sap flow and photosynthetic effective quantum yield ( $\Delta F/F_m'$ ) at the 5 stations along the gradient during the months of October and November.

The results of this study stress the importance of the non-rainfall precipitation (dewfall) as a reasonably small but continuous source of water for some arid and semiarid environments, as well as its important contribution to the local water equilibrium, especially during dry periods. The non-rainfall precipitation gains make water available as an additional source of water for vegetation in dry summers.

The present study revealed that significantly higher sap flow rates of *P. repens* occurred at higher elevations and concomitant lower temperatures, compared to those at lower elevations and higher temperatures. *P. repens* at the lowest elevation (ST5) displayed significantly lower sap flow rates and peaked earlier in the day than those at highest elevations, particularly under moderately moist and dry conditions. Under wet conditions especially after good rainfall, sap flow of plants at highest elevation was inhibited (August, May). This could be due to soil saturation and consequent inhibition of root metabolism. The effective quantum yield ( $\Delta F_v/F_m'$ ) exhibited higher values during October under lower temperature at the highest monitoring station (ST1) and lower values during November under higher temperatures at ST1 during November. At the lowest station (ST5) slightly lower  $\Delta F_v/F_m'$  values under lower T in October than November were showed.

*P. repens* showed characteristics of whole plant water use common to other drought adapted woody plants. In the present study, *P. repens* plants, proved to be highly tolerant of water stress/drought and is well adapted to Mediterranean climatic conditions. Warmer and drier conditions in the next decades, as a results of climate change, may alter the fitness of *Protea repens* L. as a shrub species within the Fynbos Biome.

*Keywords: Climate, diurnal, drought, effective quantum yield, elevation, humidity, sap flow, stem diameter, temperature, water*

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## 2.1. Introduction

Several studies have confirmed that the ecological distribution of vegetation types are regulated by climatic conditions and precipitation is one of the most significant components driving the occurrence and diversity of species (Archibold, 2012). The availability of water is significant in influencing photosynthesis, development and survival of plants in dry and semi-dry areas, such as the Mediterranean (Bacelar *et al.*, 2007), and therefore is one of the main selective forces in plant evolution and therefore the plant's ability to tolerate drought stress is a paramount controller of natural plant distribution and production (Fischer & Turner, 1978).

Plants are regularly exposed to episodes of drought during their life cycles. Frequency of such droughts is projected to increase outside the present dry or semi-dry areas. The effects of drought or water shortage on plants are complicated and it involves adverse (plastic) or adaptive (elastic) changes. These effects can be synergistically or antagonistically adapted by the other pressures under field conditions (Chaves *et al.*, 2002). Plants adopt strategies of avoidance or tolerance that involve diverse plant mechanisms to respond and survive droughts (Xiong, 2006). A mechanism of drought avoidance occurs in species with high stomatal sensitivity to drought. Such species possess a high level of stomatal regulation that allows them to retain high plant water potentials for extended periods of drought. In contrast drought-tolerating species exhibit decreased stomatal sensitivity and experience a concurrent decrease in stomatal conductance and leaf water potential (Levitt, 1980; Picon *et al.*, 1996). The influences of drought are projected to increase with future rises in occurrence and intensity through changes in climatic conditions (Houghton *et al.*, 1995; Collins *et al.*, 2009; Ortuño *et al.*, 2010).

The Mediterranean climate is characterised by severe seasonal drought during some years that will increase resulting from climatic changes (IPCC, 2001; Martinez-Vilalta *et al.*, 2003). Therefore, a good understanding of the effects of drought is vital if predictions on the impact of climate change on Mediterranean vegetation are to be made (Borghetti, 1998). Climate change can cause several stresses on plants (Munné-Bosch & Alegre, 1999).

Long-term monitoring and understanding plant responses to rapid changing environmental conditions is crucial for exploring community dynamics and assessing the vulnerability of species to changes in climate (Mcdowell *et al.*, 2008; Hoffman *et al.*, 2009; Yates *et al.*, 2010). The Cape Floristic Region (CFR) has a Mediterranean climate and is a biodiversity hotspot. Bioclimatic modelling studies have predicted that the CFR is facing severe threats to its biodiversity as an effect of continuous changes in climate (Midgley *et al.*, 2002; Midgley *et al.*, 2003; Bomhard *et al.*, 2005; Hannah *et al.*, 2005). Proteaceae is part fynbos

vegetation growing in the CFR and the fynbos plants are facing unique challenges in terms of growth: during the winter rainy season, when moisture is abundant, growth is inhibited by low temperatures (Oliveira & Penuelas, 2002). The Mediterranean summers are described as dry and hot and moisture and heat stress are growth constraints. In Mediterranean species the growth season commence as soon as spring temperatures increase. Plants of Mediterranean-type ecosystems such as deep-rooted perennials (Proteaceae) have several drought avoiding strategies (Chaves *et al.*, 2002). According to Bohnert and Sheveleva (1998) early responses to water stress help with immediate survival.

The Mediterranean summers are described by low precipitation, high temperature, high irradiance and high water vapour pressure deficit (Di Castri, 1973; Llorens & Oliveras, 2003), while the winters can have long dry and cloudless cycles that can correspond with the coldest days (Kyparissis *et al.*, 2000; Llorens & Oliveras, 2003). The adaptability of species to environmental limitations is related to changes in functional traits such as photosynthesis and plant water status. Their link with their morphology is essential (Sandquist & Ehleringer, 1997). Mediterranean vegetation production and distribution can be limited by summer drought (Di Castri, 1973; Larcher, 2000; Llorens & Oliveras, 2003). Mitrakos (1980), Tretiach (1993) and Llorens, (2003) proposed that development and distribution of evergreen species during winter is attributed to cold stress. The contribution of non-rainfall atmospheric moisture by fog, dew and water vapour adsorption in arid and semi-arid Mediterranean-climate ecosystems is an essential component of micro-hydrology (Kosmas *et al.*, 1998; Agam & Berliner, 2006, Maphangwa *et al.*, 2012). Several studies investigated the influence of dew to the water balance in semi dry ecosystems such as the Mediterranean. Fog and dew are considered essential in improving plant water deficits by reducing transpiration water loss and thermoregulation problems (Atzema, 1992; von Willert *et al.*, 1992; Ninari & Berliner, 2002; Musil *et al.*, 2005). The significance of non-rainfall precipitation for the survival of plants should be examined during the summer period (Munné-Bosch *et al.*, 1999).

Early climate predictions for the Mediterranean regions indicate a decline in net rainfall and the amount of occasions and an increase in intensity (Christensen *et al.*, 2007; Domingo *et al.*, 2011). These predictions could reduce the availability of water in environments sensitive to desertification (Baldocchi, 2008; Janssens *et al.*, 2005, Domingo *et al.*, 2011). Olson & Dinerstein (1998) have identified non-rainfall atmospheric moisture as a crucial water source for plants in several ecosystems worldwide, where it essential in the distributions of species (Maphangwa *et al.*, 2012). Several techniques have been applied in the measuring non-rainfall atmospheric moisture for example, fog and dew with numerous difficulties (Ninari & Berliner, 2002). Lysimeters are often used in hydrological studies and they can quantify the

actual evapotranspiration from bare soil or from soil covered by vegetation (Xiao *et al.*, 2009).

Physiological parameters such as stomatal conductance, sap flow, leaf water potential (LWP), stem water potential (SWP), photosynthesis and stem diameter change (SDC) can be applied as indicators of daily plant water status change in response to environmental conditions (Cohen *et al.*, 1981; Garnier & Berger, 1985; Cohen *et al.*, 2001). Ortuño *et al.*, (2010) showed that the increase in growth is one of the most sensitive indicators of water stress in plants. Stem diameter vary diurnally due to differences in stem water content (Kramer & Kozlowski, 1979), the amount of variation depending on the rate of transpiration and the elasticity of shrinking and swelling in stem tissue (Peramaki *et al.*, 2001). Daily stem diameter change is highly sensitive to climatic conditions and has been shown to be closely related to plant water potential (Simonneau *et al.*, 1993; Cohen *et al.*, 2001). Several studies have indicated relationships between SDC and other water status indicators such as SWP (Kramer *et al.*, 1979; Milne *et al.*, 1983; Cohen *et al.*, 1999; Offenthaler *et al.*, 2001). Stem diameter change measurements are easily performed under field conditions by using a linear differential variable transformer (LDVT) probe (Ortuño *et al.*, 2010).

The irreversible radial growth, reversible living-cell dehydration/rehydration, thermal expansion and contraction, and expansion of dead conducting elements due to the increase and relaxation of internal tensions are the four component combination indicated by daily stem diameter variations (SDV) (Daudet *et al.*, 2005). The daily stem variation cycle provide three different common indices namely the maximum daily stem diameter (MXSD), minimum daily stem diameter (MNSD) and the maximum daily stem shrinkage (MDS). The latter is calculated as the difference between MXSD and MNSD (Ortuño *et al.*, 2010).

Water stress in plants is defined in many ways such as leaf or stem water potential, tissue water content and differences in stem diameter (Nadezhina, 1999). Water is generally the most significant of the environmental causes that limit plant development tempo. Water stress inhibits plant development and production (Bréda *et al.*, 2006; McDowell *et al.*, 2008; Attia *et al.*, 2015). Influences of environmental factors relating to whole-plant transpiration and photosynthesis are regularly determined from rigorous single-leaf measurements (Escalona *et al.*, 2002). These approaches have limitations due to large variations of leaf estimates across the canopy cover, and possibly to uncertainties as a result of certain settings in the gas exchange cuvettes compared to the ambient air (Goulden & Field, 1994; Smith & Allen, 1996; Sousa *et al.*, 2006). It is difficult to measure any of these parameters long term (Nadezhina, 1999). One indicator of plant water stress that can be measured automatically over a long term is sap flow through woody tissues (Granier, 1987, Köstner *et*

*al.*, 1996). The application of sap flow meters or linear variable displacement transducer sensors (LVTDs) appears to be a promising alternative to overcome these limitations (Smith & Allen, 1996, Link *et al.*, 1998). Transpiration rate in whole branches and the entire plant can be determined by measuring of the xylem sap rise in the stem. Sap flow measurement is a non-destructive method for determining continuous water use by plants in their natural environment (Allen & Smith, 1996; Schoeman, 2002). Consistent, direct estimates of plant or shoot transpiration to the leaf area are provided by sap flow measurements (Steinberg *et al.*, 1990; Ansley *et al.*, 1994; Braun & Schmid, 1999). According to Eastham and Gray (1998) sap flow measurements is an integrated indicator of plant hydraulic responses to diurnal and daily fluctuations in transpiration. In a study by Jian-Hua *et al.* (2007) it was found that the mean sap flow rate showed an important linear correlation with relation to net radiation, air temperature and relative humidity (RH). Previous studies have shown that water stress of grapevines at the leaf level reduced photosynthesis due to both stomatal and metabolic damage (Flexas *et al.*, 1999; Escalona *et al.*, 1999; Flexas, 1999). Photosynthesis and transpiration were also found to have been reduced at the whole-plant level in grapevines and other crops through measurements of trunk sap flow and LVDT measurements (Myburgh, 1996; Lovisollo & Schubert, 1998; Escalona, 1999). In a study by Eastham and Gray (1998) it was found that night time sap flow increased under water shortage conditions. High vapour pressure deficits at night would facilitate night time transpiration that would indicate partial stomatal closure and a reason for night time sap flow (Eastham & Gray, 1998). Sap flow instruments have been widely used to investigate plant-water relations and may prove to be invaluable tools for providing physiological knowledge (Ewers & Oren, 2000; Zeppel *et al.*, 2008).

Where there is a requirement to measure transpiration, tillers or entire plants the application of sap flow methods is far more advantageous over other techniques. Sap flow methods can easily be programmed and the recordings of plant water use with high time resolution can easily be automated and continuously recorded (Dugas *et al.*, 1993). Continuous recordings offer the capacity to significantly enhance our understanding of the dynamics of plant responses to rapidly changing environmental factors (Oren *et al.*, 1999; Ewers & Oren, 2000). The sap flow sensors can be duplicated and connected without disturbing the environment around the plant (Smith & Allen, 1996). These sensors can determine the changing aspects of water flow in the plant (Clearwater *et al.*, 2009). Several studies confirmed the role of tissue capacitance in buffering transpirational water (James *et al.*, 2003; Burgess & Dawson, 2008), reversal in sap flow direction, especially in large diameter roots growing in drying soil (Burgess *et al.*, 1998; Smith *et al.*, 1999) and the joining of stomatal physiology to hydraulic capacity and root performance (Clearwater *et al.*, 2004).

Advances in heat-pulse techniques have recently been made, allowing measurement of low and reverse flow rates in various stem sizes (Burgess *et al.*, 2001; Clearwater *et al.*, 2009). Techniques that have been applied to small diameter stems are heat pulse probes and constant power heat balance devices (Clearwater *et al.*, 2009). Damage can be caused to the vascular tissue through the insertion of a probe. A stem of large enough diameter is therefore needed to insert the probe so that it is not disruptive of normal sap flows. Cohen *et al.* reported that the diameters of stems were in the range of 5mm to 20 mm and the sap rates measured in these fast-growing annuals were relatively high (Cohen *et al.*, 1988, 1990, 1993; Cohen & Li, 1996; Clearwater *et al.*, 2009). An alternative to the heat balance technique for quantification of low rates of sap flow in leaf petioles and small stems have been developed recently which is probe insertion into the stem (Clearwater *et al.*, 2009).

During summer, high sunlight irradiance is linked with both high warming and atmosphere vapour pressure deficit (VPD). These together with the absence of precipitation, result in drought, with the severity of which differ among sites, lasting from one to six months (Ehleringer & Mooney, 1983). Therefore, a plant's performance is highly constrained by the combined effect of water deficit and high atmospheric water demand. Dehydration may affect plant tissues negatively, resulting in embolism and plant death (Vilagrosa *et al.*, 2003; 2010; Miranda *et al.*, 2010; Flexas *et al.*, 2014). Valuable information regarding leaf photosynthetic performance of plants under drought stress is provided by chlorophyll fluorescence (Baker & Rosenqvist, 2004). It is known that photosystem II play an essential part in photosynthetic responses to drought and during unfavourable environmental conditions (Misra *et al.*, 2006; 2011; 2012). In this study, the changes in the maximum quantum yield of photosynthesis were measured ( $\Delta F/F_m'$ ) in *P. repens* to evaluate its susceptibility to water stress and temperature.

Mediterranean climate change studies indicated that Mediterranean climate would become progressively warmer, drier, and likely less windy (IPCC, 2007) with consequent effects on vegetation. The objectives of this study were to determine whether *Protea repens* L. showed resilience to drought along a moisture gradient. We hypothesized that diurnal and seasonal stem diameter and diurnal sap flow would be directly related to environmental drivers of transpiration in *Protea repens* L. We compared the estimations of sap flow obtained from the sensors to environmental parameters such as temperature, vapour pressure deficit and to photosynthetic performance.

## 2.2. Materials and Methods

### 2.2.1. Study area and sites

The study was carried out on an altitudinal moisture gradient on the north-facing slope of Jonaskop (33°58'00 S 19°30'00 E) at altitude 1646 above sea level, in the Rivieronderend mountains of the Western Cape, South Africa. The area is recorded among 35 global biodiversity hotspots (Myers *et al.*, 2000; Mittermeier *et al.*, 2004) with a Mediterranean climate. The site is characterised by increasing precipitation and decreasing temperature with rise in altitude. Five sites (stations) were selected along the gradient at different elevations (Fig.1). The mean annual rainfall varies between 700 mm at the top (1303 m) and 400 mm at the bottom (744 m). At Jonaskop there is an average difference of 4.4 °C between the highest site (1303 m) (station 1) and the lowest site (744 m) (site 5). Five sites (stations) were selected along the gradient at different elevations (Fig.3.1). The mean daily summer temperature varies between 24.5 °C at site 1 and 29.5°C at site 5. The experiment was conducted between August 2011 and August 2012.

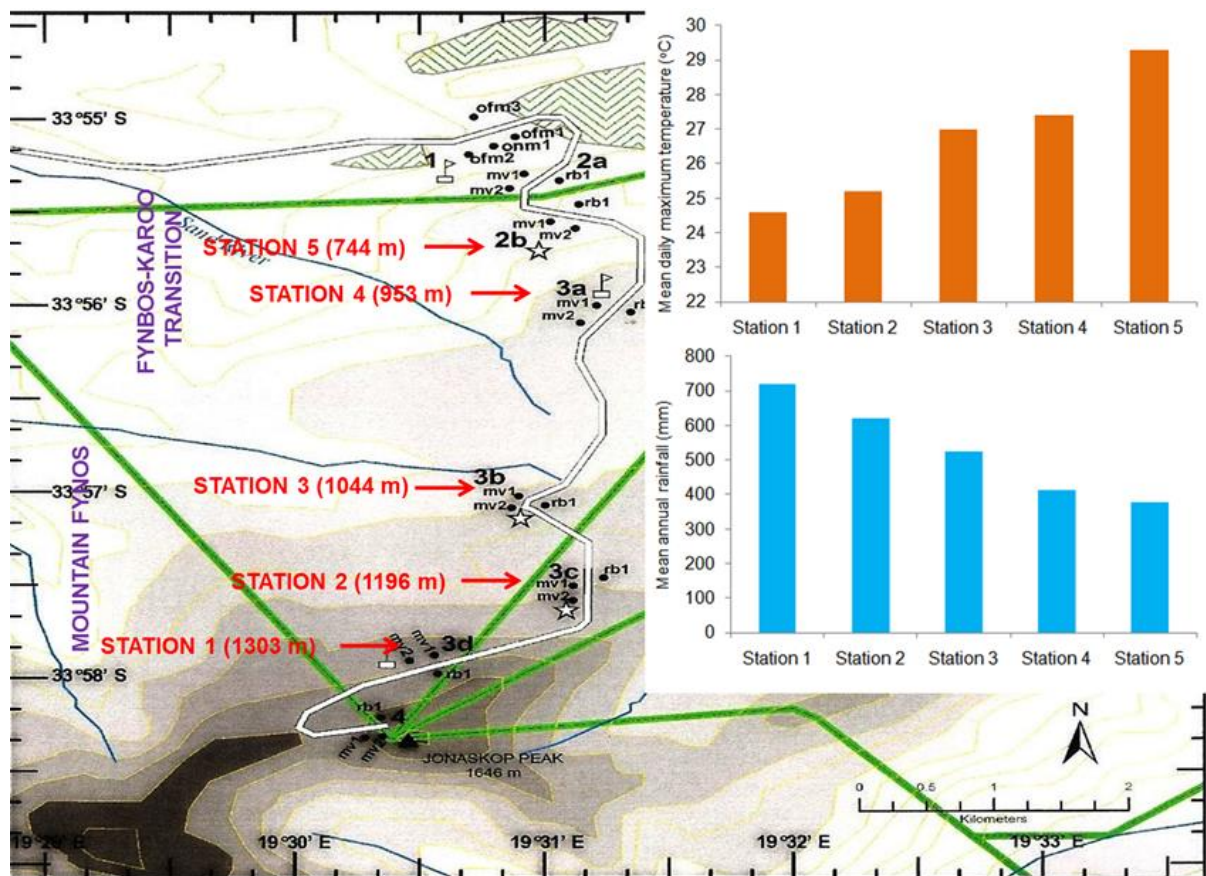


Figure 2.1. Topographical chart of the study site indicate the area of data loggers and species sampling plots. Contours represent 100 m intervals. Station 1; 1303 m; Station 2;

1196 m; Station 3; 1044 m; Station 4; 953 m; Station 5; 744 m. GIS data provided by Cape Nature, Department of Land Affairs: Surveys and Mapping and the Department of Agriculture. (Source: Lize Agenbag (adapted)).

Table 2.1. Location of weather stations along the gradient

	ST-1	ST-2	ST-3	ST-4	ST-5	ST-6
Full weather station	•			•		•
Mini weather station	▪	▪	▪	▪	▪	

The field plots were prepared at four selected altitudes (Figure 3.1): 1. 1303 m, near the top of the mountain; 2. 953 m, near the middle of the gradient; 3. 744 m, in the ecotonal zone, and 4. 545 m, in the succulent Karoo. Station 6 (ST-6) is the hottest site along the gradient. Table 3.1 indicate the location of weather stations at the different stations (sites) along the gradient

### 2.2.2. Species description

*Protea repens* belong to the Proteaceae family and is found in the Fynbos Biome in the CFR. The Fynbos Biome has strong climatic boundaries that are linked with its distribution (Rutherford, 1997). It is a large, thick-stemmed shrub (up to 300 mm in diameter) with broad leaves (Rebelo, 2001). The CFR is ranked among the 34 global biodiversity areas (Myers *et al.*, 2000; Mittermeier *et al.*, 2004). The Cape Floristic Region has a regular Mediterranean climate with hot, dry summers and cool, wet winters. *Protea repens* is a species found in the Fynbos Biome in the CFR. The Fynbos region has strong climatic boundaries associated with its distribution (Rutherford, 1997).

Fynbos is not found where the annual rainfall is below 200 mm (Rebelo, 1998), and is endemic to areas where more than 40% of annual rainfall occurs during the winter season (Cowling *et al.*, 1997). Cape fynbos grows in highly leached, extremely nutrient deprived soils that originated from quartzites and sandstone of the Cape Supergroup. Transitions to other vegetation types often occur with borders between sandy, nutrient poor soils and less sandy and more fertile soils such as those derived from shales (Cowling & Homes, 1992). *Protea repens* (L) L. (Sugarbush) is an upright, much branched shrub with a large, thick stem (up to 300 mm in diameter) with broad leaves and reaches a height of 2.5 metres but

can grow to a height of 4.5 m. *P. repens* (L.) is found at altitudes up to 1500 metres and is scattered in between other fynbos plants or in dense stands (Rebelo, 2001).

### 2.2.3. Environmental measurements

Temperature, rainfall and humidity were obtained hourly with thermocouples, relative humidity and tipping gauge sensors interfaced with small data loggers enclosed in ventilated radiation shields (Watch Dog 450, Spectrum Technologies Inc., Plainfield, Illinois, USA). Soil moisture was measured at hourly intervals using 5TE moisture sensors buried at 10 cm depth, which were interfaced with EM50 data loggers (Decagon Devices Inc., Pullman, USA) at each site along the climate gradient. Atmospheric vapour pressure deficits (VPD) were computed from the measured air temperature (T) and relative humidity (RH) applying the following formulae (Snyder & Paw, 2006): where VPD is vapour pressure deficit (KPa), T is air temperature (°C), RH is relative humidity (%).

$$VPD = [100 \times 0.6108 \exp((17.27T/T)+265.5)] / RH - [0.6108 \exp(17.27T/T+265.5)] \quad (1)$$

$$e = 0.6108 \exp(17.27T/T + 265.5) \dots\dots\dots (2)$$

$$es = 100e/RH \dots\dots\dots (3)$$

$$VPD = es - e \dots\dots\dots (4)$$

Where e = vapour pressure (kPa), T is air temperature (°C), es = saturation vapor pressure (kPa), RH = relative humidity (%) and VPD = vapour pressure deficit (kPa).

Daily amplitudes (fluxes) in atmospheric vapour pressure deficit amounts were estimated as the differences between the recorded diurnal maximum and minimum values over each 24-hr period.

Hourly records of soil volumetric water contents (ECHO-TM sensor, EM-50 logger, Decagon Devices Inc., USA), air temperatures, relative humidity and rainfall amounts were obtained with small data loggers enclosed in ventilated radiation shields (Watch Dog 450, Spectrum Technologies Inc., Plainfield, Illinois, USA) at each site along the climate gradient. Dewfall was measured with a portable lysimeter. Dew is part of the precipitation at a site. In semi-arid and arid regions dew play an important role in the water balance and dew can

significantly lower the evaporative loss of soil water during some periods of the day (Monteith, 1957).

### 2.2.3. Plant measurements

#### 2.2.3.1. Soil lysimeters

Portable soil lysimeters (Fig. 2.2 A-B) with measuring accuracy of 0.002 mm were used to quantify changes in water mass of mountain fynbos soil in response to precipitation events and dewfall. One lysimeter was installed at each site along the gradient. Soil cores (240 mm diameter x 45 mm deep) were excavated and transferred into lysimeter weighing pans. The pans were located 35 cm above the soil surface at each of the 5 stations. Changes in mass of soil substrates and load cell temperatures were logged at hourly intervals by a programmable circuit board installed in each lysimeter over 8 months starting in mid-winter (August) and terminating at the end of autumn (April). Replication of the lysimeters and other automated environmental sensors at the study site were prohibited by their cost.



Figure 2.2. (A) and (B) Portable lysimeter comprising plain fynbos topsoil in a measuring pan.

#### 2.2.3.2. Stem diameter measurements

Changes in stem diameter in response to precipitation events were measured with stem diameter variation sensors with a resolution of 5  $\mu\text{m}$  interfaced with loggers (LS-SD-56-L, Phytech Ltd, Israel). The loggers were installed on the woody stems of *P. repens* that varied in size between 400  $\mu\text{m}$  to 450  $\mu\text{m}$  and set to record at hourly intervals at each of the five sites along the climatic gradient. One stem diameter logger (2.3 A-B) was installed on *P. repens* at each station. Stem micro variation sensors are accurate incremental sensors that consist of a linear variable displacement transducer sensor (LDVT) probe mounted in a

special fixing bracket and a DC-powered signal conditioner for monitoring micro-variations of stem diameter in micron range (Ortuño *et al.*, 2010). Differences in stem diameter are translated via the LDVT to the conditioner, which allows stem diameter to be read to the nearest 5  $\mu\text{m}$ .

$\Delta\%$  Change in stem diameter was calculated as follows:  $\Delta\% \text{ Change} = (\text{MX} - \text{MN} / \text{MN}) * 100$   
where:

MX= maximum stem diameter change for the day

MN= minimum stem diameter change for the day

The  $\Delta\%$  change in stem diameter was calculated for two consecutive days during the four seasons of the year.



Figure 2.3. (A) and (B) Stem diameter sensor connected to plant.

#### 2.2.3.3. Sap flow

Sap flow sensors measure the velocity of sap flow by monitoring the changes in sap temperature when heat is applied to the stem. These measures are used to measure transpiration rates in plants. Relative sap flow sensors use heat as a tracer. Stem heat balance (SHB) are highly precise incremental sensors that functions by measuring heat transmission due to the movement of sap when the stem is continuously heated. As heat is applied to the stem, the flow of sap is resolved by balance of the fluxes of heat from vertical loss by conduction in the stem, radial heat loss by conduction and by heat loss from sap flow (White & Raine, 2008). The operation of SF-4M sensors is created on a constant heat method with symmetric disposition of thermo-sensitive elements. A small heater warms a

part of a stem to 2-3 degrees above the ambient temperature. Junctions of the thermopile are located at identical distance above and below the heater. Sap flow was measured using the stem heat balance technique (White & Raine, 2008) in the same plants used for stem diameter measurements throughout the experimental period. Changes in stem sap flow rates were measured at hourly intervals with relative rate sap flow sensors (SF-4M Phytech Ltd., Israel) interfaced with loggers installed on the same terminal branches of *P. repens* at each site along the climate gradient. One sensor was mounted on a woody stem per station ranging from 1-5 mm in diameter.



Figure 2.4. Sap flow sensor attached to the plant.

#### 2.2.3.4. Chlorophyll fluorescence measurements

Chlorophyll *a* fluorescence is a useful, non-destructive tool for energy screening of plant photosynthetic reactions to temperature (Bilger *et al.*, 1989) and provides a sensitive indicator of photosystem II (PS II) efficiency (Golding & Johnson, 2003). A portable modulated fluorimeter (OSI-F1: Opti-Sciences Inc, Hudson, NH) measured effective quantum yields of PSII at a steady state ( $Y_s = F_{ms} - F_s / F_{ms} = \Delta F / F_m'$ ). Diurnal hourly measurements were conducted on young, randomly selected leaves of *P. repens*. The leaves of *Protea repens* were exposed to 0.8-s saturating light pulse of  $15,000 \mu\text{mol m}^{-2} \text{s}^{-1}$

under clear sky conditions. The diurnal fluorescence measurements taken at hourly intervals from 09h00 to 16h00 were conducted monthly, over an 8-month period from August (winter) through to February (late summer). Five replicated fluorescence measurements taken on five randomly selected young leaves of four *P. repens* plants at each station along the gradient (20 measurements per hour per station per month).

The photochemical quantum yield normally corresponds to the 'optimal quantum yield', which is lowered by illumination to an 'effective quantum yield'. Therefore, the following fluorescence expressions for determination of PSII quantum yield are obtained (Schreiber, 2004):

Optimal quantum yield:  $F_v/F_m = (F_m - F_o)/F_m$ ; Effective quantum yield:  $\Delta F/F_m' = (F_m' - F)/F_m'$ ;  $Y_s = F_{ms} - F_s/F_{ms} = \Delta F/F_m'$

where,

$F_s$  = steady state of fluorescence

$F_v, F_v'$  = variable yield of fluorescence (i.e.  $F_m - F_o$ )

$F_v/F_m$  = optimal quantum yield of PS II

PS II = photosystem II

$F_m, F_m'$  = maximal yield of fluorescence

### 2.3. Statistical Analysis

The net amounts of water gained weekly by the soil were computed as the differences between the recorded maximum and minimum water masses over 7 day period. These weekly net fluxes in water content were divided by the lysimeter pan surface area to convert them to mm of precipitation intercepted. Daily changes (fluxes) in stem diameter were calculated as the differences between the recorded daily maximum and minimum values over each 24-h period.

Measured effective quantum yields of PSII at a steady state were normally distributed. Pearson correlations tested for significant correspondence between daily sap flow, temperature, vapour pressure deficit amplitudes and  $\Delta F/F_m'$ .

## 2.4. Results

Rainfall (mm) was recorded only at the wetter site (ST-1) and the drier stations (ST-6) (Figure 2.5). Soil moisture (kPa) was recorded at all the stations (Figure 4.2). Some data are missing because of malfunctioning of the sensors.

### 2.4.1. Soil lysimeter

The highest weekly net amounts of precipitation over an 11 month period were intercepted by fynbos soil at station 1 (167 mm in May), station 2 (144 mm in Jan), station 3 (146 mm in Apr), station 4 (131 mm in Apr) and station 5 (131 mm in Apr) (Fig. 2.3). The lowest weekly net precipitation over the same period were intercepted at stations 2 and 5 (9 mm Mar and Apr), station 3 (10 mm in Feb), station 4 (12 mm in Oct) and station 1 (14 mm in Mar). The highest precipitation at all the stations was intercepted during the wetter mid-winter (June-September) and the lowest were intercepted during the drier months (December- February). The amounts of non-rainfall precipitation decreased along the gradient from the highest at station 1 to the lowest at station 5.

Although dewfall was not measured, the weekly moisture input into the soil measured by the lysimeters without any rainfall event, could be attributed to fog and dew events. At all 5 sites along the gradient (Fig. 2.6) this was evident and the amount of volumetric soil moisture measured during non-rainfall events were between 10-20 mm per week . The peaks on the graphs gave an indication of the rainfall events that took place and just after a rainfall event the dewfall is higher than normal (soil moisture is higher than 20 mm). The rainfall events is also evident from the weekly rainfall from 2011-2012 (Fig 2.5). Higher amounts of dewfall were recorded in late winter and early spring at all the stations along the gradient. This is also the time when vegetation starts active growth in the CFR. Dewfall was the lowest during the summer and autumn at all the stations. The highest dewfall occurred directly after a rainfall event (Fig. 2.6).

### 2.4.2. Changes in stem diameter

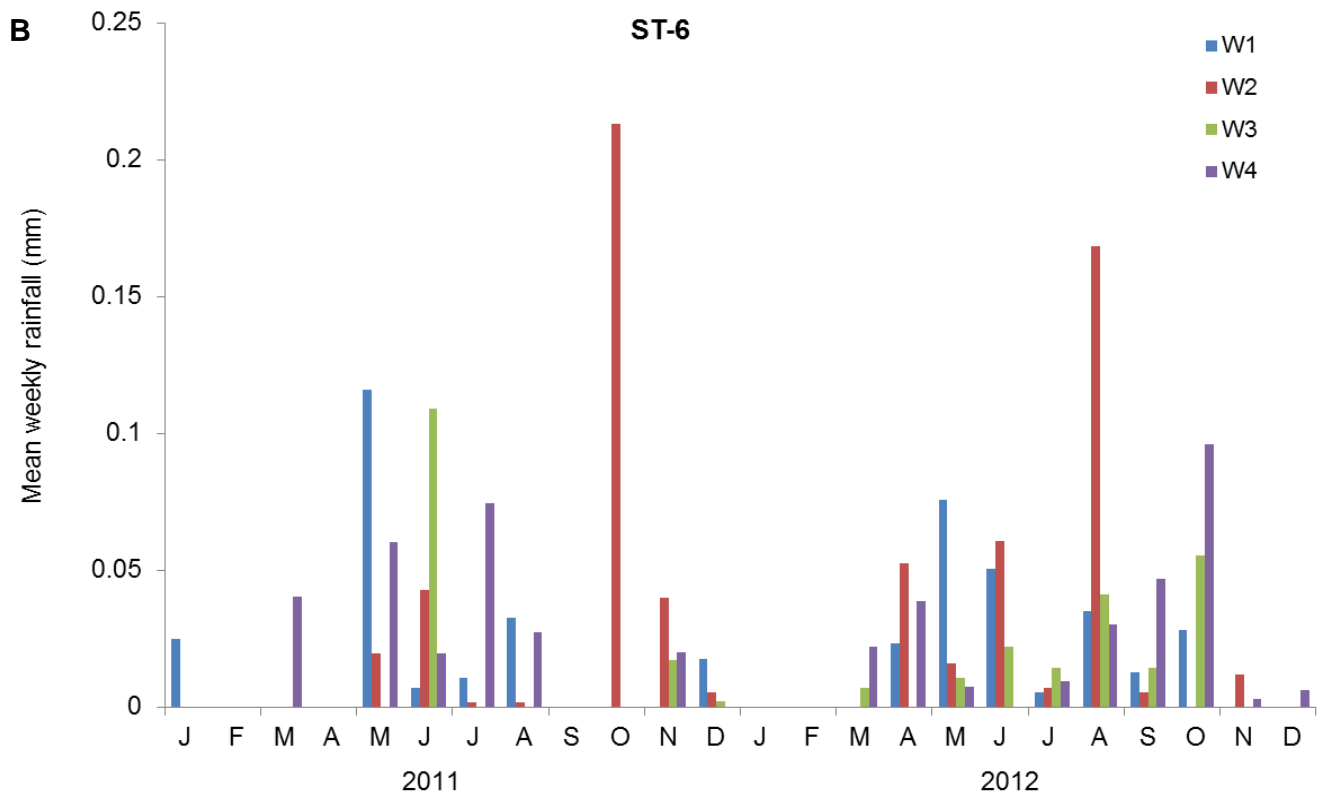
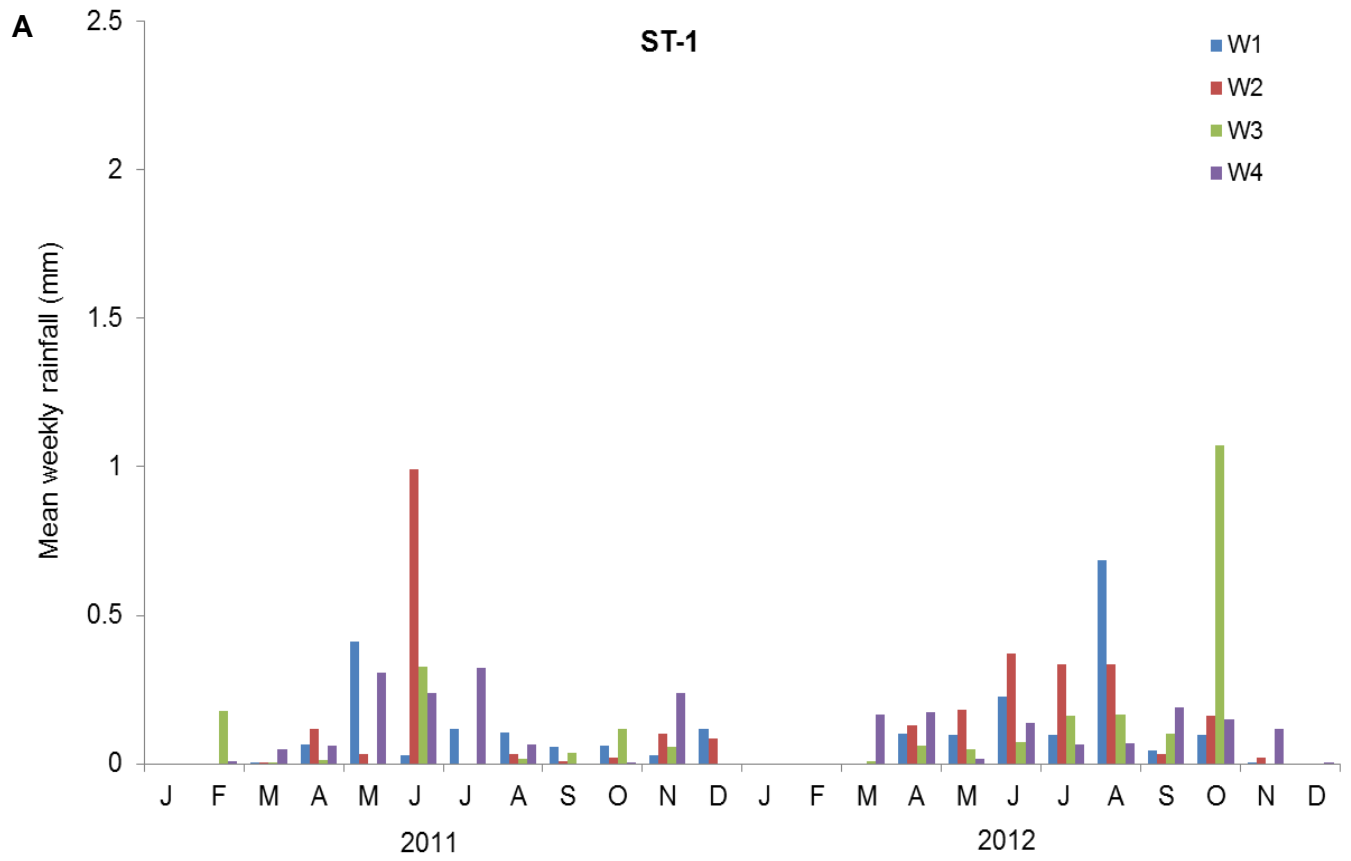
Tree stems, shrink due to decreased xylem water potential. The reason for the shrunk in the stems is that as the stomata open each morning, the transpiration stream runs to substitute the water lost. Then gas exchange between the leaves and the environment starts, and the leaves partially dehydrate. In the afternoon, as stomata close and transpiration tempo

decreases, root water uptake exceeds tree water loss leading to increased xylem pressure potential and the stem diameter starts to increase (Ferreles *et al.*, 2003).

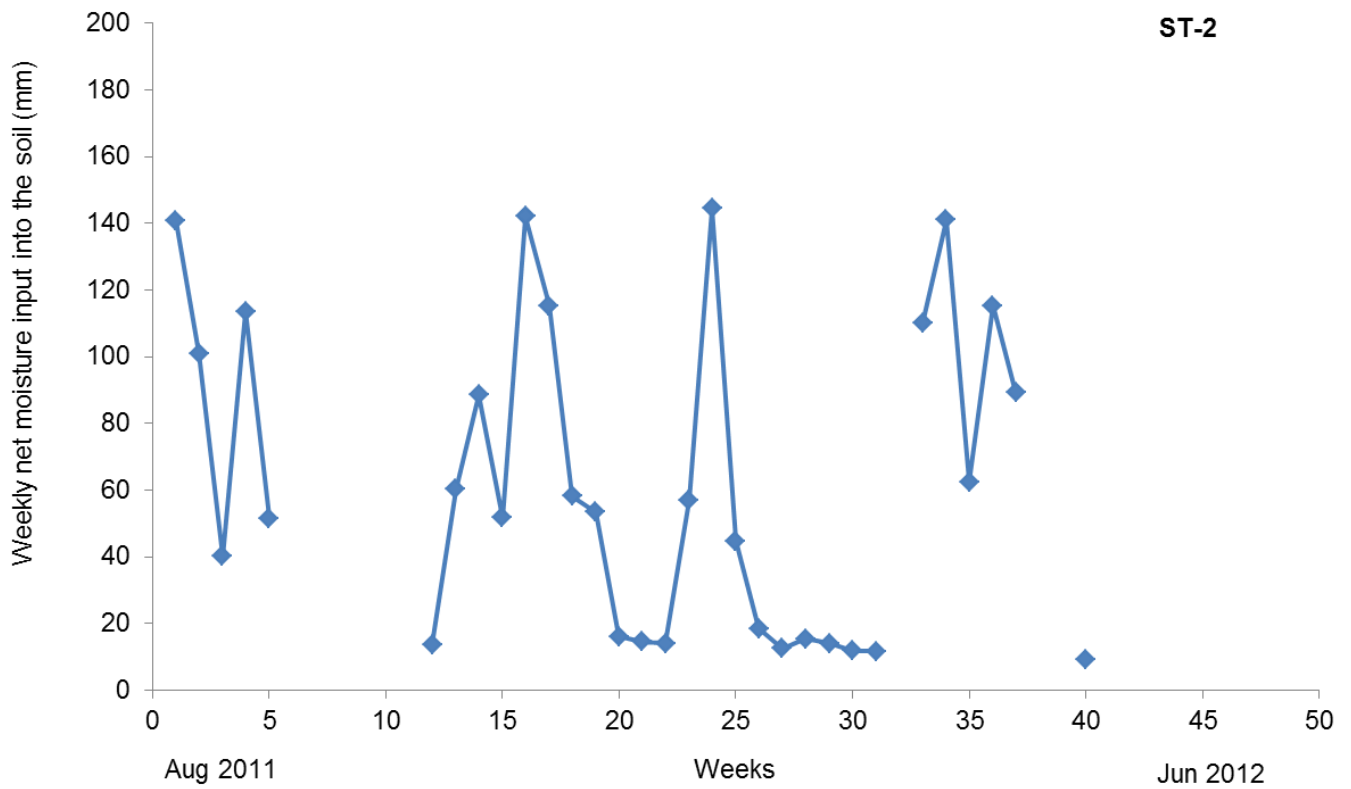
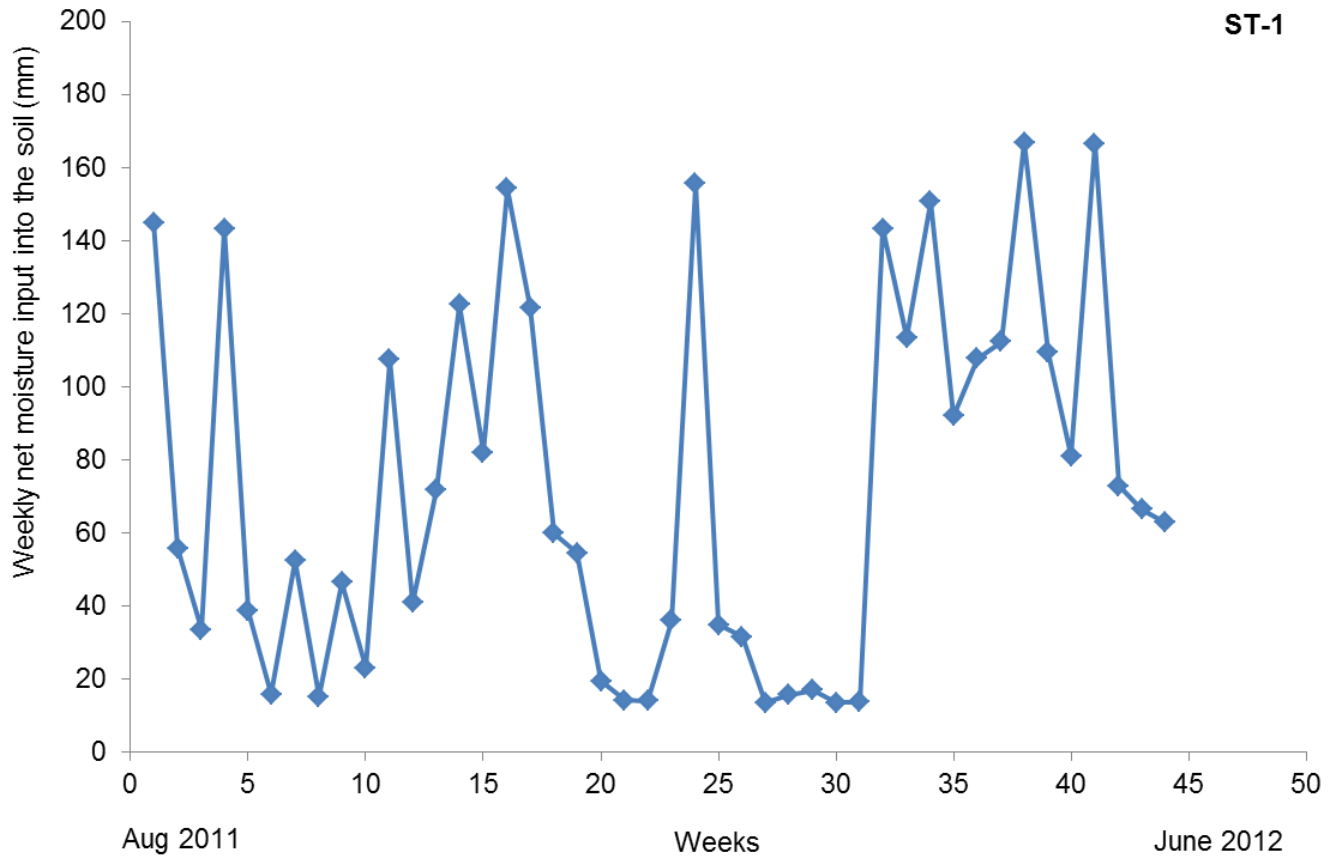
The results of the present study show that in August (winter) the stem diameter change was 0% between consecutive days and 8% in September at ST-1. Station 2 indicated a 5% increase between consecutive days during August and a 1% decrease between consecutive days (Fig. 2.7 A). No data for St-3 during August was available. At ST-3 during September there was a 0.2% increase in stem diameter between consecutive days (Fig. 2.7 B). At ST-4 during August a 0.3% increase was found between two consecutive days while a 0% change was indicated between two consecutive days during September. During August and September ST-5 showed 0% change between two consecutive days (Fig. 2.7C).

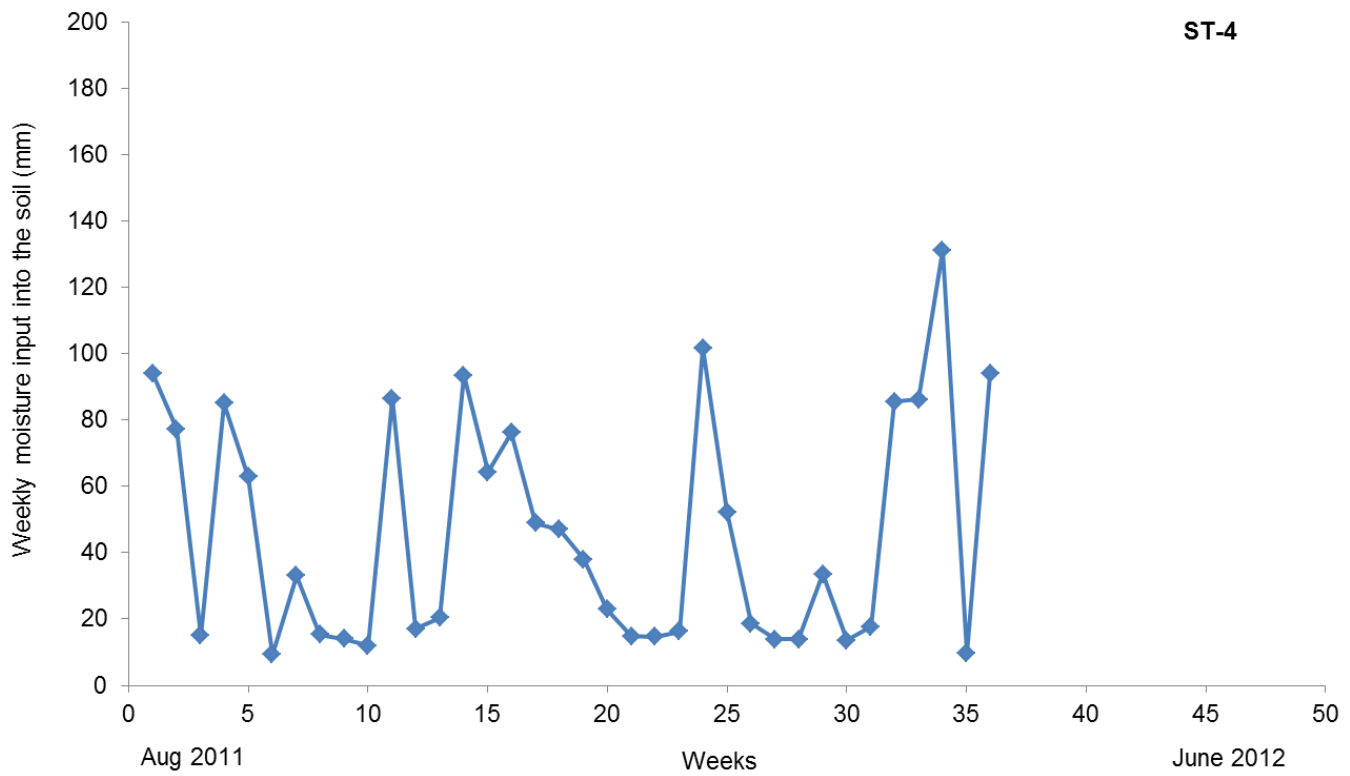
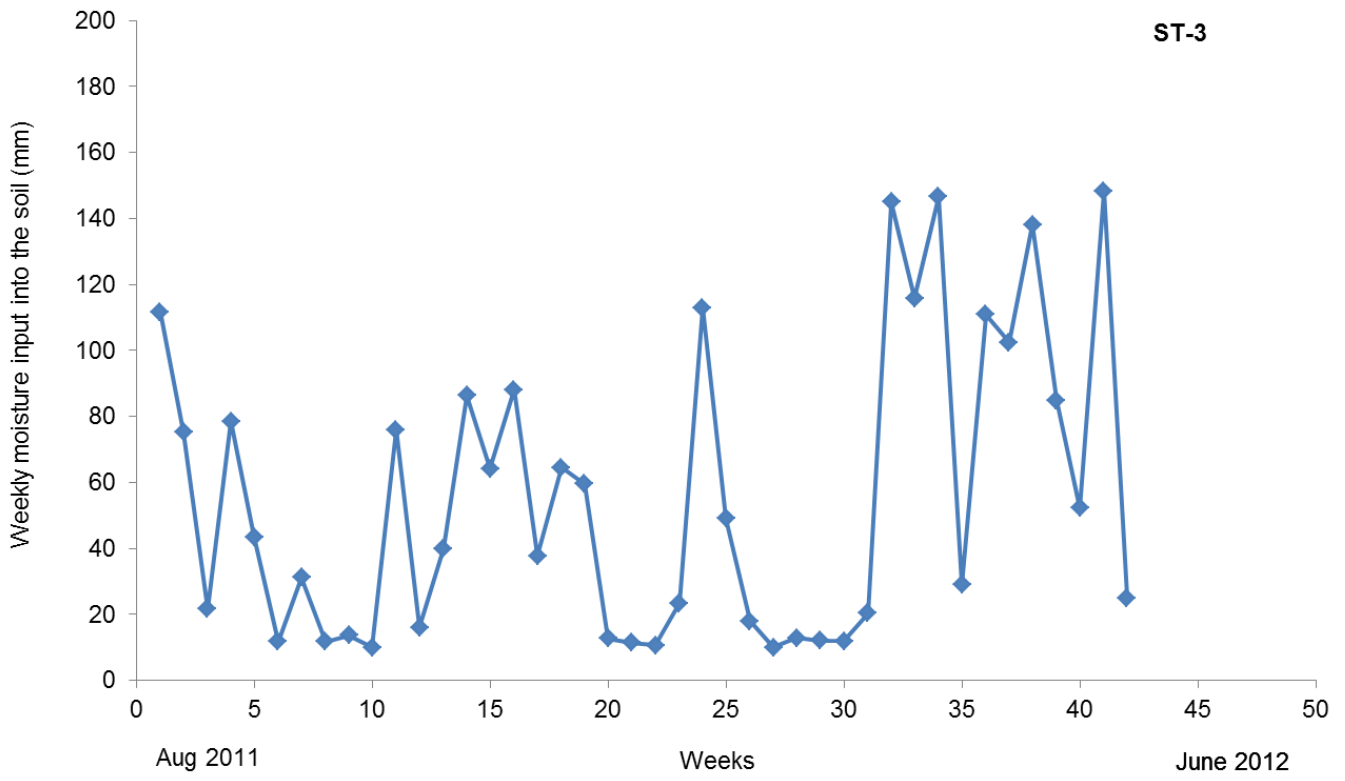
During October and November (spring) at ST-1 a 1.1% and a 1.9% increase in stem diameter was found between consecutive days (Fig. 2.7 D). Station 2 reported a 0.1% and 0.4% increase in stem diameter, ST-3 showed a 0.3% decrease during October and a 0.71% increase in stem diameter during November during consecutive days (Fig. 2.7 D-E). Station 4 showed a 1.3% increase in stem diameter during consecutive days during October and a 0.4% decrease during November (Fig. 2.7E). Station 5 showed a 0.35% increase in stem diameter during October and 0.03% increase during November during consecutive days (Fig. 2.7F).

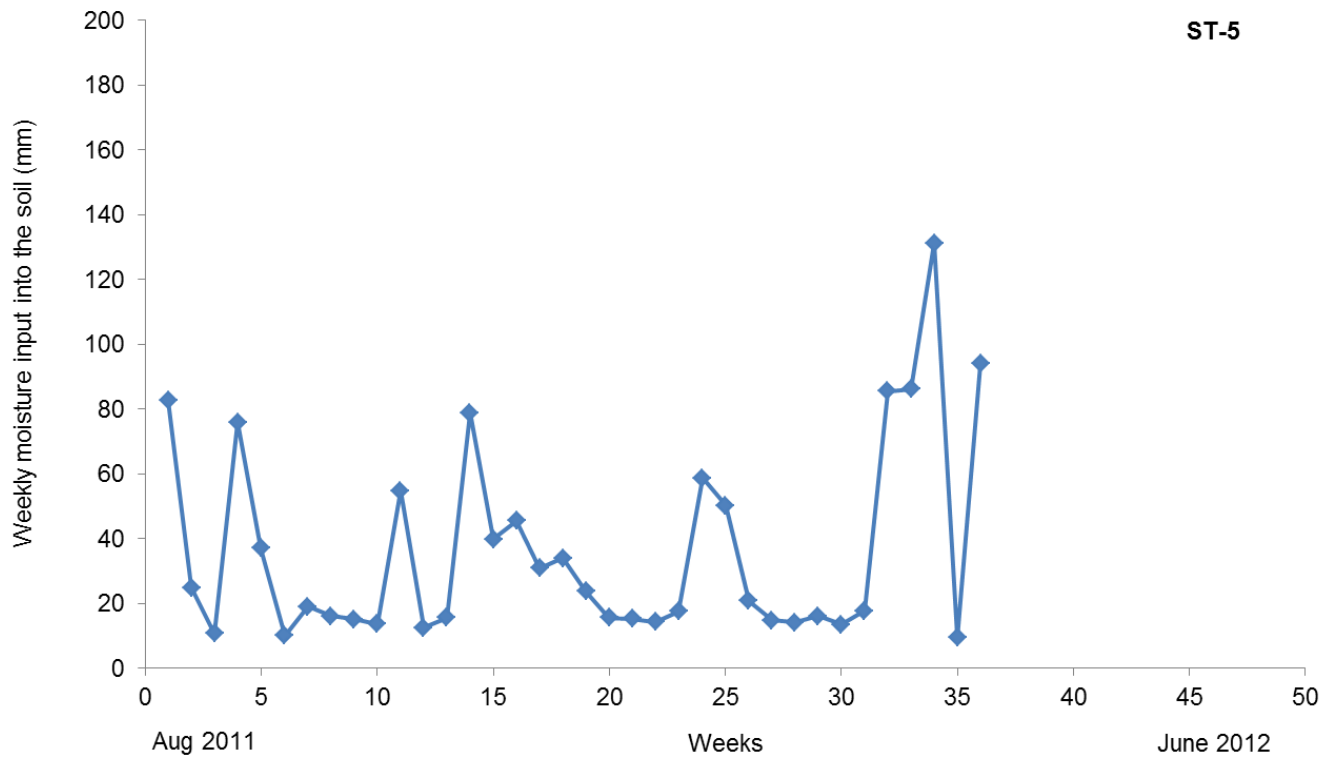
During summer (December, January and February), ST-1 showed a 1.1% increase in stem diameter during two consecutive in December, a 1.9% decrease in January and a 2.2% decrease during February (Fig. 2.7G). Station 2 showed a 0.6% decrease in December and January and a 1.3% decrease in February in stem diameter (Fig. 2.7G). Station 3 showed a 1.2% decrease in December, a 2.1% decrease in January and a 2% decrease in stem diameter during February. Station 4 showed a 0% change in December, a 2 % decrease in January and a 0.2% decrease in February stem diameter between two consecutive days. Station 5 showed 0.5% increase in December and February, 0% change in January in stem diameter during two consecutive days (Fig. 2.7H). During autumn (March and April), ST-1 showed a 5.4% increase in stem diameter in March and a 5.0% decrease in April during two consecutive days. Station 2 showed a 0.2% decrease in stem diameter during March and a 1.0% decrease April between two consecutive days. Station 3 showed a 2.2% decrease in March and 8.7% decrease in April in stem diameter during two consecutive days (Fig.2.7K). Station 4 showed a 0.4% decrease in stem diameter during March and a 3.6% increase in stem diameter in April. Station 5 indicated a 0.1% decrease in stem diameter March and a 0.9% decrease in April during two consecutive days (Fig. 2.7K).



**Figure 2.5. Mean weekly rainfall (mm) at (A) station 1 and (B) station 6**

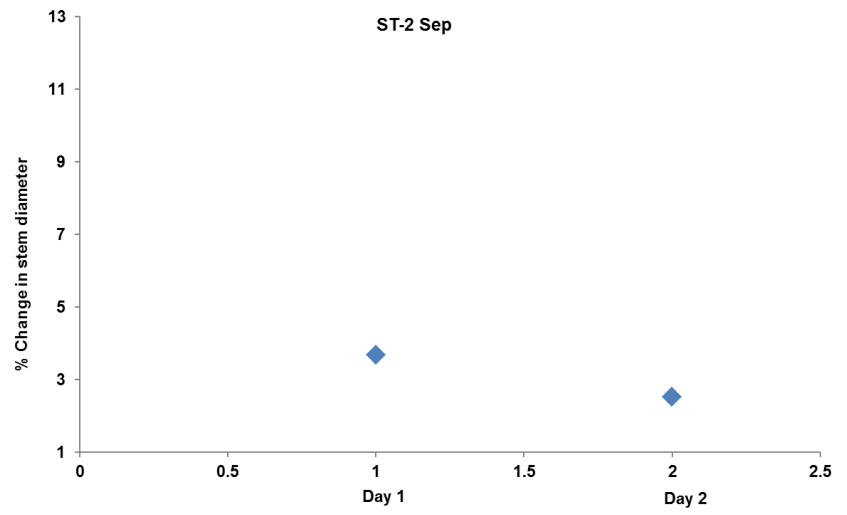
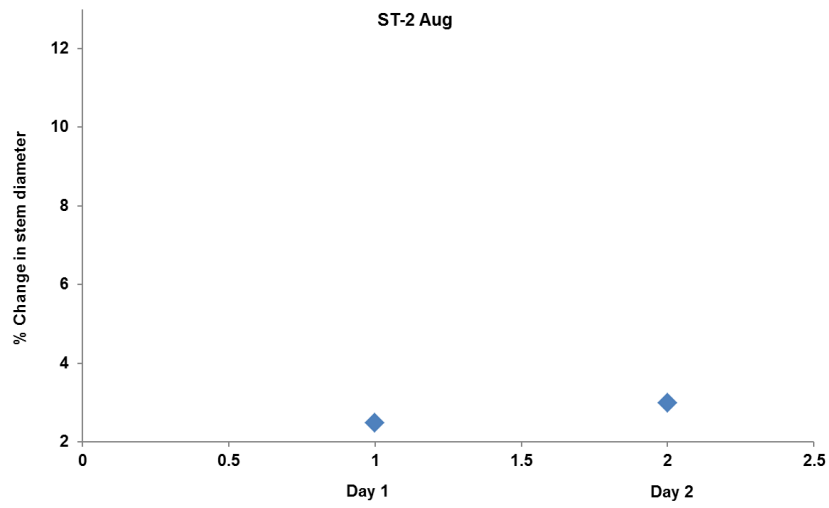
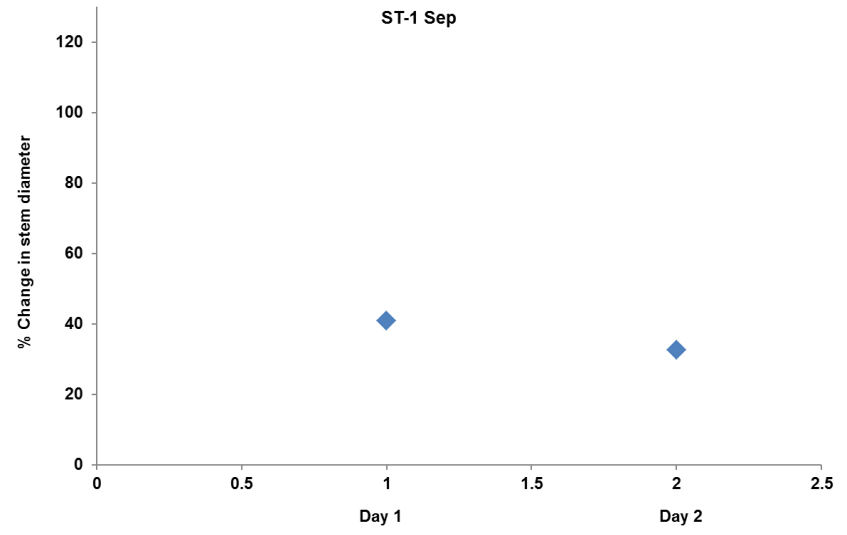
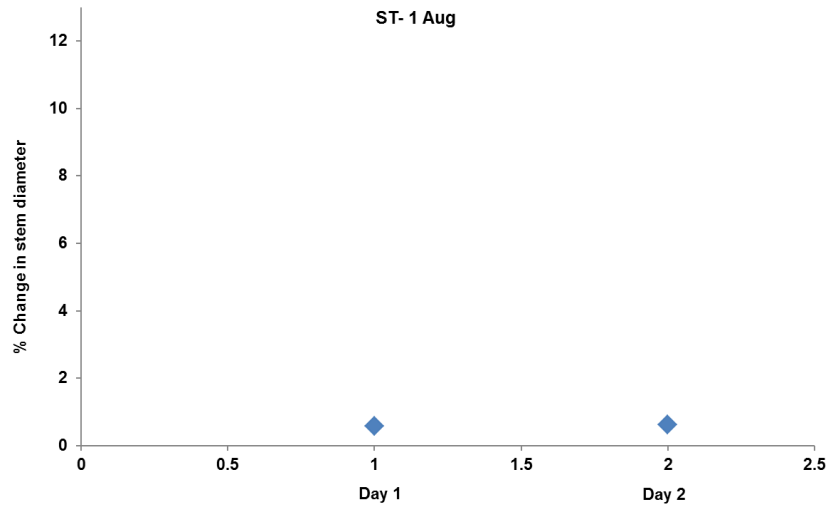




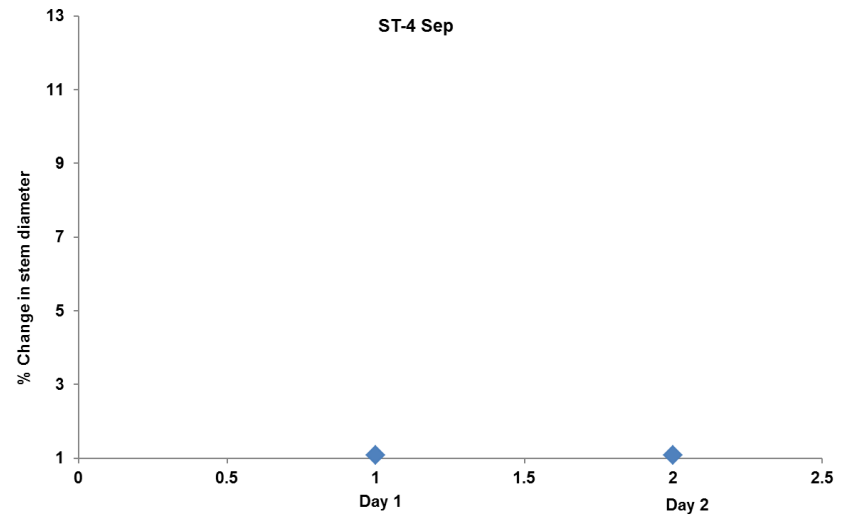
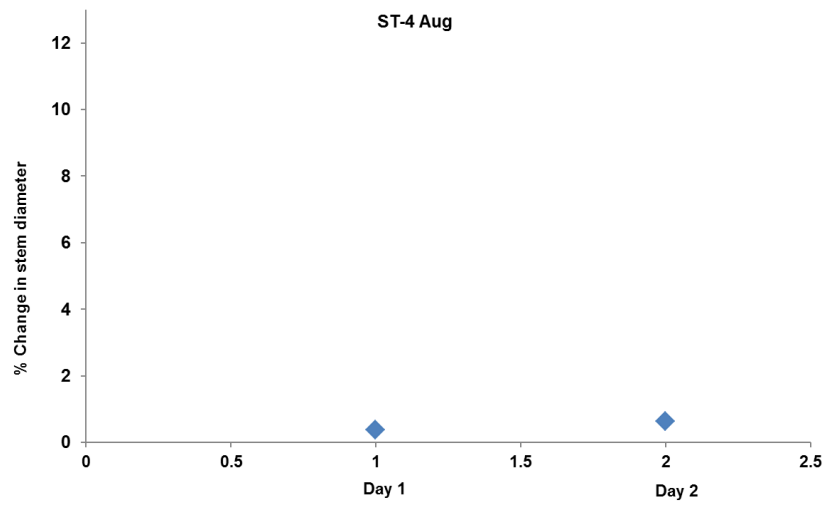
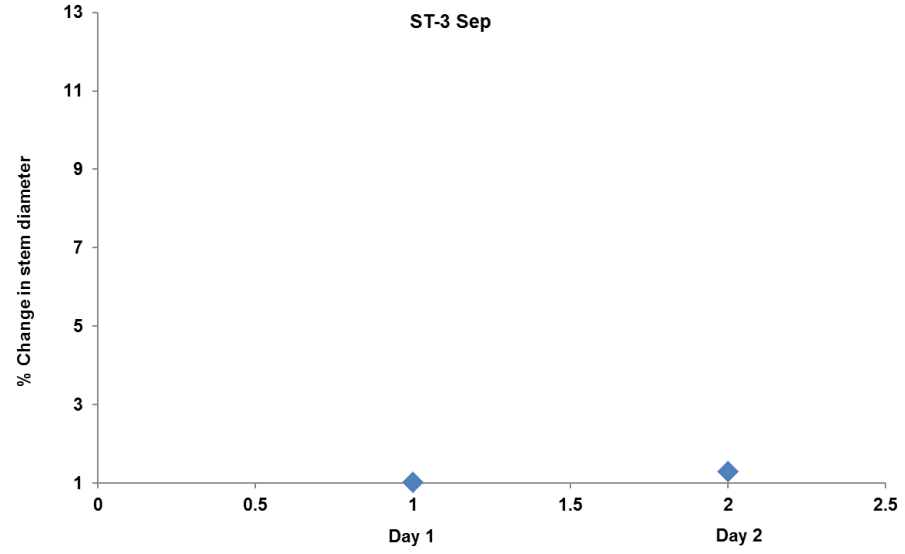


**Figure 2.6. Weekly net fluxes in volumetric soil water content (mm) at stations 1-5.**

**A**



**B**



C

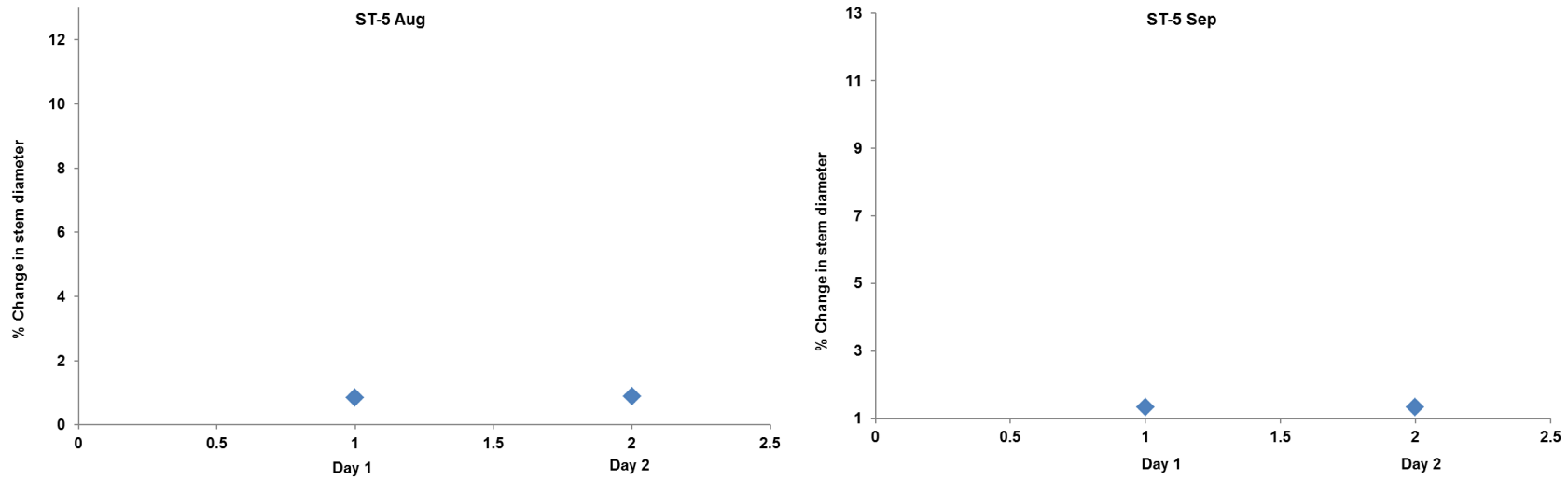
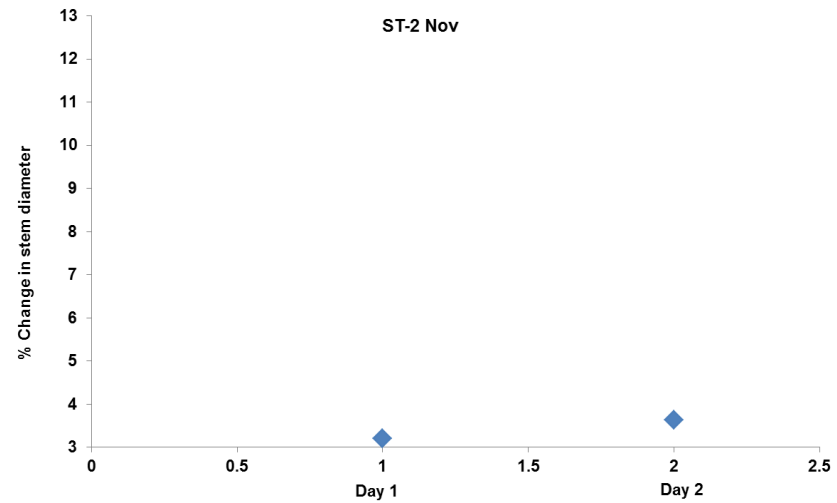
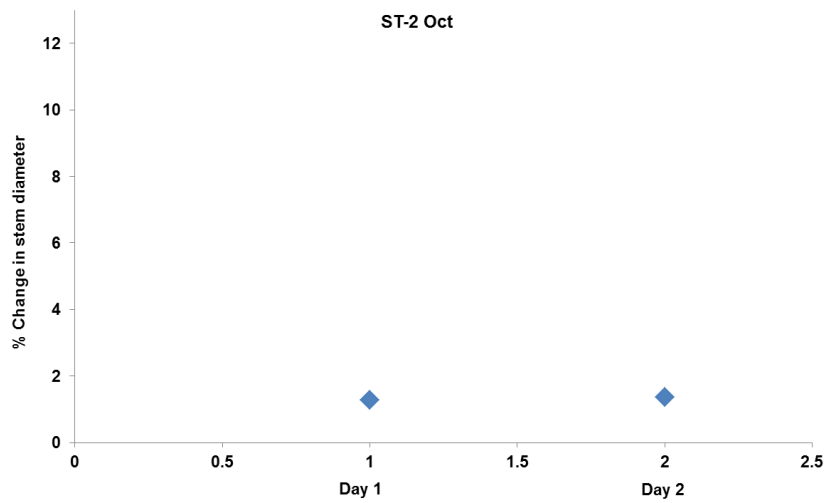
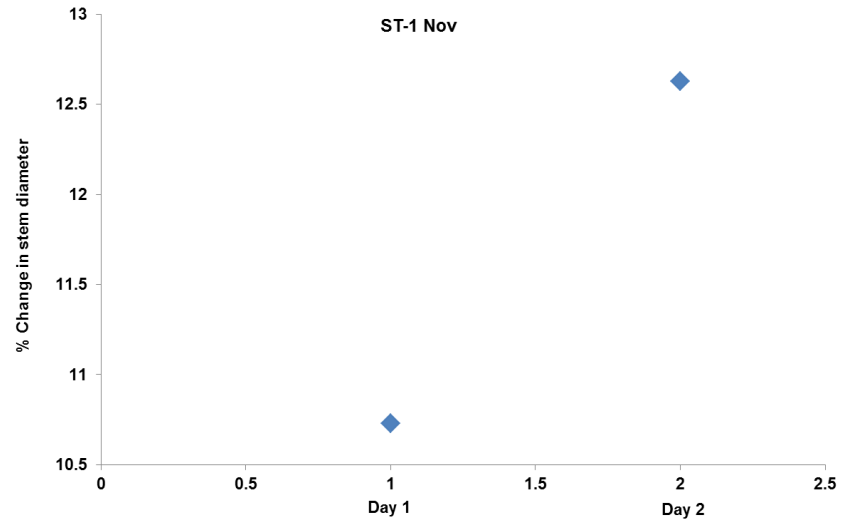
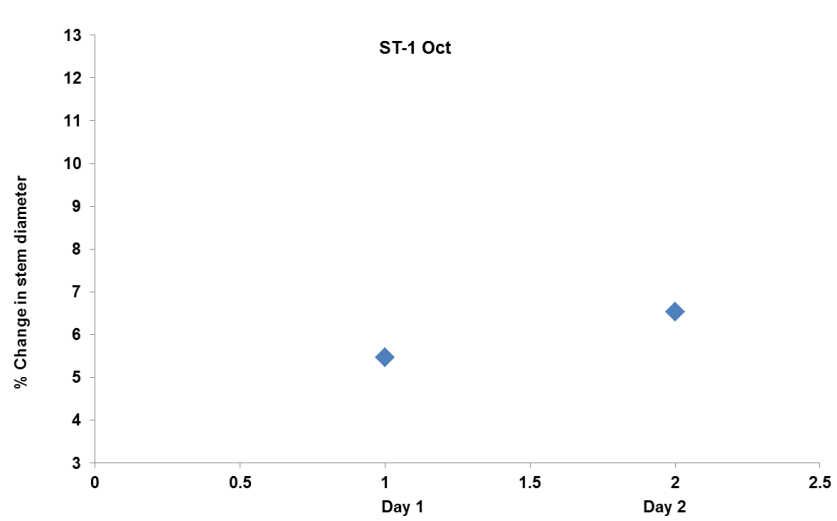
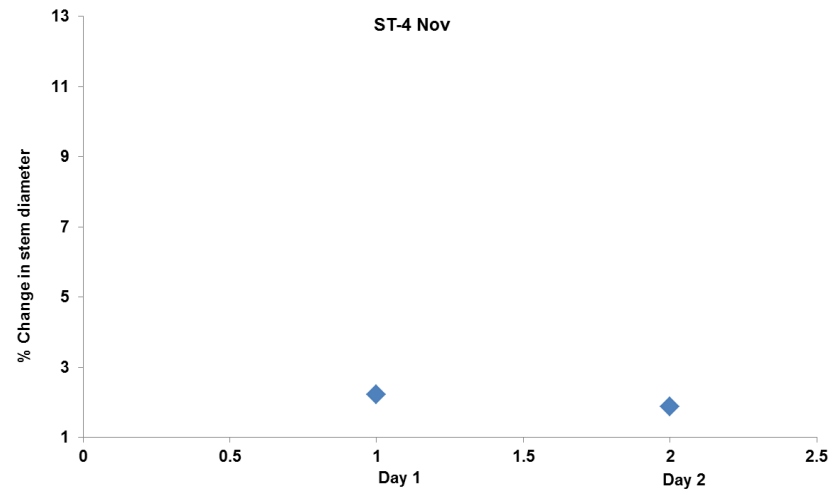
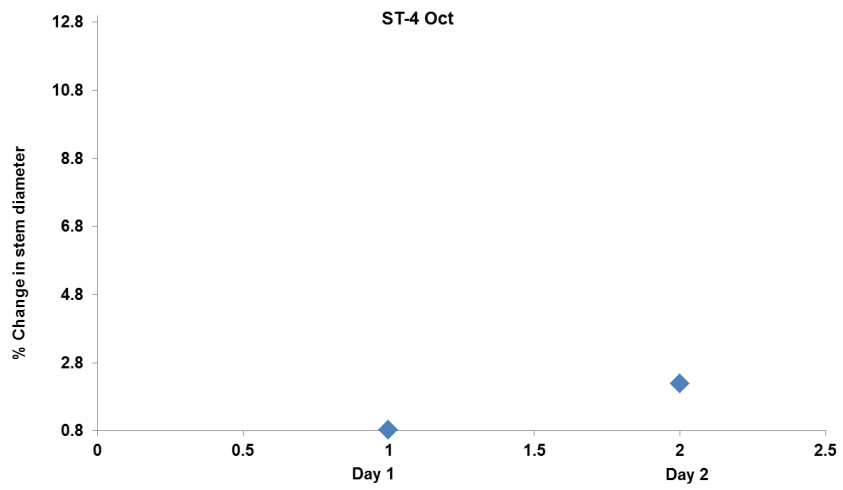
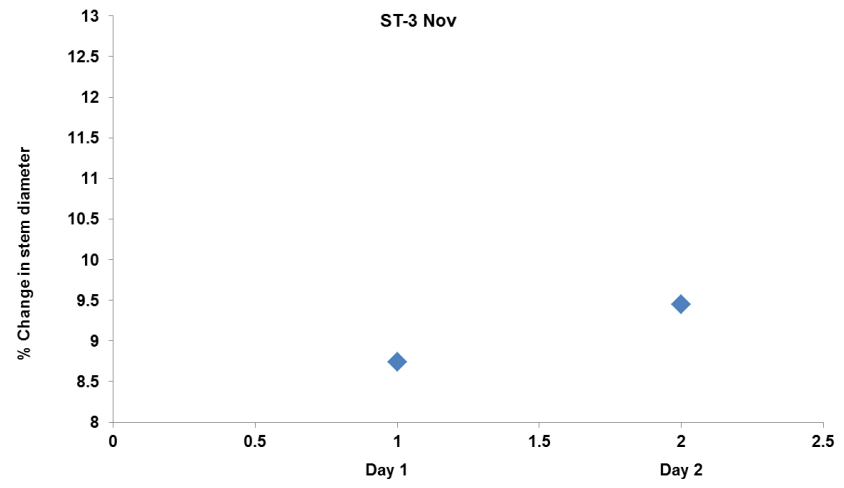
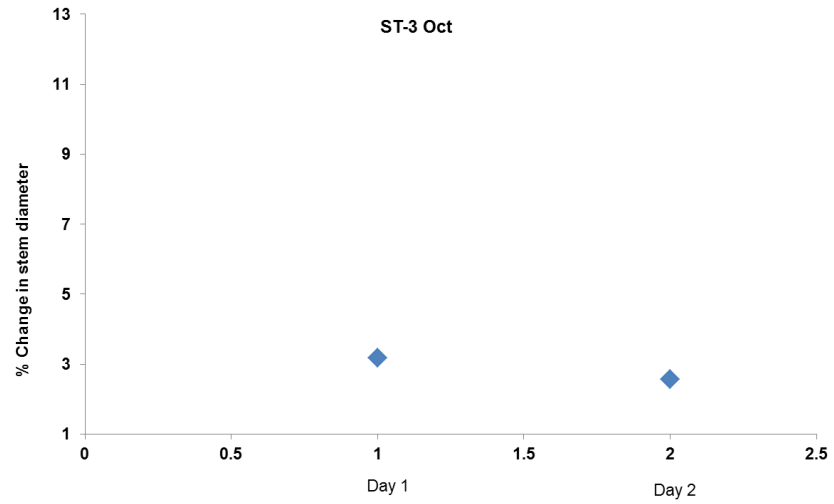


Figure 2.7(A-C). Percentage change in stem diameter on two consecutive days in *P. repens* for spring (August and September) at five different stations along a moisture gradient.

**D**



**F**



**F**

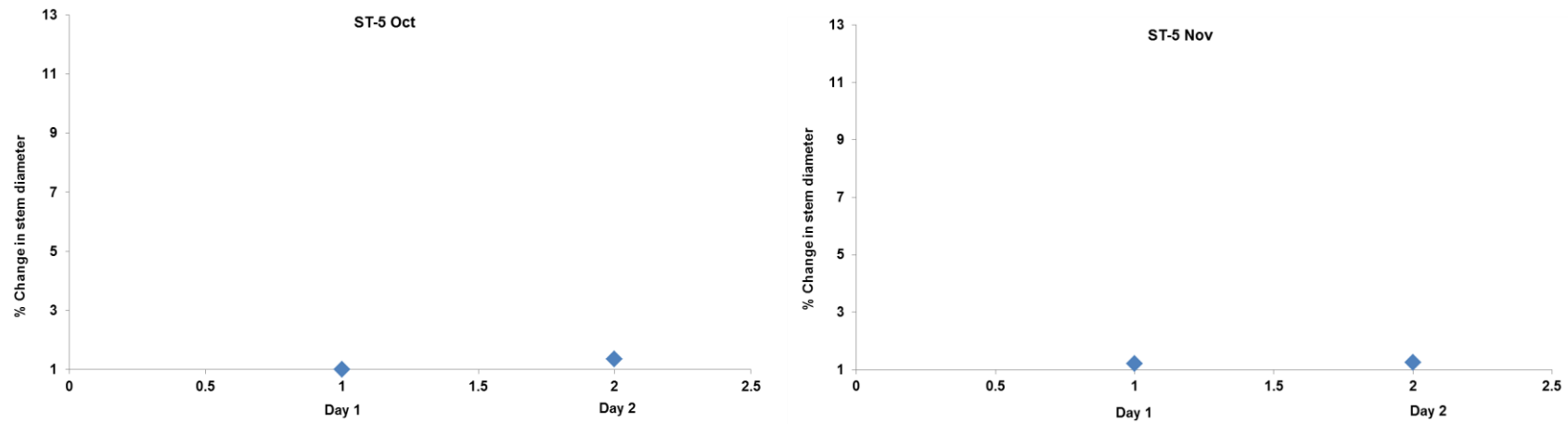
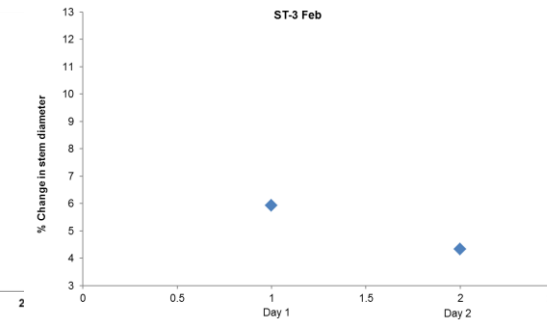
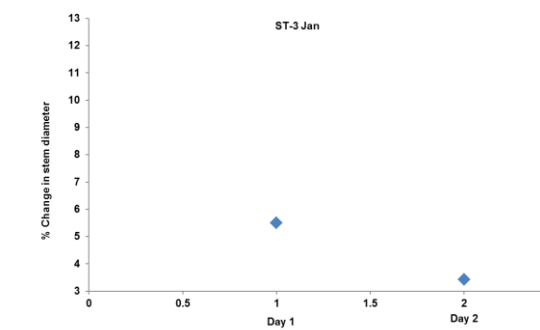
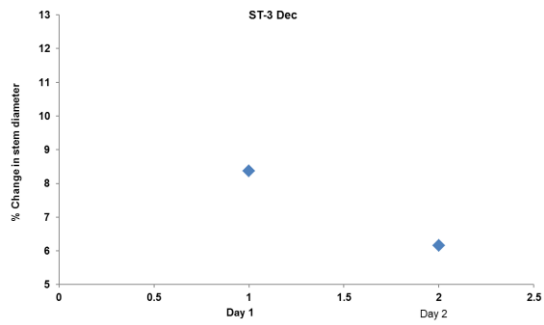
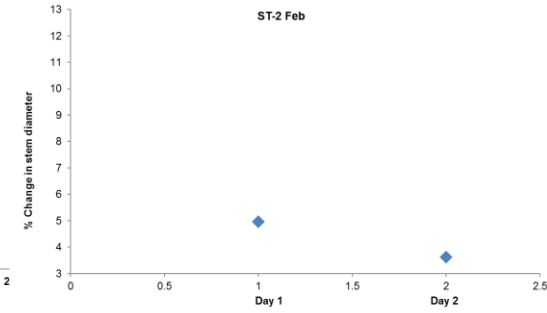
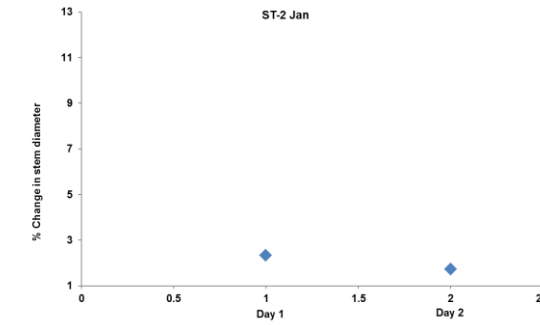
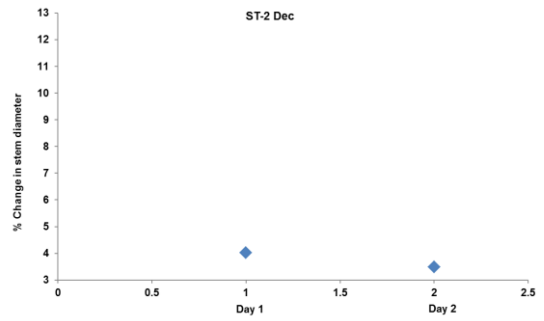
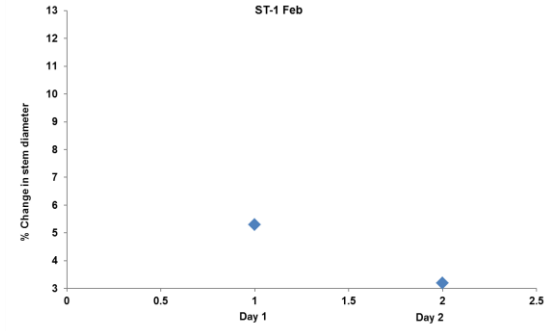
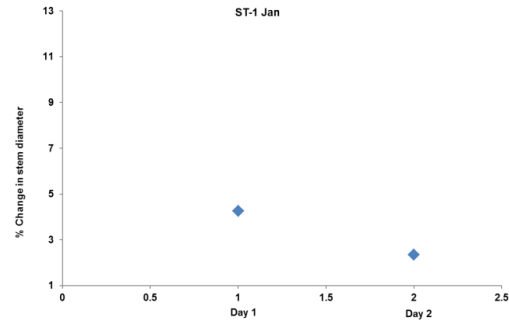
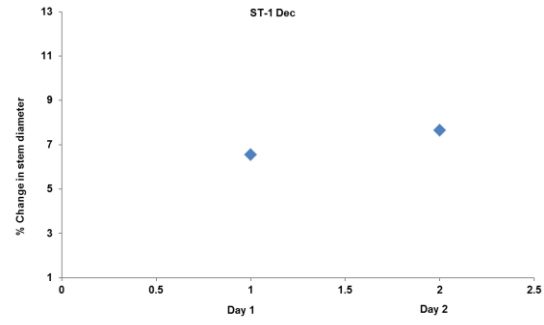


Figure 2.7(D-F). Percentage change in stem diameter on two consecutive days in *P. repens* for spring (October and November) at five different stations along a moisture gradient.

**G**

H

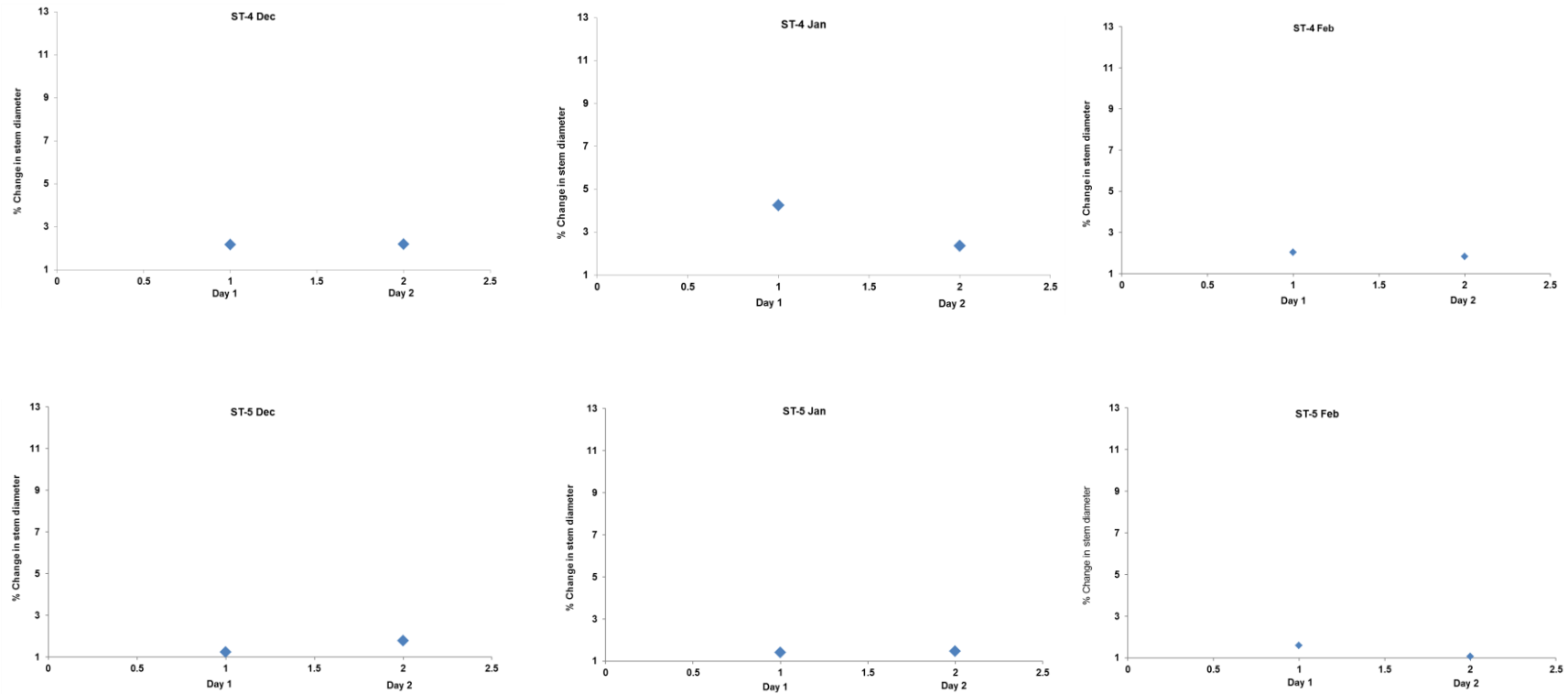
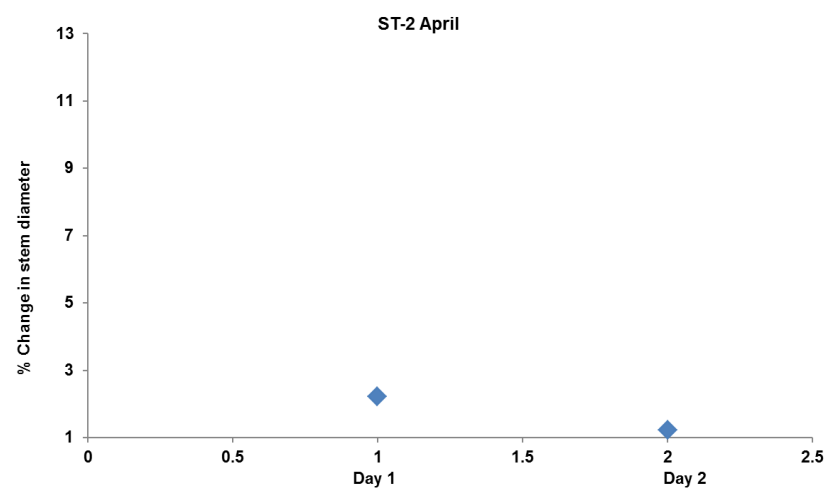
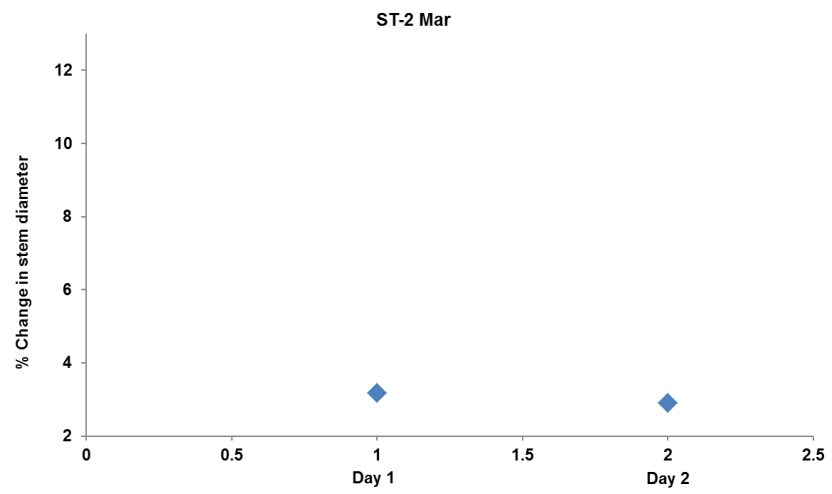
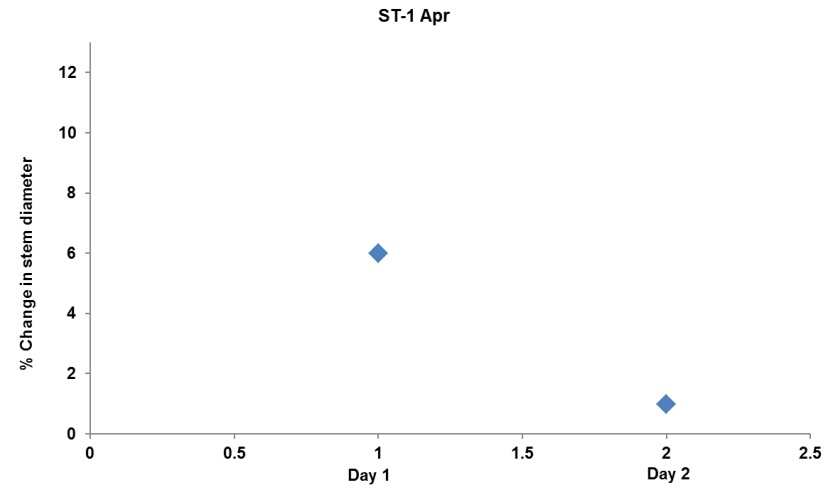
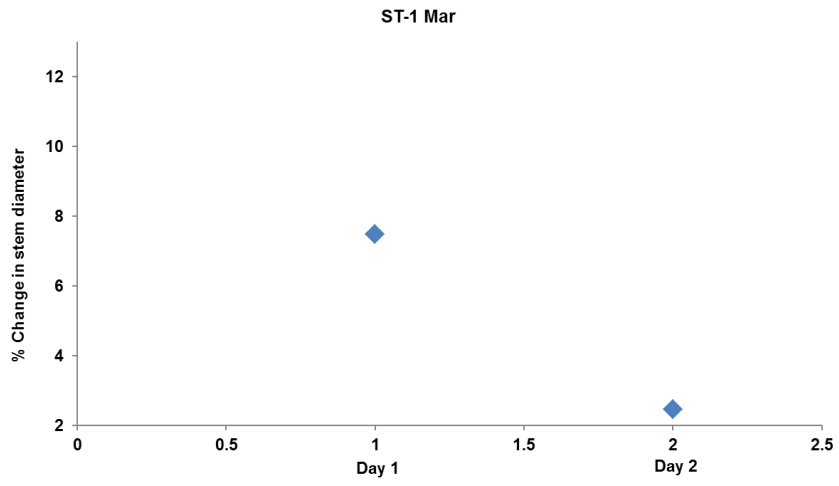
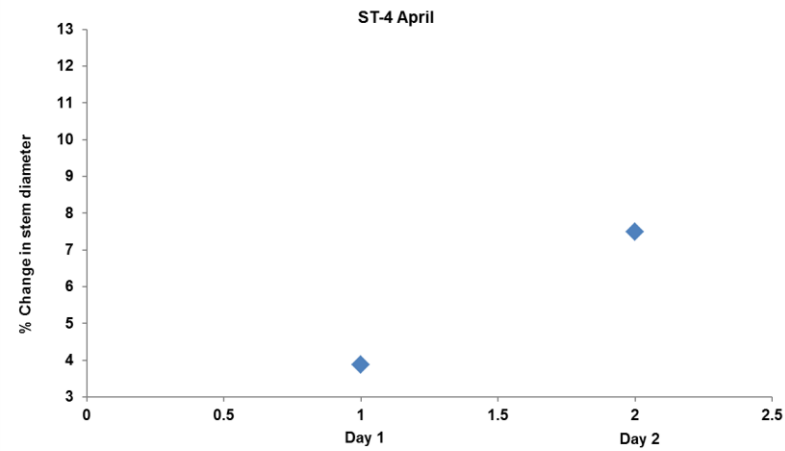
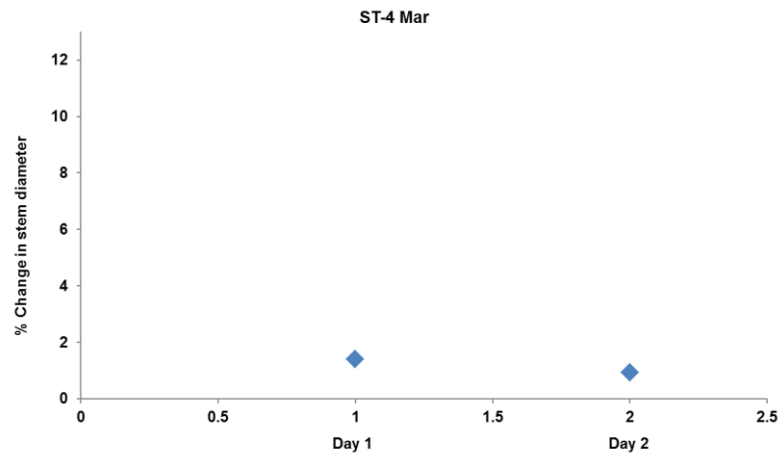
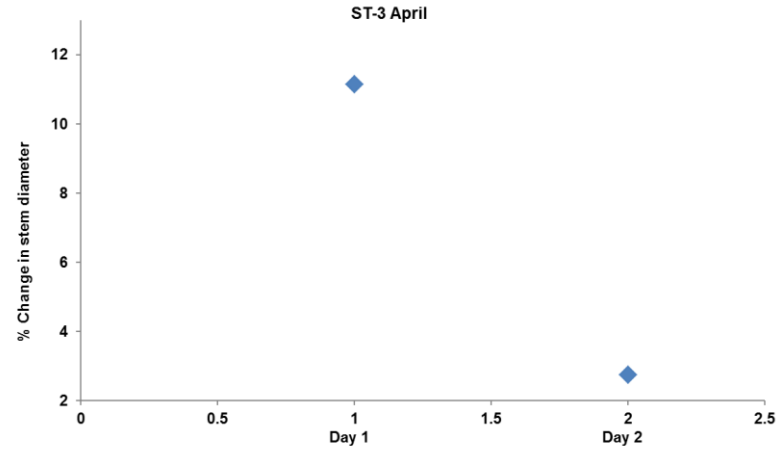
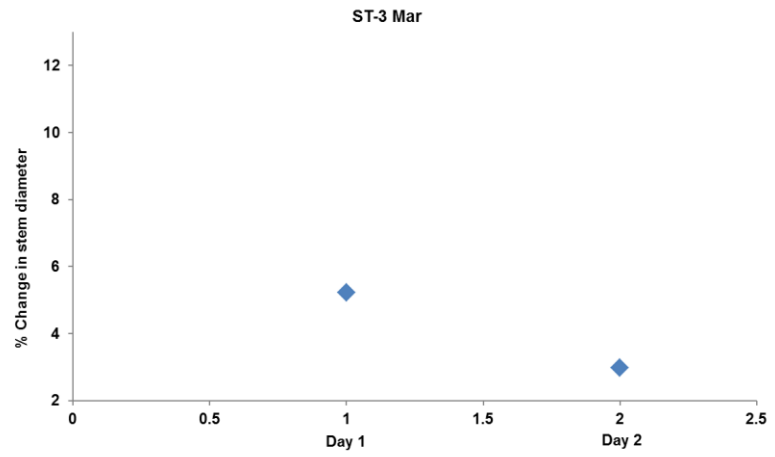


Figure 2.7(G-H). Percentage change in stem diameter for two consecutive days in *P. repens* for summer (December, January and February) at five different stations along the moisture gradient.

**J**



K



L

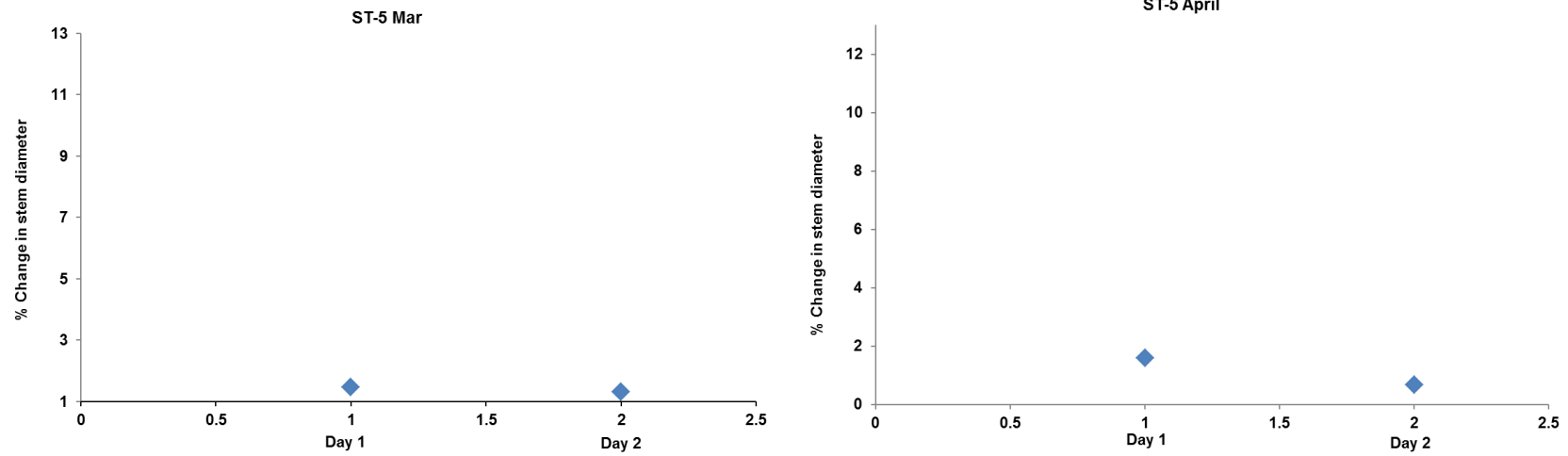


Figure 2.7(J-L). Percentage change in stem diameter on two consecutive days in *P. repens* for autumn (March and April) at five different stations along a moisture gradient

### 2.4.3. Sap flow

The test species were examined to determine whether its daily amplitudes in sap flow were consistently or intermittently positively correlated with daily amplitudes in temperature (T) and vapour pressure (VPD) deficit. *P. repens* L. displayed significantly ( $P \leq 0.05$ ) negative correlations between their total daily amplitudes in sap flow and station maximum daily temperature both in winter (August), spring (September – November ( $P \leq 0.001$ )), summer (December – February ( $P \leq 0.001$ )) and autumn (March ( $P \leq 0.001$ ) – April ( $P \leq 0.01$ )) (Figures 2.8-2.10). No significant correlations between temperature and sap flow amplitudes were found for the month of May ( $P \geq 0.05$ ). The highest total daily sap flow ( $\text{mm d}^{-1}$ ) and lowest temperature ( $^{\circ}\text{C}$ ) was found at station 1 (ST1) and the lowest total sap flow and highest temperature was found at station 5 (ST5). These trends were also displayed between daily maximum amplitudes in total daily sap flow and station maximum daily vapour pressure. The highest total daily sap flow and the lowest daily vapour pressure deficit (KPa) were found at the highest elevation (ST1). The lowest daily sap flow and the highest maximum daily vapour pressure deficit (KPa) were found at the lowest elevation (ST5) for all months. Station 2 had consistently depressed sap flow rates throughout the year. Regression functions project a mid-summer 9% reduction in sap flow for each one degree rise in temperature and 23% reduction in sap flow for each 10 kPa increase in total daily VPD. Figure 2.11 show the diurnal sap flow rates (hourly means) for *P. repens* along the elevation gradient at Jonaskop in different months. Sap flow diurnal patterns showed a steep morning increase leading to maximum rates reached at about midday, followed by a continuous gradual decrease until late in the afternoon (Figure 2.11). The plants at the lowest elevation (ST5) display lower sap flow rates which peaked earlier in the day than those at the highest elevations, especially under moderately moist and dry conditions. Under wet conditions especially after good rainfall, the sap flow of plants at the highest elevation (ST1) was inhibited.

The leaves of *P. repens* L. displayed significantly ( $P \leq 0.001$ ) negative correlations between their total daily amplitudes in sap flow and photosynthetic effective quantum yield ( $\Delta F/F_m'$ ) at the 5 stations along the gradient during the months of October and November (Figure 2.12). During the month of October ST1 showed the highest daily sap flow rate of  $5.6 \text{ mmd}^{-1}$  and the lowest  $\Delta F/F_m'$  (0.65) compared to ST5 with a total daily sap flow rate of  $2.3 \text{ mmd}^{-1}$  and the highest  $\Delta F/F_m'$  of 0.72. ST 3 and ST4 also displayed significantly higher sap flow rates and lower  $\Delta F/F_m'$  compared to ST5 ( $P \leq 0.001$ ). The results for November indicate that ST1 showed the highest daily sap flow rate of  $6.8 \text{ mmd}^{-1}$  and the lowest  $\Delta F/F_m'$  (0.54) compared to ST5 with a total daily sap flow rate of  $2.2 \text{ mmd}^{-1}$  and the highest  $\Delta F/F_m'$  of 0.74. Station 3

displayed significantly higher sap flow rates and lower  $\Delta F/F_m'$  compared to ST4 and ST5 ( $P \leq 0.001$ ). In figure 2.12 photosynthetic effective quantum yield was positively correlated with maximum daily temperature at all 5 stations along the gradient during October and November. During the month of October  $\Delta F/F_m'$  was significantly higher at ST1, ST2 and ST3 at lower temperatures than during higher temperatures during November. The photosynthetic quantum yield at ST4 and ST5 were significantly lower at lower temperatures during October compared the month of the November when the temperatures were higher (Figure 2.12).

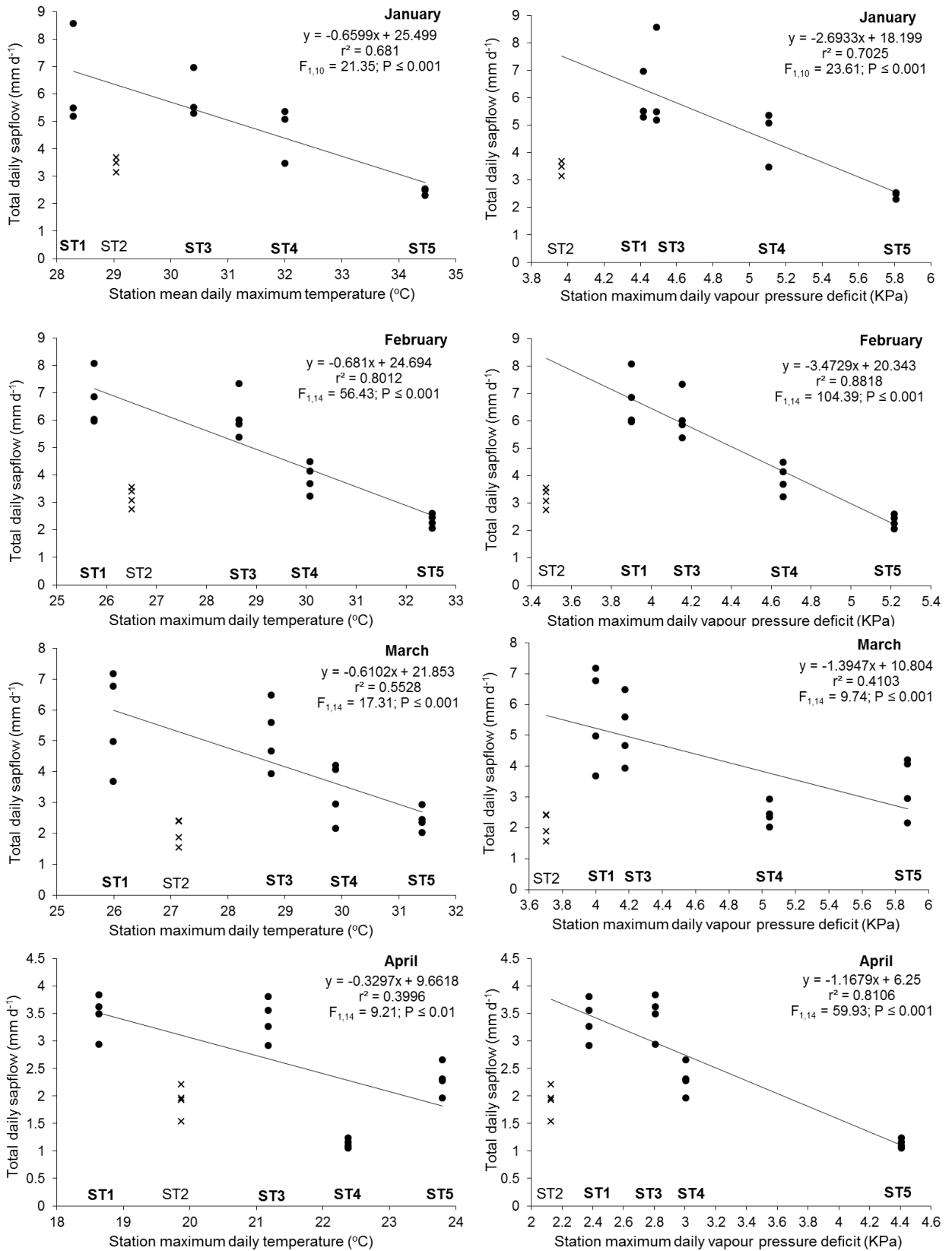


Figure 2.8. Relationships between daily amplitudes in total daily sap flow and station maximum temperature (T) and between daily amplitudes in total daily sap flow and station maximum daily vapour pressure deficit (VPD) of *P. repens* L. from January to April

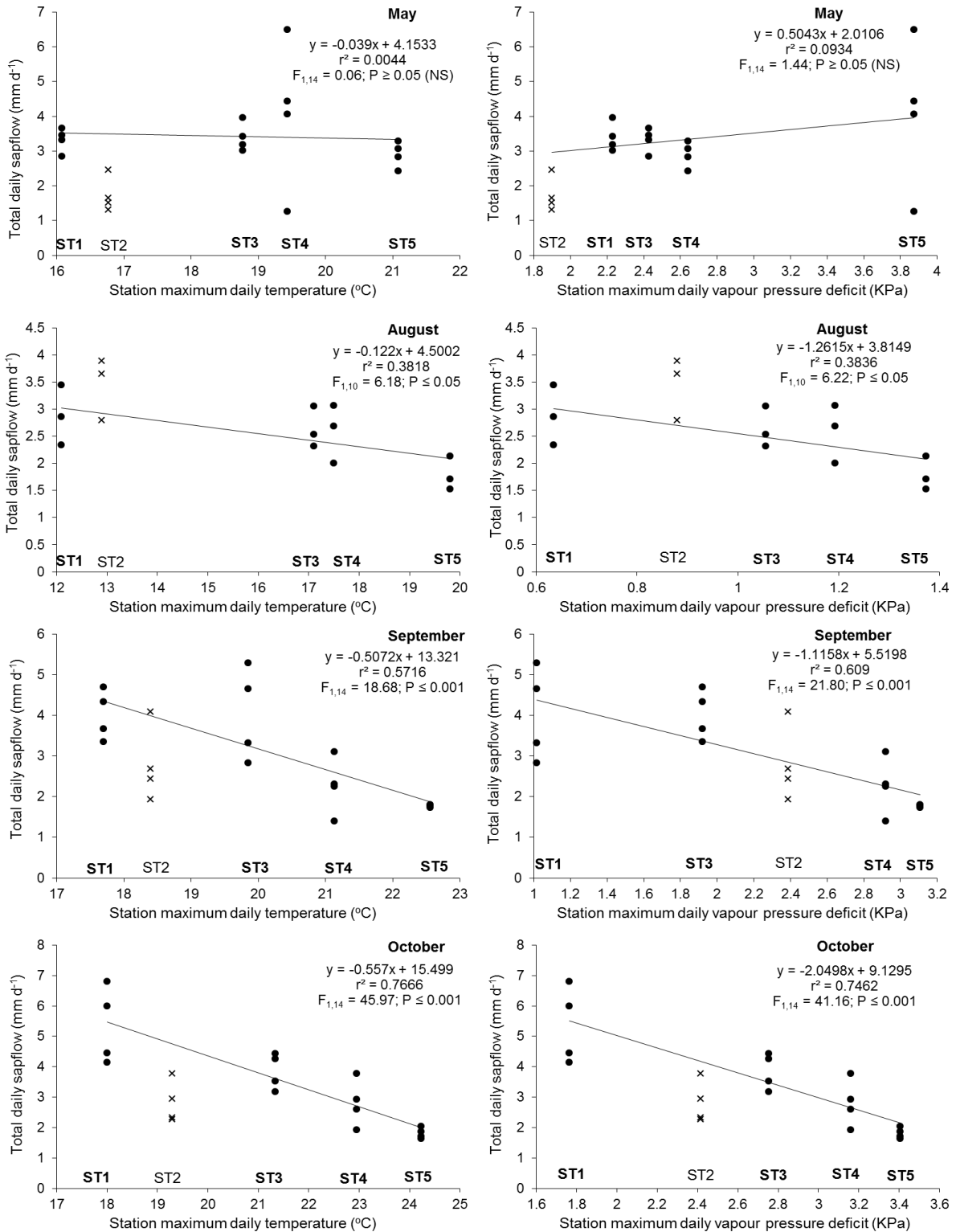


Figure 2.9. Relationships between daily amplitudes in total daily sap flow and station maximum temperature (T) and between daily amplitudes in total daily sap flow and station maximum daily vapour pressure deficit (VPD) of *P. repens* L. from May to October

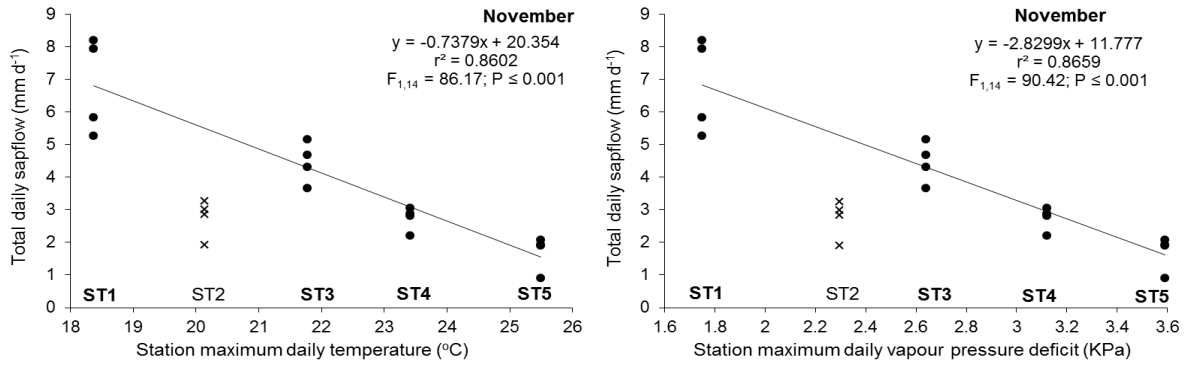


Figure 2.10. Relationships between daily amplitudes in total daily sap flow and station maximum temperature (T) and between daily amplitudes in total daily sap flow and station maximum daily vapour pressure deficit (VPD) of *P. repens* L. for November

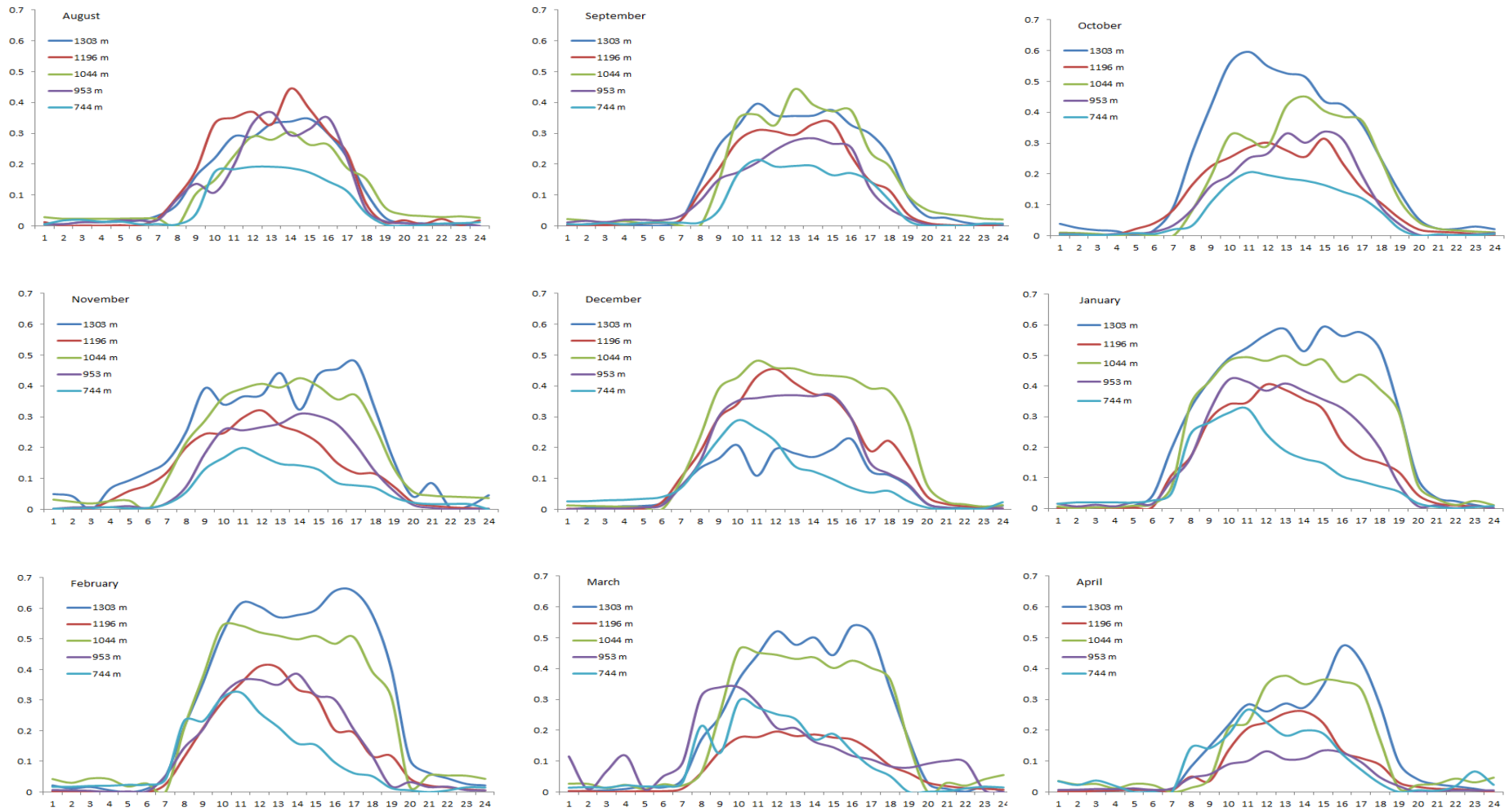


Figure 2.11. Diurnal sap flow rates (hourly means) for *P. repens* along an elevation gradient at Jonaskop in different months

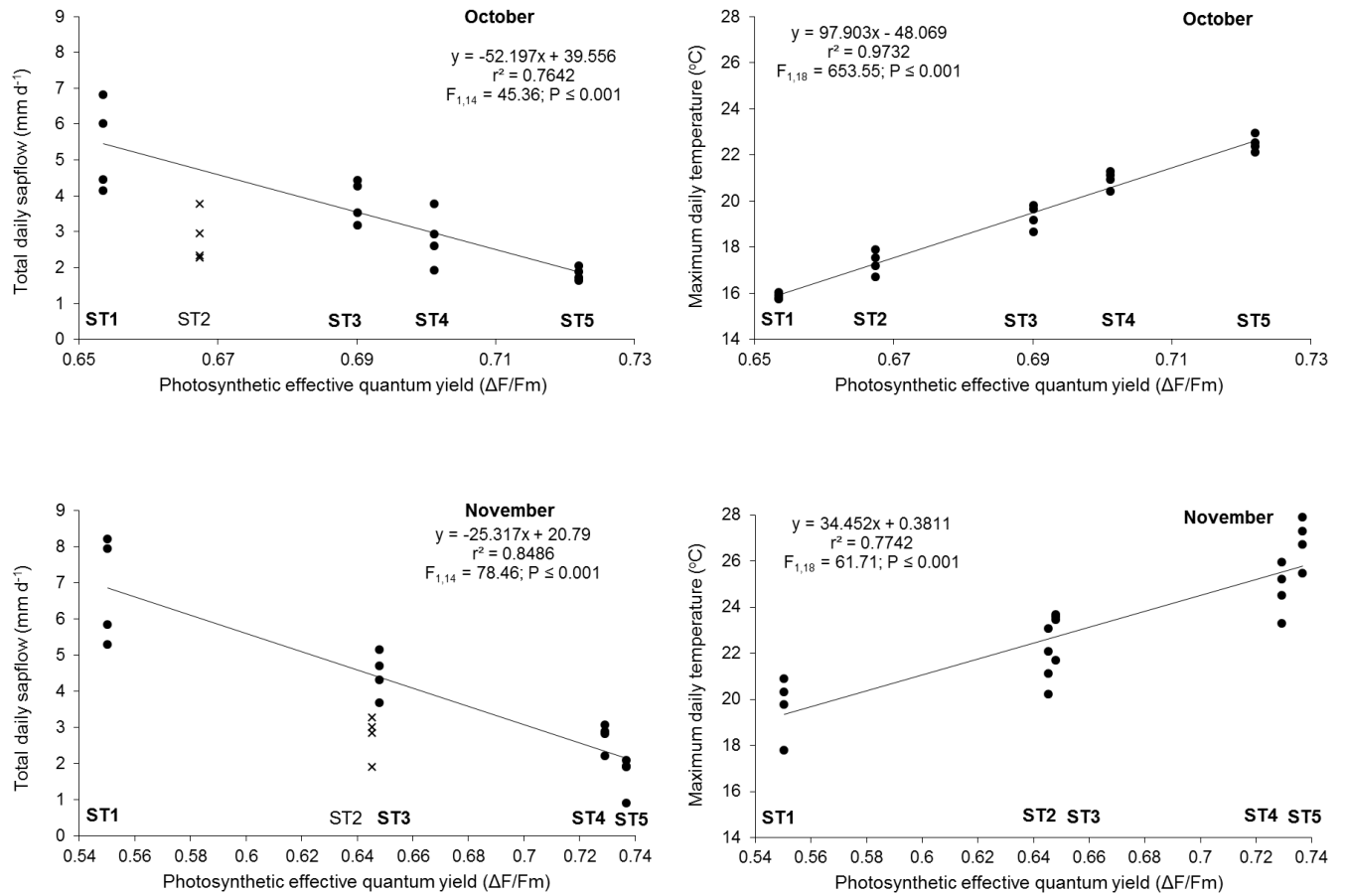


Figure 2.12. Relationships between daily amplitudes in total daily sap flow and photosynthetic effective quantum yield ( $\Delta F/F_m'$ ) and between maximum daily temperature (T) and photosynthetic effective quantum yield ( $\Delta F/F_m'$ ) in *P. repens* L. for October and November at all five stations along the gradient

## 2.5. Discussion

Vegetation in Mediterranean climate regions is known to be generally drought resilient and has a wide variety of drought avoidance strategies associated with different plants species (Chaves *et al.*, 2002). Leaf anatomical traits such as reduced leaf size, sclerophylly, trichome layers and ericoid leaves are considered to be adaptations against excessive transpirational water loss during summer months (Dickison, 2000; Lamont *et al.*, 2002).

Non-rainfall atmospheric moisture capture by soil at this semi-arid study area was big compared to the rainfall over the 8-month period (Figures 2.5 and 2.6). Greater non-rainfall atmospheric moisture inputs relative to rainfall have been reported in other semi-arid and arid ecosystems (Matimati *et al.*, 2012). Recent lysimeter based measurements of non-rainfall atmospheric moisture interception by bare gypsum soils in a South African coastal desert indicated an annual deposition  $510\text{mmy}^{-1}$  that is over ten times greater than the rainfall amount of  $43\text{mmy}^{-1}$  (Maphangwa *et al.*, 2012), a consequence of the higher frequency of fog at this coastal site and the hygroscopic nature of gypsum soils (Pavlík *et al.*, 2008).

This study investigated stem diameter variations and sap flow of *Protea repens* growing at five different sites along a moisture gradient in a Mediterranean climate during four seasons (winter, spring, summer and autumn). Daily values of MXSD (daily maximum stem diameter/thickness) and MNSD (daily minimum stem diameter) are achieved just before sunrise and sometime during the afternoon, respectively (Goldhamer & Ferres, 2001; Ortuño *et al.*, 2010). This pattern indicates the combined effects of soil water supply and evaporative demand on maximum shrinkage and the re-hydration process. The re-hydration process depends mainly on soil water supply and might be indirectly affected by the previous day's evaporative requirement (Goldhamer & Ferres, 2001; Ortuño *et al.*, 2010). Zweifel *et al.* (2000) concluded that in addition to soil water availability and plant transpiration, the degree to which plant storage tissues were saturated was also a factor that influenced the diurnal course of stem diameter changes, because stored water plays an essential role not only during periods of drought, but whenever water transport takes place within the tree.

Diurnal variations in plant stem diameter, which are related to the difference in total water potential between living bark cells and xylem vessels, and structural radial growth is the two main components of the observed daily changes of stem diameter. Carbon and water limitations can occur concurrently in the process of radial growth (Daudet *et al.*, 2005). Results obtained by Simonneau *et al.*, (1993) indicated that in peach trees (*Prunus persica* (L.) Batsch) showed that the changes in stem diameter are closely related to changes in

water content in the whole plant. Three different patterns of stem diameter variations during the growing season have been described for various tree species (Lassoie, 1979; Antonova *et al.*, 1995; Fernández *et al.*, 2010). First, with low evaporative demand and high soil-water availability, the stem diameter increased from one morning to the next, with, during the day, often either no decrease or just a reduction in the rate of diameter increase. This pattern corresponded to periods of active growth. Secondly, with high evaporative demand and high soil-water availability, stem diameter increased from one morning to the next, but a substantial decrease could take place during the day. This pattern is characterised by a mixture of growth and tissue rehydration. Thirdly, during periods of high evaporative demand and low soil-water availability, there was a reduction in stem diameter, with only partial recovery during the night. This pattern was due solely to changes in hydration (Fernández *et al.*, 2010). The results of this study show that stem diameter variation could be influenced by the amount of water from the soil, temperature and humidity. Diurnal stem diameter variation is a sensitive indicator of drought and recovery in *Protea repens*.

The results of the present study show that at station 1 with low dewfall during the summer months the stem diameter variations were lower compared to the readings for winter with higher dewfall or non-rainfall precipitation. Station 5 exhibited lower dewfall over the entire experimental period compared the dewfall at station 1. The stem diameter variations remained constant over the four seasons with the lowest dewfall recorded at station 5 during spring (9.5 mm). This was also true for station 2. The highest recorded stem diameter measurements recorded at station 3 were during winter and the lowest stem diameter measurements were recorded during summer. The lowest dewfall for station 3 were 10 mm recorded during the summer. The highest stem diameter measurements for station 4 were recorded during autumn and lowest readings were recorded during winter and spring. The lowest dewfall for station 4 was recorded during spring. In winter (August and September)

The results of previous studies have indicated that sap flow of plants has a relationship to climatic variables, such as VPD (Huang *et al.*, 2015). Data of the present study convincingly demonstrated that higher sap flow rates occurred in *P. repens* at higher elevations and low temperatures, than at lower elevations and higher temperatures. It was also found that *P.repens* at the lowest elevation (ST5) display significantly lower sap flow rates and peak earlier in the day than those at highest elevations, particularly under moderately moist and dry conditions. Under wet conditions especially after good rainfall, sap flow of plants at highest elevation is inhibited (August, May), which may have been due to soil saturation and consequent inhibition of root metabolism. This is in contrast with studies by Schwinning and Sala (2004) and Zeppel *et al.* (2008) who have found that rainfall pulses can rapidly increase

sap velocity and change the water-use efficiency (WUE) of vegetation. According to Huang *et al.* (2015) sap flow depend on temperature, relative humidity and wind speed.

Daily peak sap flow occurs simultaneously with net radiation, but a few hours before peak temperature and the minimum RH (Huang *et al.*, 2015). In the present study, the sap flow of *P. repens* decreased significantly with lower altitude, higher temperature and higher VPD. *P. repens* displayed significant decreases in sap flow during the summer months (Jan-Feb). The sap flow in *Salix psammophila* increased almost linearly with temperature but decreased with RH. A multiple regression of sap flow of *S. psammophila* and climatic variables can account for more than 80% of variations in diurnal sap flow fluctuations (Huang *et al.*, 2015). This is contrary to what was found in our study where sap flow decreased with lower elevation and higher temperature. The reduction in sap flow with lower elevations and higher temperature could be the result of stomatal closure to reduce water loss (Attia *et al.*, 2015). From this study it is apparent that *P. repens* is sensitive to VPD. VPD decreased with an increase in altitude and lower temperature. *P. repens* is an isohydric species and appears to be more sensitive to VPD. Attia *et al.* (2015) have found that in isohydric species, stomata conservatively regulate plant water status by controlling the rate of water loss to the atmosphere such that it matches the capacity of the soil–plant hydraulic system to supply water to leaves.

Jones and Sutherland (1991) indicated that greater sensitivity VPD rather than soil moisture could provide an early signal to close stomata and to consequently, avoid cavitation. Isohydric plants maintain a constant, or nearly constant, minimum daily leaf potential and relative water content by reducing stomatal conductance and transpiration under water stress to decrease the risk of hydraulic impairment and leaf dehydration, (Attia *et al.*, 2015). Anisohydric plants allow leaf potential to decline during drought conditions compared to a well-watered area, therefore reaching a lower leaf potential and relative water content with increasing evaporative demand and maintaining the driving force for water flow to leaves (Moshelion *et al.*, 2014; Attia *et al.*, 2015). Therefore, *P. repens*, might not suffer drought-induced hydraulic failure during severe drought events or rely heavily on summer rainfall to maintain hydraulic conductance.

A projected 4.5 °C future rise in temperature will result in about 40% decrease in sap flow rate and might lead to further reductions in stomatal conductance and transpiration. The sap flow-activity period showed an increase from October to February and a decrease from March to September. Plants under high rainfall (ST1) indicated lower daytime sap flow rates than those at lower elevations (ST5) during winter. A possible explanation for the inhibition of

sap flow of plants under wet conditions (high rainfall) could be due to soil saturation and consequent inhibition of root metabolism (Figure 2.15).

Chlorophyll fluorescence is considered as a reliable indicator of photosynthesis and is a critical factor in stress physiology (Misra *et al.*, 2001; 2006; 2012). In the present study  $\Delta F/F_m'$  exhibited higher values during October under lower temperature (T) at ST1 and lower values during November under higher T at ST1 during November. Station 5 showed slightly lower  $\Delta F/F_m'$  values under lower T in October than November. Under higher T and low transpiration *P. repens* at ST4 and ST5 showed higher  $\Delta F/F_m'$ . These stations (ST4 and ST5) are much lower down the gradient with higher T, lower water availability and lower rate of transpiration compared to the other 3 stations (ST1, ST2, ST3) with lower T, higher water availability and a higher rate of transpiration. Drought stress often results in stomatal closure which is the main mechanism through which plants avoid or delay tissue dehydration. Stomatal closure causes a decrease in photosynthesis and as a consequence it compromises the plant's carbon balance further (McDowell, 2011, Flexas *et al.*, 2014). An early response of Mediterranean plants to water stress is stomatal closure (Martínez-Ferri *et al.*, 2000; Gullás *et al.*, 2002; Gallé & Feller, 2007). Stomatal closure is known as the prominent factor limiting photosynthesis under drought stress (Flexas *et al.*, 2014). In this study, despite the higher T during the month of November at ST5 and ST4 the  $\Delta F/F_m'$  values for these stations were slightly higher compared to those  $\Delta F/F_m'$  values of October. ST1 experienced a significant decrease in  $\Delta F/F_m'$  during November compared to the month of October under higher T. The results of  $\Delta F/F_m'$  could have been influenced by other factors such as solar radiation in addition to T and water availability. The leaves of *Quercus ilex* and other Mediterranean species exposed to high solar radiation during winter cold were more photoinhibited and exhibited lower photochemical efficiency of PS II a study by Oliveira and Peñuelas (2002) revealed. According to Larson (1980) plants exhibit changes in their photosynthetic capacity which could partly be interpreted as an acclimation to current climatic conditions.

## 2.6. Conclusion

The results of this study stress the importance of the non-rainfall precipitation (dewfall) as a reasonably little but continuous source of water for some dry and semi dry environments, as well as its important influence on the local water equilibrium, especially during episodes of drought. Dew is an important source of moisture in dry and semi-dry environments. In water-limited areas and during periods of drought, dewfall can exceed the amount of rainfall or

frequency, or even be the only source of liquid water for plants (Richards, 2004). The phenomenon of non-rainfall precipitation profits in semiarid ecosystems should receive more attention, investigating different substrates on water gains from atmospheric water adsorption by soil (AWAS) or dew in Mediterranean semiarid areas. The non-rainfall water profits would provide an extra supply of water for vegetation in dry summers. Higher temperatures and drought conditions in the next century, as a consequence of climate change, could affect the fitness of *Protea repens* L. as a shrub species within the Fynbos Biome. Fog and dew events in semi-arid and arid areas are significant to the survival of plants in the absence of rainfall events.

Mediterranean ecosystems are complex and several abiotic environmental factors can affect plants species simultaneously. The continuous recording of sap flow reveals information on transpiration dynamics over hourly, daily, monthly and seasonal time scales. *P. repens* show characteristics of whole plant water use common to other drought adapted woody plants. In the present study, *P. repens* plants, was highly tolerant of water stress/drought and is well adapted to Mediterranean climatic conditions. However, under extreme drought conditions, *P. repens*, might operate at the limits of safety, which maybe exceeded and prolonged by climatic change effects such as temperature, rainfall and droughts. These effects might have negative effects on the survival of these species. *Protea repens* has a large root system (1-3m) (Smith & Higgings, 1990) that might be buffering reductions in relative extractable water, thus maintaining sufficient water supply with minor effects on leaf water relations.

Pinheiro *et al.* (2008) and Guidi and Calatyud (2014) revealed that in Mediterranean plant species photosynthesis is limited by environmental constraints either directly (as the diffusion limitations through the stomata and the mesophyll and the changes in photosynthetic metabolism) or indirectly, such as by the oxidative stress arising from the superimposition of multiple stresses. Chlorophyll fluorescence allows collecting a large set of data regarding photosynthetic processes in Mediterranean species like *P. repens* subjected to multiple environmental stresses. The present study revealed that,  $\Delta F/F_m'$  decreased under higher T and higher elevations and increased slightly under higher T and lower elevations. Photosynthetic effective quantum yield increased under lower sap flow rates at lower elevations and decreased under lower sap flow rates at higher elevations. The fluorescence data provided us with information on the potential photosynthetic performance of *P. repens* under high T and on its possible effect on sap flow rates.

These analyses maybe useful to increase our understanding of the physiological mechanisms in *Protea repens* as a species of drought tolerance in a Mediterranean ecosystem.

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## CHAPTER 3

**Experimental climate warming enforces seed dormancy in South African Proteaceae but seedling drought resilience exceeds summer drought**



## Experimental climate warming enforces seed dormancy in South African Proteaceae but seedling drought resilience exceeds summer drought periods

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**Abstract** Two hypotheses—that elevated night-time temperatures due to climate warming would enforce post-fire dormancy of Proteaceae seed due to low moisture, and that periods without rain during summer would exceed desiccation periods tolerated by Proteaceae seedlings—were tested empirically. Enforced dormancy, i.e., the inability to germinate due to an environmental restraint, was tested by measuring seed germination in 11 Proteaceae species in experimental mesocosms whose soils were artificially elevated by 1.4 and 3.5 °C above ambient by far-red wavelength filtered infrared lamps. Diminished totality of germination and velocities were observed in 91 and 64 %, respectively, of the Proteaceae species tested. Drought resilience was tested in one-year-old seedlings of 16 Proteaceae species by withholding water from potted plants during summer in a greenhouse. The most drought-resilient Proteaceae species displayed the lowest initial transpiration rates at field capacity, the smallest declines in transpiration rate with decreasing soil water content, and the lowest water losses

by transpiration. Projected drought periods leading to the complete cessation of transpiration in all Proteaceae species greatly exceeded the number of days without rain per month during summer in the current distribution ranges of those species. It was therefore concluded that enforced seed dormancy induced by elevated night-time temperatures is the post-fire recruitment stage of Proteaceae that is most sensitive to climate warming.

**Keywords** Experimental mesocosms · Seed germination · Seedling transpiration · Soil temperature · Soil moisture

### Introduction

The accumulation of CO<sub>2</sub> and other greenhouse gases in the atmosphere since pre-industrial times has already had a discernible influence on global temperature, and is predicted to cause further warming this century (IPCC 2007). On the African continent, various climate futures focusing on regional mean temperatures and rainfall changes in different seasons have been presented (Hulme et al. 2001). These draw upon different draft emission scenarios prepared for the Intergovernmental Panel on Climate Change (IPCC 2007). The scenarios predict mean annual temperature increases of between 1.3 and 4.5 °C and an up to 30 % decline in winter rainfall in the Southern African Mediterranean-climate ecosystem (Hulme et al. 2001). This ecosystem is characterized by summer drought, low soil fertility, and natural fires occurring at frequencies of 4–40 years (Kruger and Bigalke 1984).

Climate-controlled differences in seedling recruitment and survival are considered the dominant influence on plant community distribution, extent, internal structure, and function (Cornelius et al. 1991). Indeed, recruitment processes

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are crucial to plant species persistence, especially in the presence of regular disturbances (Klinkhamer and de Jong 1988). Although there are several processes associated with recruitment, seed germination and seedling establishment have received less attention than seed production, dispersal, and competition for limiting resources (Landhauser and Wein 1994). The Cape Floristic Region is adapted to recurrent fire cycles and characteristically experiences intense recruitment by resprouting and seed germination immediately after fire, with little or no recruitment occurring between fires (Kruger and Bigalke 1984). Fire stimulates seed release and seed germination in serotinous Proteaceae through various direct and indirect cues (Brown and Botha 2004). Serotiny is defined as an ecological adaptation in which canopy-stored seed is released in response to an environmental trigger, rather than spontaneously at seed maturation (Heelemann et al. 2008). Canopy-stored seeds of serotinous Proteaceae are dormant until activated by low temperatures (Deall and Brown 1981). Soil-stored seeds of nonserotinous Proteaceae have an additional dormancy mechanism; they can be activated by high temperatures, in order to take advantage of the early post-fire situation (Brits 1986). Increased diurnal soil temperature amplitudes resulting from the removal of the insulating vegetation layer in the immediate post-fire environment provide an important cue for some nonserotinous Proteaceae species to germinate (Brits 1987).

A crucial stage following seed germination in Mediterranean-climate ecosystems is seedling establishment and persistence over the dry summer period following wintertime seedling recruitment. Germination and recruitment are critical stages in the life cycles of arid plants, with seedling survival especially vulnerable to water stress and dependent on adequate postgermination rainfall (Hoffman et al. 2009). Proteas are unique in the Cape Floristic Region in their ability to continue growing during the summer drought period, since adult plants, unlike their seedlings, are able to tap deep water. Plant sensitivity to drought is dependent upon its intensity and duration, and differs with plant species, growth stage, and organizational level (Chaves et al. 2002). Four field studies and three greenhouse studies, as summarized by Hoffman et al. (2009), have measured plant responses to drought in the Southern African Mediterranean-climate region. Field studies showed that drought reduces species richness and individual plant abundance and cover. Greenhouse studies showed that relatively short desiccation periods of 21–26 days lead to complete mortality of the evergreen shrub species *P. vulgaris*, *L. pubescens*, and *G. africana*.

Clearly, an understanding of seed germination responses to elevated temperatures and seedling responses to drought is becoming increasingly important, as climate change scenarios suggest increases in temperature and aridity in many

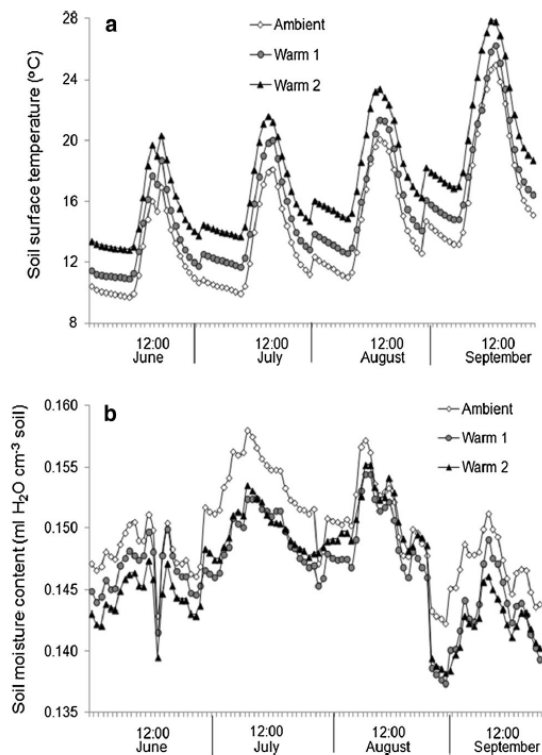
regions of the globe (Petit et al. 1999). Also, knowledge of seed germination and seedling survival characteristics may assist in the selection of species for reintroduction into disturbed vegetation in climate mitigation programs. In view of this, we tested the following hypotheses: (1) the dormancy of Proteaceae seed is enforced by especially elevated night-time temperatures; (2) drought periods tolerated by Proteaceae seedlings are exceeded by periods without rain during summer.

## Methods and materials

### Experiment 1: seed germination responses to experimental climate warming

The experimental design comprised 15 mesocosms located outdoors in a natural setting in the Kirstenbosch National Botanical Gardens. Each mesocosm, which measured 1.2 m in length, 0.8 m in width, and 0.5 m in depth, was filled with sterile aeolian sand typical of lowland fynbos. Four infrared lamps (PAR38 IR 175R, Philips, Amsterdam, The Netherlands) were suspended in banks above each of the 15 chambers. Far-red wavelengths emitted by the lamps were filtered with a 150- $\mu\text{m}$ -thick polyethylene film (Solatrol, BP Visqueen Horticultural Products, London, UK), the average transmission of which was 38 % between 650 and 670 nm and 4 % between 720 and 740 nm (Paul et al. 2005). Silicon pyranometers interfaced with data loggers (WatchDog Series 400, Spectrum Technologies Inc., Plainfield, IL, USA) monitored radiant fluxes (range 300–1100 nm) at the soil surface in the mesocosms at hourly intervals. Decagon 5TE sensors interfaced with Decagon E-20 loggers (Decagon Devices Inc., Pullman, WA, USA) monitored temperatures at the soil surface and volumetric moisture contents in the upper 100-mm soil layer in the mesocosms at hourly intervals.

Lamps were not energized in five of the chambers; those served as ambient temperature controls. In the remaining ten chambers, which alternated with the controls, two lamps were energized in five chambers and all four lamps were energized in the remaining five chambers to provide supplementary radiant fluxes of 40 and 90  $\text{W m}^{-2}$ . These elevated average daily soil surface temperatures by  $1.4 \pm 0.03$  °C in the first warming treatment (Warm 1) and  $3.5 \pm 0.03$  °C in the second warming treatment (Warm 2), respectively, with daytime increases of a similar magnitude to the night-time increases (Fig. 1). Increases in photosynthetic photon flux densities (PPFD) at the soil surface beneath the energized infrared lamps in the mesocosms, measured with a quantum sensor (LI-189, Li-Cor, Lincoln, NE, USA), were small. These ranged between 0.8 and 1.8  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in the first and second warming treatments, respectively.



**Fig. 1** Average **a** soil temperatures and **b** soil moisture contents measured hourly under ambient soil temperature and average increases of 1.4 °C (Warm 1) and 3.5 °C (Warm 2) in soil temperature

The mesocosms received natural precipitation supplemented by an automated irrigation system. Reductions in soil volumetric moisture content (Fig. 1b) in the infrared lamp-energized mesocosms were small. They averaged  $0.0030 \text{ cm}^3 \text{ H}_2\text{O cm}^{-3}$  (2.0 % decrease) in the first warming treatment (Warm 1) and  $0.0031 \text{ cm}^3 \text{ H}_2\text{O cm}^{-3}$  (2.1 % decrease) in the second warming treatment (Warm 2). The average night-time reductions in soil volumetric moisture content were slightly smaller than the average daytime reductions in both the first warming treatment ( $0.0029 \text{ cm}^3 \text{ H}_2\text{O cm}^{-3}$  night compared with  $0.0031 \text{ cm}^3 \text{ H}_2\text{O cm}^{-3}$  day) and the second warming treatment ( $0.0025 \text{ cm}^3 \text{ H}_2\text{O cm}^{-3}$  night compared with  $0.0038 \text{ cm}^3 \text{ H}_2\text{O cm}^{-3}$  day).

Seeds of 11 different Proteaceae species representing three genera that were widespread in mountain and/or lowland fynbos communities were supplied by the seed division of the Kirstenbosch National Botanical Gardens. The seeds comprised composite batches collected from several different natural populations in the Mediterranean-climate Cape Floristic Region of South Africa (Mucina and

Rutherford 2006). The test species comprised the partly or totally serotinous *Leucadendron coniferum* (L.) Meisn., *L. eucalyptifolium*, H. Buek ex Meisn., *L. laureolum* (Lam.) Fourc., *L. meridianum* I. Williams, *L. rubrum* Burm. F., *L. salicifolium* (Salisb.) I. Williams, *L. spissifolium* subsp. *spissifolium* (Salisb. ex Knight) I. Williams, *Protea magnifica* Link., *P. neriifolia* R. Br., and *P. repens* L. as well as the nonserotinous, myrmecochorous (ant-dispersed) *Leucospermum conocarpodendron* (L.) H. Buek subsp. *conocarpodendron*. Temperature, rainfall, and elevation regimes over the natural distribution ranges of the Proteaceae test species are presented in Table 1.

Only seed batches of the test Proteaceae species whose absolute germination percentages exceeded 25 % were included in the study. All seeds were initially imbibed for 12 h in diluted (1:9) smoke extract solution to promote germination (Brown and Botha 2004). The smoke extract only enhances germination once primary dormancy is overcome by seasonal temperature changes (Ooi et al. 2006). The imbibed seeds of each species were sown at the beginning of winter (June) at depths of 10 mm at 30 mm intervals into one of 11 randomized rows (25 seeds per row) spaced 70 mm apart in each of the 15 chambers. The number of seedlings of each species that emerged from each chamber was recorded at two-day intervals spanning a 90-day period after seed sowing. Mean daily germination and peak germination rates were computed for each species from sequential twice-daily counts of emerging seedlings in each treatment. Mean daily germination was calculated as the average number of seedlings emerging per day over the test period, which ended soon after the attainment of complete (absolute) germination. This provided an index of total germination that expressed both relative germination vigor and the length of the test period. Peak germination expressed the velocity of germination. This was calculated as the maximum quotient obtained from the computed cumulative germination percentage divided by the number of days from the commencement of the test. The quotients characteristically decline progressively on either side of the peak day (Czabator 1962).

#### Experiment 2: seedling responses to drought

The test seedlings comprised one-year-old seedlings, with similar heights and sizes, of 16 Proteaceae species representing four genera that were widespread in mountain and/or lowland fynbos. The seedlings were cultivated from composite seed batches collected from several different natural populations in the Mediterranean-climate Cape Floristic Region of South Africa (Mucina and Rutherford 2006) by the seed division of the Kirstenbosch National Botanical Gardens. They included the wind-dispersed *Leucadendron microcephalum* (Gand.) Gand. and Schinz, *L. sessile* R.

**Table 1** Ranges of temperature, rainfall, and elevation (105 × 105 km grid cell means) over the natural distributions of the Proteaceae test species used in experiment 1 (seed germination responses to soil warming) and experiment 2 (seedling responses to drought)

Species	Experiment nos.	Minimum daily temperature range (°C)	Maximum daily temperature range (°C)	Rainfall range (mm a <sup>-1</sup> )	Elevation range (m)
<i>Leucadendron</i>					
<i>L. coniferum</i>	1 and 2	4–8	21–30	275–2,217	1–797
<i>L. eucalyptifolium</i>	1	–1–8	22–33	129–1,858	2–1,817
<i>L. laureolum</i>	1 and 2	2–8	18–32	223–2,298	1–1,548
<i>L. meridianum</i>	1	3–8	22–28	256–948	1–703
<i>L. microcephalum</i>	2	2–8	19–30	318–2,298	7–1,520
<i>L. muirii</i>	2	5–7	24–29	256–618	1–436
<i>L. rubrum</i>	1	–3–8	18–33	110–2,877	9–1,959
<i>L. salicifolium</i>	2	1–8	19–34	123–2,815	6–1,942
<i>L. sessile</i>	1	2–8	19–32	442–2,373	8–1,460
<i>L. spissifolium</i>	1	1–8	20–31	264–1,522	13–1,558
<i>Leucospermum</i>					
<i>L. catherinae</i>	2	2–7	24–31	235–1,312	187–1,360
<i>L. conocarpodendron</i> subsp <i>conocarpodendron</i>	1 and 2	6–8	22–27	485–963	8–924
<i>L. conocarpodendron</i> subsp <i>viridum</i>	2	6–8	22–27	485–963	8–924
<i>L. oleifolium</i>	2	2–8	18–32	219–3,152	1–1,445
<i>L. reflexum</i>	2	2–8	23–33	207–1,493	1–91,339
<i>L. truncatum</i>	2	3–8	20–30	342–1,703	1–5,144
<i>Mimetes</i>					
<i>M. cucullatus</i>	2	0–8	18–32	167–2,373	1–1,616
<i>M. fimbriifolius</i>	2	4–8	18–28	474–1,501	3–936
<i>Protea</i>					
<i>P. cynaroides</i>	2	–2–9	18–32	208–3,345	1–1,956
<i>P. magnifica</i>	1	–1–8	19–32	167–3,228	8–2,061
<i>P. mundii</i>	2	–1–9	18–33	123–3,345	7–1,705
<i>P. neriifolia</i>	1	–1–8	18–32	170–3,345	2–1,729
<i>P. punctata</i>	2	–3–8	20–32	129–3,138	51–1,973
<i>P. repens</i>	1	–2–9	18–33	94–3,228	1–2,025

Species distributions were sourced from the ACKDAT, PRECIS (Mucina and Rutherford 2006), and Protea Atlas (Rebello 2010) databases, and corresponding environmental data are from Schulze and Maharaj (2007), Lynch and Schulze (2007), and Schulze and Horan (2007)

Br., *L. laureolum* (Lam.) Fourc., *L. coniferum* (L.) Meisn., and *L. muirii* E. Phillips, the serotinous *Protea punctata* Meisn., *P. mundii* Klotzsch and *P. cynaroides* (L.) L., and the nonserotinous, myrmecochorous *Leucospermum conocarpodendron* (L.) H. Buek subsp *conocarpodendron*, *L. conocarpodendron* (L.) H. Buek subsp *viridum* Rourke, *L. reflexum* H. Buek ex Meisn., *L. catherinae* Compton, *L. truncatum* (H. Buek ex Meisn.) Rourke, *L. oleifolium* (P.J. Bergius) R. Br., *Mimetes fimbriifolius* Salisb. ex Knight, and *M. cucullatus* (L.) R. Br. Temperature, rainfall, and elevation regimes over the natural distribution ranges of the Proteaceae test species are presented in Table 1.

Six seedlings of each of the 16 Proteaceae species (with similar ages and dimensions) were planted individually into 180 wide × 200 mm deep pots containing equivalent

volumes of a fynbos soil medium comprising a mixture of aeolian sand, typical of lowland fynbos, loam, and organic material (3:1:1). Pots were randomized during summer in a passively ventilated greenhouse at the Kirstenbosch National Botanical Gardens, where the summer air temperatures—monitored hourly with a radiation-shielded thermocouple interfaced with a miniature Watch Dog 450 data logger (Spectrum Technologies Inc.)—averaged 31.5 ± 0.7 °C during daytime and 21.4 ± 0.3 °C during night-time. Seedlings were allowed to acclimate in the greenhouse for a period of two weeks, during which time they were irrigated with 750 ml of water twice weekly to maintain the soils in the pots at about 50 % of field capacity. At commencement of the drought studies, all fynbos soil media in the pots were flushed with 2 L of water and

allowed to drain for 48 h to their field capacity of 28 % of soil volume (0.28 ml H<sub>2</sub>O cm<sup>-3</sup> of soil), after which irrigation was withheld and measurements commenced.

Soil volumetric moisture contents were measured at hourly intervals throughout the study in randomly selected pots containing each of the 16 Proteaceae test species with Decagon 5TE moisture sensors interfaced with Decagon E-20 loggers (Decagon Devices Inc.). The moisture sensors were pre-calibrated against the fynbos soil medium to improve measurement accuracy to ±1–2 % using published methods (Czarnomski et al. 2005).

Transpiration rates (in mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) were measured twice weekly in a 2-h period before solar noon (11:00–13:00 hours) on five fully expanded leaves of each individual plant of each Proteaceae test species with a portable leaf porometer (SC-1, Decagon Devices Inc.). Measured transpiration rates at each interval were averaged for each plant, converted to ml H<sub>2</sub>O dm<sup>2</sup> h<sup>-1</sup>, and terminated when soil moisture contents fell below 5 % of soil volume (0.05 ml H<sub>2</sub>O cm<sup>-3</sup> of soil).

Rainfall frequency and Proteaceae distributions

Long-term (1963–1999) averages of the number of days with rainfall exceeding 1 mm per month were obtained from 45 meteorological stations distributed in the south-western Cape. Monthly rainfall frequencies were converted to days without rainfall per month and summertime (December to February) monthly averages mapped with GIS, over which were superimposed recorded distributions (105 × 105 km grid cells) of the 16 Proteaceae test species sourced from the ACKDAT, PRECIS (Mucina and Rutherford 2006), and Protea Atlas (Rebello 2010) databases.

Data synthesis and statistical analyses

Measured mean daily germination and peak germination rates were log transformed to correct for non-normality in proportions. An analysis of variance tested for significant effects of elevated temperatures on the measured components of germination in each Proteaceae test species. Significantly different treatment means were separated with a Duncan’s multiple range test.

Least-squares linear regressions quantified the relationship between transpiration rate (*E<sub>DD</sub>*) and desiccation period (DD) in days (Fig. 5a) and between transpiration rate (*E<sub>sw</sub>*) and volumetric water content (SW) of the soil (Fig. 5b) for each Proteaceae test species. Analyses of variance tested the regressions for statistical significance. The regression equations were used to compute the following transpiration parameters for each Proteaceae test species: (1) transpiration rate at soil field capacity (*E<sub>FC</sub>*), (2) average transpiration rate per drought day (*AE<sub>DD</sub>*), (3) average

transpiration rate per ml of soil water loss (*AE<sub>sw</sub>*), (4) total water transpired on a drought day basis (*TE<sub>DD</sub>*), (5) total water transpired on a soil water basis (*TE<sub>sw</sub>*), and (6) the drought period (in days) leading to complete cessation of transpiration (*DD<sub>CT</sub>*). These values were derived as follows:

$$DD_{CT} = \text{intercept/slope of regression function of } E_{DD} \text{ versus drought days} \tag{1}$$

$$AE_{DD} (\text{mlH}_2\text{O dm}^{-2} \text{ h}^{-1} \text{ d}^{-1}) = \text{slope of regression function of } E_{DD} \text{ versus drought days} \tag{2}$$

$$AE_{sw} (\text{mlH}_2\text{O dm}^{-2} \text{ h}^{-1} \text{ ml}^{-1} \text{ soil water loss}) = [E_{swt1} - E_{swt2}] / [(SW_{t1} \times 5,090) - (SW_{t2} \times 5,090)], \tag{3}$$

where *E<sub>swt1</sub>* and *E<sub>swt2</sub>* are the computed transpiration rates at the commencement (*t<sub>1</sub>*) and termination (*t<sub>2</sub>*) of transpiration measurements. *SW<sub>t1</sub>* and *SW<sub>t2</sub>* are the measured soil volumetric water contents at the commencement (*t<sub>1</sub>*) and termination (*t<sub>2</sub>*) of transpiration measurements. 5,090 is the volume of fynbos soil medium in a pot (in cm<sup>3</sup>). From the above computed values of *AE<sub>DD</sub>* and *AE<sub>sw</sub>*, the total quantities of water transpired on a drought day basis (*TE<sub>DD</sub>*) and on a soil water basis (*TE<sub>sw</sub>*) were calculated for each Proteaceae test species as follows:

$$TE_{DD} (\text{mlH}_2\text{O dm}^{-2}) = AE_{DD} \times 8 \times DD_{CT} \tag{4}$$

$$TE_{sw} (\text{mlH}_2\text{O dm}^{-2}) = AE_{sw} \times 8 \times 1,440.8, \tag{5}$$

where *DD<sub>CT</sub>* is the number of drought days needed for the cessation of transpiration, 8 corresponds to the presumed 8-h daily transpiration period, and 1,440.8 is the total volume of water (in ml) present in the fynbos soil medium in each pot at field capacity.

*TE<sub>DD</sub>* and *TE<sub>sw</sub>* were also expressed as percentages of the total volume of water present in the fynbos soil medium at field capacity. The correspondence between all of the computed transpiration parameters was tested with the Pearson correlation coefficient and tested for significance with Student’s *t* test.

Results

Experiment 1: seed germination responses to experimental warming

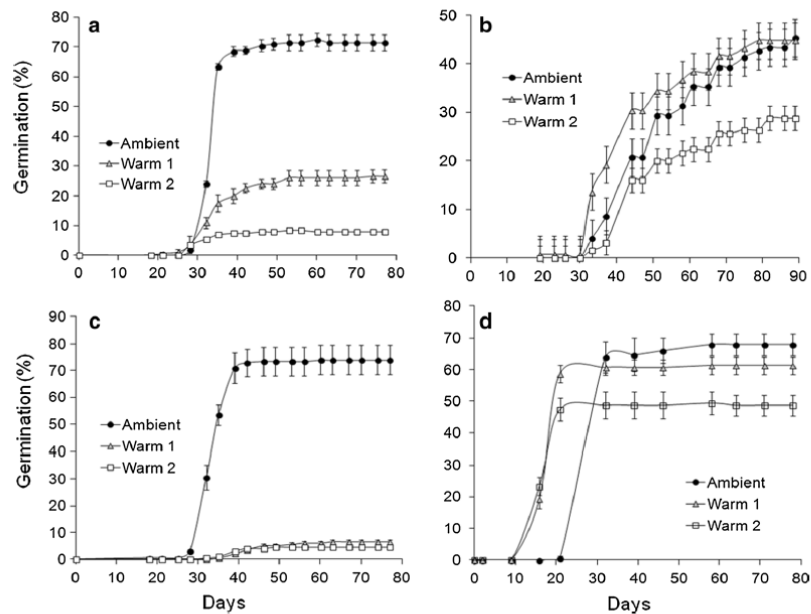
Significantly (*P* ≤ 0.05) diminished mean daily germination (Table 2) and absolute germination (Figs. 2, 3, 4) at a

**Table 2** Effects of experimentally enhanced soil temperatures on germination totality and velocity of 11 South African Proteaceae species

Genus/species	Mean daily germination			ANOVA ( $F_{2,11}$ )	Peak germination			ANOVA ( $F_{2,11}$ )
	Ambient	Warm 1 (+1.4 °C)	Warm 2 (+3.5 °C)		Ambient	Warm 1 (+1.4 °C)	Warm 2 (+3.5 °C)	
<i>Leucadendron</i>								
<i>L. coniferum</i>	0.929a	0.344b	0.104c	186.4***	1.814a	0.536b	0.200c	391.4***
<i>L. eucalyptifolium</i>	0.432a	0.411a	0.259b	4.7*	0.673a	0.756a	0.479b	5.8*
<i>L. lauroolum</i>	0.961a	0.084b	0.058b	75.9***	1.821a	0.107b	0.095b	89.3***
<i>L. meridianum</i>	0.872a	0.786a	0.624b	10.9**	2.000a	2.794b	2.254a	7.6*
<i>L. rubrum</i>	0.604a	0.344b	0.227b	11.1**	0.667a	0.418b	0.306b	8.1**
<i>L. salicifolium</i>	0.475a	0.360b	0.396b	4.0*	0.934a	0.786a	0.748a	0.4
<i>L. spissifolium</i>	0.889a	0.923a	0.855a	0.3	1.207a	2.375b	1.958c	24.5***
<i>Leucospermum</i>								
<i>L. conocarpedendron</i> subsp <i>conocarpedendron</i>	0.299a	0.013b	0.007b	71.6***	0.348a	0.022b	0.022b	151.2***
<i>Protea</i>								
<i>P. magnifica</i>	0.792a	0.513b	0.234c	14.1**	0.990a	0.762a	0.359b	6.5*
<i>P. nerifolia</i>	0.513a	0.591a	0.331b	9.7**	0.917a	1.077a	0.607b	8.7**
<i>P. repens</i>	0.246a	0.159b	0.159b	4.3*	0.380a	0.288a	0.302a	1.6

Treatment values with dissimilar letters are significantly different with \*  $P \leq 0.05$ , \*\*  $P \leq 0.01$ , \*\*\*  $P \leq 0.001$

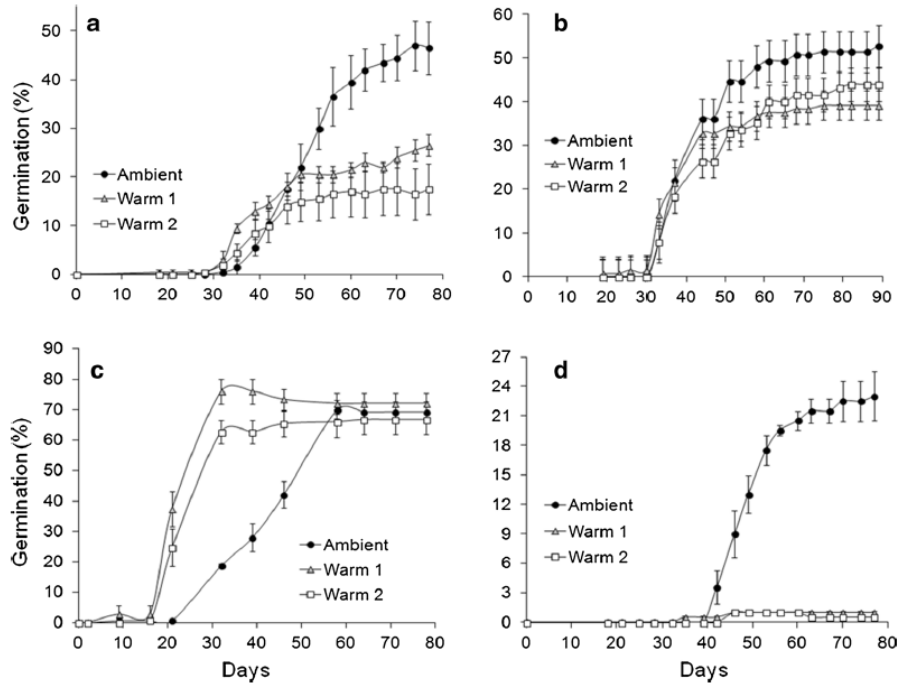
**Fig. 2** Absolute germination percentages  $\pm$  standard errors measured at two-day intervals in **a** *Leucadendron coniferum*, **b** *L. eucalyptifolium*, **c** *L. lauroolum*, and **d** *L. meridianum* under ambient and average 1.4 °C (Warm 1) and 3.5 °C (Warm 2) increases in soil temperature



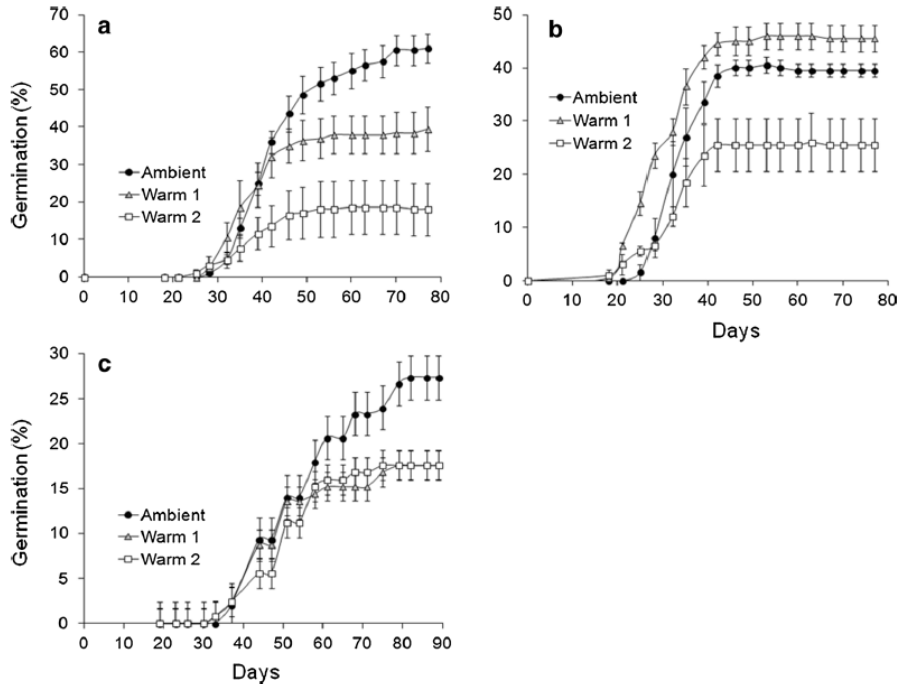
soil temperature elevation of 3.5 °C were observed in 91 % (10) of the species, the exception being *L. spissifolium*, but in only 64 % (7) of the species at a soil temperature elevation of 1.4 °C (Table 2). Also, significant ( $P \leq 0.05$ ) reductions in peak germination at a soil temperature elevation of

3.5 °C were observed in 64 % (7) of the species but in only 36 % (4) of the species at a soil temperature elevation of 1.4 °C (Table 2). The exceptions were *L. meridianum*, which displayed a significantly increased ( $P \leq 0.05$ ) peak germination at 1.4 °C; *L. spissifolium*, which exhibited significantly

**Fig. 3** Absolute germination percentages  $\pm$  standard errors measured at two-day intervals in **a** *Leucadendron rubrum*, **b** *L. salicifolium*, **c** *L. spissifolium*, and **d** *Leucospermum conocarpodendron* subsp *conocarpodendron* under ambient and average 1.4 °C (Warm 1) and 3.5 °C (Warm 2) increases in soil temperature



**Fig. 4** Absolute germination percentages  $\pm$  standard errors measured at two-day intervals in **a** *Protea magnifica*, **b** *P. neriifolia*, and **c** *P. repens* under ambient and average increases in soil temperature of 1.4 °C (Warm 1) and 3.5 °C (Warm 2)



increased ( $P \leq 0.05$ ) peak germination at both 1.4 °C and 3.5 °C; and *L. salicifolium* and *P. repens*, which exhibited non-significantly ( $P \geq 0.05$ ) altered peak germination at both 1.4 and 3.5 °C (Table 2). The dormancies of *L. conocarpodendron*

subsp *conocarpodendron* and *L. laureolum* seeds were most severely enforced by the elevated temperatures, with a >90 % reduction in their absolute germination (Figs. 2, 3), mean daily germination, and peak germination (Table 2).

## Experiment 2: seedling responses to drought

Measured transpiration rates declined linearly ( $P \leq 0.05$ ) with increasing duration of drought and decreasing soil volumetric water content in all 16 Proteaceae test species (Table 3). Computed initial transpiration rates at soil field capacity ( $E_{FC}$ ) and average transpiration rates per drought day ( $AE_{DD}$ ) and per ml of soil water loss ( $AE_{SW}$ ) were all significantly ( $P \leq 0.05$ ) positively correlated among the 16 Proteaceae species (Table 4), as were the total quantities of water transpired on a drought day basis ( $TE_{DD}$ ) and a soil water loss basis ( $TES_W$ ) (Fig. 5a). However, only average transpiration rates per drought day ( $AE_{DD}$ ) were significantly ( $P \leq 0.05$ ) inversely correlated with the computed drought periods in days leading to complete cessation of transpiration ( $DD_{CT}$ ), which averaged 62.8 (range 52.3–74.4) days in *Leucadendron* species, 42.1 (range 36.2–50.1) days in *Leucospermum*, 40.6 (range 27.0–54.1) days in *Mimetes*, and 52.5 (range 49.2–61.5) days in *Protea*. These computed drought periods for each Proteaceae species greatly exceeded the average monthly periods without

rain during summer in the current distribution of that species (Fig. 6a–d).

## Discussion

## Experiment 1: seed germination responses to experimental warming

The average soil temperature increases of 1.4 and 3.5 °C recorded in the experimental warming chambers were in the range of mean annual temperature increases predicted by the SRES B1-low and A2-high climate sensitivity scenarios, respectively (means of 7-GCM experiments) for the southwestern Cape in the year 2080: between 1.3 and 4.5 °C (Hulme et al. 2001). These temperature increases resulted in significantly diminished values for absolute and mean daily germination in 91 % of the Proteaceae species tested. These were unlikely to be due to the small (2.0–2.1 %), ineffectual decreases in soil volumetric water content measured in the experimental warming chambers.

**Table 3** Least-squares regressions of transpiration rate ( $E_{DD}$ ) against drought days (DD) and transpiration rate ( $E_{SW}$ ) against soil water content (SW) for 16 South African species of one-year-old Proteaceae

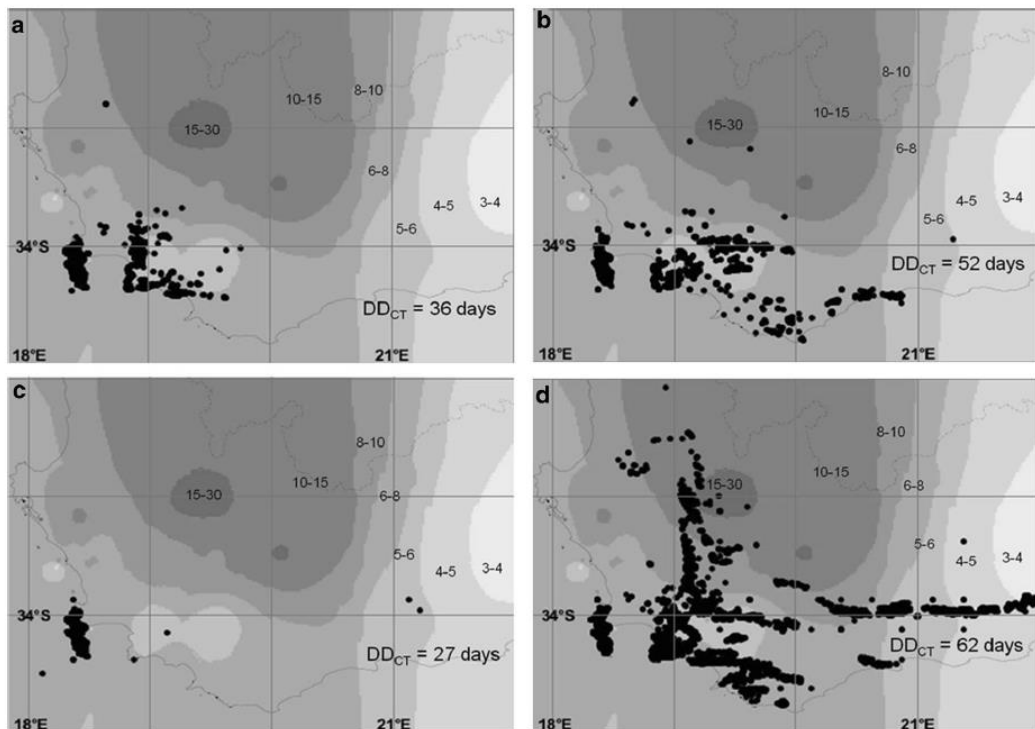
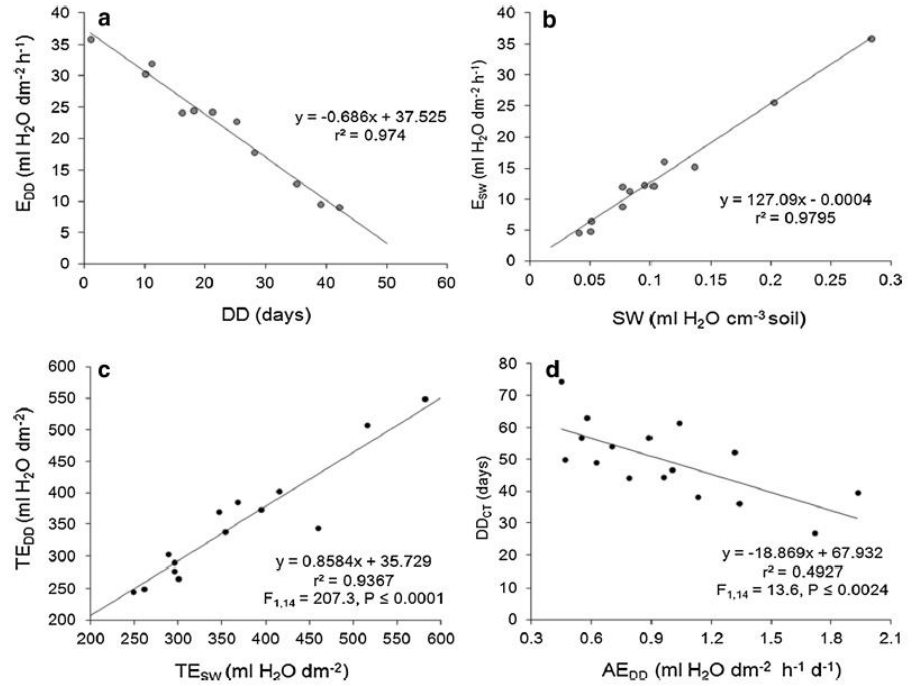
Genus/species	Transpiration versus drought days			Transpiration versus soil water content				
	Regression function ( $E_{DD} = a \times DD + b$ )		$r^2$	ANOVA $F$ ratio	Regression function ( $E_{SW} = c \times SW + d$ )		ANOVA $F$ ratio	
	$a$	$b$			$c$	$d$		
<i>Leucadendron</i>								
<i>L. laureolum</i>	-1.3,131	68.666	0.9706	$F_{1,16} = 231.5^{***}$	256.59	$7 \times 10^{-6}$	0.9410	$F_{1,16} = 111.7^{***}$
<i>L. microcephalum</i>	-0.8841	50.435	0.6573	$F_{1,21} = 15.3^{**}$	183.05	0.0004	0.7708	$F_{1,21} = 26.9^{***}$
<i>L. coniferum</i>	-0.5767	36.471	0.9240	$F_{1,16} = 121.5^{***}$	130.19	0.0004	0.9087	$F_{1,16} = 99.5^{***}$
<i>L. muiirii</i>	-0.5582	31.288	0.9783	$F_{1,17} = 180.7^{***}$	114.98	0.0003	0.6894	$F_{1,17} = 8.9^*$
<i>L. sessile</i>	-0.4493	33.440	0.8932	$F_{1,23} = 58.6^{***}$	132.04	$-3 \times 10^{-5}$	0.9564	$F_{1,23} = 153.7^{***}$
<i>Leucospermum</i>								
<i>L. catherinae</i>	-1.9236	76.11	0.9088	$F_{1,10} = 39.8^{**}$	302.98	0.0044	0.8703	$F_{1,10} = 26.8^{**}$
<i>L. conocarpodendron</i> subsp <i>viridum</i>	-1.3345	48.266	0.6596	$F_{1,6} = 9.7^*$	161.99	-2.1682	0.7195	$F_{1,6} = 12.8^*$
<i>L. reflexum</i>	-1.1279	43.098	0.8048	$F_{1,12} = 33.0^{***}$	202.48	0.0013	0.8324	$F_{1,12} = 39.7^{***}$
<i>L. conocarpodendron</i> subsp <i>conocarpodenron</i>	-0.9565	42.451	0.8828	$F_{1,14} = 45.2^{***}$	155.86	$2 \times 10^{-5}$	0.8910	$F_{1,14} = 49.0^{***}$
<i>L. truncatum</i>	-0.7871	34.897	0.8045	$F_{1,11} = 32.9^{***}$	130.05	0.0004	0.8126	$F_{1,11} = 34.7^{***}$
<i>L. oleifolium</i>	-0.4673	23.419	0.8706	$F_{1,11} = 47.9^{***}$	84.981	0.0002	0.8308	$F_{1,11} = 44.2^{***}$
<i>Mimetes</i>								
<i>M. fimbriifolius</i>	-1.7162	49.278	0.9118	$F_{1,9} = 72.4^{***}$	189.99	0.0004	0.8918	$F_{1,9} = 57.7^{***}$
<i>M. cucullatus</i>	-0.7037	38.044	0.9669	$F_{1,10} = 233.7^{***}$	254.17	-0.0005	0.8765	$F_{1,10} = 56.8^{***}$
<i>Protea</i>								
<i>P. cynaroides</i>	-1.0341	63.571	0.8875	$F_{1,21} = 47.3^{***}$	227.38	0.0010	0.9145	$F_{1,21} = 64.1^{***}$
<i>P. mundii</i>	-0.9982	46.816	0.8848	$F_{1,16} = 69.1^{***}$	174.02	0.0003	0.9240	$F_{1,16} = 109.4^{***}$
<i>P. punctata</i>	-0.6231	30.631	0.9554	$F_{1,15} = 149.8^{***}$	109.57	0.0002	0.9582	$F_{1,15} = 160.6^{***}$

$F$  ratios derived from analysis of variance (ANOVA) of the regressions were significant at \*  $P \leq 0.05$ , \*\*  $P \leq 0.01$ , \*\*\*  $P \leq 0.001$

**Table 4** Initial transpiration rate at soil field capacity ( $E_{FC}$ ), average transpiration rates per drought day ( $AE_{DP}$ ) and per ml of soil water loss ( $AE_{SW}$ ), total water transpired on either a drought day ( $TE_{DP}$ ) or a soil water ( $TE_{SW}$ ) basis, and predicted drought periods for complete cessation of transpiration (DDCT) in 16 South African species of one-year-old Proteaceae

Genus/species	Sclerophylly index g dry mass $dm^{-2}$	$E_{FC}$ (ml $H_2O$ $dm^{-2}$ $h^{-1}$ at $SW_{FC}$ )	$AE_{DP}$ (ml $H_2O$ $dm^{-2}$ $h^{-1}$ $day^{-1}$ )	$TE_{DP}$ (ml $H_2O$ $dm^{-2}$ total SW in parentheses)	DDCT (days)	$AE_{SW}$ (ml $H_2O$ $dm^{-2}$ $h^{-1}$ $ml^{-1}$ SW)	$TE_{SW}$ (ml $H_2O$ $dm^{-2}$ , % total SW in parentheses)
<i>Leucadendron</i>							
<i>L. laurcolom</i>	0.6584	72.6	1.3131	549.4 (38.1)	52.3	0.0504	581.1 (48.3)
<i>L. microcephalum</i>	0.8356	51.8	0.8841	403.2 (28.0)	57.0	0.0360	414.5 (28.8)
<i>L. confierum</i>	1.1492	36.8	0.5767	291.6 (20.2)	63.2	0.0256	294.8 (24.5)
<i>L. muiirii</i>	0.3607	32.5	0.5502	250.5 (17.4)	56.9	0.0226	260.4 (18.1)
<i>L. sessile</i>	0.6669	37.4	0.4493	267.4 (18.6)	74.4	0.0259	299.0 (20.8)
<i>Leucospermum</i>							
<i>L. catharinae</i>	0.9107	85.7	1.9236	609.4 (42.3)	39.6	0.0595	686.1 (47.6)
<i>L. conocarpodendron</i> subsp <i>viridum</i>	0.7576	43.7	1.3345	386.5 (26.8)	36.2	0.0318	366.8 (25.5)
<i>L. reflexum</i>	0.3118	57.3	1.1279	344.7 (23.9)	38.2	0.0398	458.5 (31.8)
<i>L. conocarpodendron</i> subsp <i>conocarpodendron</i>	0.8545	44.1	0.9565	339.7 (23.6)	44.4	0.0306	353.0 (24.5)
<i>L. truncatum</i>	0.9737	36.8	0.7871	278.9 (19.4)	44.3	0.0256	294.5 (20.4)
<i>L. oleifolium</i>	0.8224	24.1	0.4673	187.3 (13.0)	50.1	0.0167	192.4 (13.4)
<i>Mimetes</i>							
<i>M. fimbriifolius</i>	0.9243	53.8	1.7162	370.7 (25.7)	27.0	0.0300	346.0 (24.0)
<i>M. cucullatus</i>	0.7890	36.0	0.7037	304.6 (21.1)	54.1	0.0250	287.8 (20.0)
<i>Protea</i>							
<i>P. cynaroides</i>	0.5366	64.4	1.0341	508.8 (35.3)	61.5	0.0447	514.9 (35.7)
<i>P. mundtii</i>	0.8979	49.2	0.9982	374.5 (26.0)	46.9	0.0342	394.1 (27.4)
<i>P. punctata</i>	0.6507	31.0	0.6231	245.3 (17.0)	49.2	0.0215	248.1 (17.2)

**Fig. 5** Least-squares linear regressions of **a** transpiration rate ( $E_{DD}$ ) against drought days (DD) for *M. cucullatus* juveniles, **b** transpiration rate ( $E_{SW}$ ) against soil moisture content (SW) for *M. cucullatus* juveniles, **c** total water transpired on a drought day basis ( $TE_{DD}$ ) against total water transpired on a soil water basis ( $TE_{SW}$ ) in 16 one-year-old Proteaceae species, and **d** average daily transpiration rate ( $AE_{DD}$ ) against number of drought days leading to cessation of transpiration ( $DD_{CT}$ ) in 16 one-year-old Proteaceae species



**Fig. 6** Average number of days without rain per month during summer, as derived from long-term (1969–1990) monthly rainfall frequencies, along with superimposed distributions of the most drought-sensitive Proteaceae test species in four different genera: **a**

*Leucospermum conocarpodendron* subsp. *viridum*, **b** *Leucadendron laureolum*, **c** *Mimetes fimbriifolius*, and **d** *Protea cynaroides*.  $DD_{CT}$  is the number of drought days required for cessation of transpiration

Comparison of the diurnal soil temperature minima and maxima recorded in the experimental warming chambers during the first month of germination with base and ceiling soil temperatures reported for optimum Proteaceae germination (Brits 1986) clearly indicated that the measured reductions in seedling recruitment were more closely associated with increases in diurnal soil temperature minima than maxima. The recorded average diurnal soil temperature minima of 11.0 and 12.8 °C in the first and second warming treatments both exceeded the reported base soil temperatures of 7–9 °C required for optimum Proteaceae germination (Brits 1986), as well as the highest diurnal temperature minima of between 7 and 9 °C that were evident over the natural distribution ranges of all Proteaceae test species (Table 1). This contrasted with the diurnal soil temperature maxima of 18.7 and 20.4 °C recorded in the first and second warming treatments, which were mostly below the ceiling soil temperatures of between 20 and 24 °C above which Proteaceae germination is inhibited (Brits 1986). These findings supported the proposition that the dormancy of Proteaceae seed would be enforced by especially elevated diurnal temperature minima accompanying climate warming. The ecological role of the low-temperature requirement for initiating seedling recruitment in Proteaceae has been explained as a moisture stress avoidance measure in which seed dormancy is maintained during transiently favorable rainy periods in dry Mediterranean-climate summer months (Brits and Van Niekerk 1986). The consequences of such elevated daily temperature minima with climate warming would be the enforcement of primary dormancy in dispersed Proteaceae seeds following fire, resulting in reduced seedling recruitment during favorable wet winter months. Furthermore, the decreased germination velocity (peak values) observed with experimental warming in 64 % of the Proteaceae test species suggests that climate warming could also hinder rapid en masse seed germination after fire, a proposed means by which establishing seedlings avoid inter- and intraspecific competition with rapidly regenerating fynbos (Bond and van Wilgen 1996). Movement of dispersed Proteaceae seed to more favorable climatic locations for germination—one potential self-adaptive response to climate warming—seems highly improbable. Protea Atlas database records show that distinct Proteaceae species populations with immensely different densities exist in close proximity across elevation gradients, which is indicative of constrained seed dispersal and environmental heterogeneity. These records are supported by analyses of post-fire seeding recruitment patterns around skeletons of burnt adult Proteaceae, which show relatively short dispersal distances of only up to 17 m for ant-dispersed (myrmecochorous) seeds of some nonserotinous Proteaceae (Slingsby and Bond 1985), and between 15 and 40 m for wind-dispersed seeds of some serotinous

Proteaceae (Bond 1988; Hammill et al. 1998). More recently, empirically validated mechanistic models have demonstrated that secondary dispersal of seed along the ground by wind may also contribute significant distances to the dispersal patterns of some serotinous Proteaceae seeds after fire, with projected seed dispersal distances of up to 59 km for *Protea repens*, although these distances can be as short as 3 m for other serotinous species such as *Leucadendron salignum* (Schurr et al. 2005). However, these models assumed spatially homogeneous wind profiles and obstacle patterns. In reality, seed movement over distances of several kilometers would be restrained by impervious topographical barriers such as steep slopes, boulder fields, rivers, natural vegetation, and extensive areas that have been transformed for agricultural and forestry purposes.

It is worth noting that seeds of most of the Proteaceae species sampled showed appreciable germination at both 1.4 and 3.5 °C above ambient, except for *L. laureolum*, which displayed less than 5 % germination at 3.5 °C above ambient, and *L. conocarpodendron*, with less than 2 % at 3.5 °C above ambient. Consequently, there seems to be sufficient plasticity within seeds of most of the Proteaceae species sampled to accommodate adequate seedling recruitment under the warmest climatic scenario tested. The nonserotinous myrmecochorous *L. conocarpodendron* differs from the serotinous Proteaceae species in that its recruitment niche (a soil-stored seed bank) probably contains a fair proportion of seeds that have remained dormant through 2–3 fire cycles (i.e., 30–80 years) in the wild, and dry stored seed 200 years old has been recorded in dry stored seed (Daws et al. 2007). However, with climate warming, these dormant seeds could gradually become less adapted to suitable germination cues. Currently, seedling germination of nonserotinous species under increases of 1.4 and 3.5 °C is exceptionally low, and effectively nonexistent for *L. conocarpodendron*. However, a gradual increase in temperature accompanying climate warming could allow a greater proportion of the nondormant seed bank to recruit and thus effectively select for an increasing minimum temperature threshold cue. Indeed, it took less than 50 years to inadvertently eliminate the minimum temperature threshold in cultivated *Leucospermum cordifolium* (G. Brits, pers. comm.), so at least some species have sufficient variability to rapidly adapt to projected temperature changes. The fact that the Cape flora has a high species richness, 9,086 species in an area of 87,892 km<sup>2</sup> (Cowling et al. 1998), suggests that most of these species have weathered the previous climatic changes during the past glacial and interglacial. This is borne out by genetic data which suggest that the modal age for Protea is 1.4 million years (Valente et al. 2010). It also fits the scenario where most of the sister species are allopatric (Rebello 2010), suggesting that migration was not a mechanism for coping with past climatic

changes. This is feasible in the Cape Floristic Region, with its high local relief, especially for the mountain flora, where many species would merely have to “migrate” a few hundred meters horizontally to topographically track their climatic niche.

#### Experiment 2: seedling responses to drought

The most drought-resilient Proteaceae species identified in the present study within each of the four genera displayed the lowest initial transpiration rates at field capacity, the smallest declines in transpiration rate with decreasing soil water content, and the least quantities of water transpired, and vice versa. Similarly, in comparative desiccation experiments conducted on leaves of chaparral and coastal sage species in Mediterranean-climate California, chaparral species with low initial photosynthetic and transpiration rates were also found to be more resilient to drought than coastal sage species with high initial gas and moisture exchange rates (Harrison et al. 1971). As observed in the chaparral species (Harrison et al. 1971), Proteaceae species did not display a sharp initial decrease in transpiration indicative of rapid stomatal closure, but rather a slow decline in transpiration rate with increasing soil desiccation. Such stomatal control, which assists plants in maintaining a favorable water balance during periods of drought stress (Jones 1998), has been reported in “isohydric” plants such as cowpea (Bates and Hall 1981) and maize (Tardieu et al. 1993), as well as in adult baobab tree species, even during short drought events during the rainy season (Chapotin et al. 2006). Also, it has been proposed that a high sclerophylly index—an indication of the amount of vascular and sclerenchyma tissue in the leaf—may play a role in water economy. A high concentration of mechanical cells and thick cell walls give Mediterranean sclerophyllous leaves a high leaf mass per area ratio (LMA) (Cowling and Campbell 1983), with several studies demonstrating an increase in LMA during drought (Salleo and Lo Gullo 1990; Yin 2002). Higher LMAs can be produced by increasing leaf density, leaf thickness, or both; thick leaves are often linked to a higher tolerance of drought (Chandra et al. 2004), and an increase in leaf density was also reported under low soil water availability (Groom and Lamont 1997; Niinemets 2001). However, no significant correlation was found between leaf sclerophylly and rate of water loss through transpiration by Proteaceae in this study.

The projected average drought period of  $49.7 \pm 2.9$  days required for the complete cessation of transpiration in the one-year-old Proteaceae species included in this study exceeded the mean survival periods of 26 days in sandstone soils and 22 days in shale soils reported for *L. pubescens* and *P. vulgaris* seedlings, as well as 21 days for *G. africana* seedlings (Hoffman et al. 2009). The capacity of the

organically rich, finer-textured soils in which the one-year-old Proteaceae seedlings were cultivated in this study to retain large amounts of water may partly explain their high drought resilience. Indeed, lower levels of seedling emergence in four proteoid species were reported on coarse-textured sandy soils than on adjacent fine-grained clay soils (Mustart and Cowling 1993a). This was attributed to the higher moisture capacity of the fine-grained clay soils, as corroborated by subsequent laboratory experiments where differences in the survival patterns of newly emerged seedlings of two proteoid species were found in soils with different moisture capacities (Mustart and Cowling 1993b). However, under field conditions, the recharging of soil moisture via capillary action and overnight distillation processes (Francis et al. 2007) as well as the absorption of non-rainfall moisture sources such as dew, fog, and atmospheric water vapor (Agam and Berliner 2006) could also provide additional sources of water that are captured by the extensive subsurface proteoid root system. Such moisture sources were unavailable to potted plants in the greenhouse environment. Despite this, it should be noted that there are several factors that may potentially enhance seedling resilience to drought under greenhouse conditions. These include a lower maximum transpiration rate and higher leaf water potential under greenhouse rather than field conditions due to the reduced solar irradiance and consequent smaller evaporative demand in greenhouses. This gives less weight to hydraulic than chemical mechanisms of stomatal control in the greenhouse than in the field (Kramer 1988). Also, the lower unsaturated hydraulic conductivities of natural soils in the field compared to those of soil substrates in the greenhouse increases the probability of short and local water deficits in the roots, even in wet soil and at relatively low evaporative demands (Jones and Tardieu 1998).

Despite differences in drought responses between plants in natural and greenhouse environments, the projected desiccation periods leading to complete cessation of transpiration in the 16 one-year-old Proteaceae species tested in this study all greatly exceeded the number of days per month without rain in summer in their current distribution ranges (Fig. 6). Also, it was recently reported that desiccation periods of 21 days, which exceeds the average monthly period without rain during summer in over 95 % of the Cape Floristic Region (Fig. 6), still allowed complete seed germination of all 25 Proteaceae species tested in the pre-radicle emergent phase (Mustart et al. 2012). Even in the more drought-sensitive post-radicle emergent phase, a 13-day desiccation period—which exceeds the average monthly period without rain during summer in over 75 % of the Cape Floristic region (Fig. 6)—only resulted in a 50 % reduction in germination in 58 % of the Proteaceae species tested (Mustart et al. 2012). These findings of the present study lead us to conclude that Proteaceae seedlings

are tolerant of summer desiccation periods over a large portion of the South African Cape Floristic region, and that enforced seed dormancy induced by elevated night-time temperatures is the post-fire recruitment stage among Proteaceae that is most sensitive to climate warming.

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## CHAPTER 4

### Germination of three Proteaceae species along a moisture gradient in a Mediterranean ecosystem under drought conditions



## ABSTRACT

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In Mediterranean ecosystems, like the Cape Floristic Region (CFR), temperature and the recording of rainfall is essential for effective establishment of seedlings and also the surviving of the first year's summer drought is important for population continuity. Climate change is changing the environmental signals that seeds receive causing compromised seedling emergence. Here, we examine how temperature and moisture will affect germination in three Proteaceae species. An altitudinal gradient of temperature and moisture was used to simulate climate change effects. Climate change niches were constructed along the gradient and seeds of three Proteaceae species collected from the study site were planted in soil from the study site. Germination of the three species was also tested in a greenhouse. Under present climate change conditions germinations of the three species did not occur at the lowest altitude (545 m) because of higher temperature and low soil moisture. Germination of the three species occurred 227 days after sowing in the field and germination occurred after 74 days in the greenhouse. The highest germination percentages were found at the two closest stations (933m and 744 m) along the gradient. *Protea repens* had the highest germination percentage at both of the sites. Germination of the three species occurred at a temperature of between 6.7 °C to 10.4 °C. Temperature and moisture play an essential role in the germination process of Proteaceae. The results of this study suggest that under higher temperatures and low moisture Proteaceae in the Fynbos Biome may be at risk.

*Keywords: Germination, moisture, Proteaceae, temperature*

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## 4.1. Introduction

The processes of recruitment such as seed germination and seedling emergence are crucial for plant species persistence and long-term survival (Klinkhamer & de Jong, 1988; Clark *et al.*, 1998; Peters, 2000; Silvertown & Charlesworth, 2001; Holloway-Phillips *et al.*, 2015). Climate controlled differences in seed production, seedling recruitment and persistence are considered the main reason that cause the distribution and size of plant communities as well as their internal structure and function (Cornelius *et al.*, 1991; Bowman & Panton, 1993; Hanson & Wertz, 2000). Success during early life-cycle stages is mostly dependent on environmental conditions and water proves to be a major limitation in arid and semi-arid environments (Bond & van Wilgen, 1996; Holloway-Phillips *et al.*, 2015). Temperature is crucial in controlling sprouting and following seedling appearance with ecological conditions ideal for seedling formation (Luna *et al.*, 2012; Holloway-Phillips *et al.*, 2015). Several plant species have survived climatic fluctuations such as moisture and temperature in their adaptability and regeneration success (Doescher *et al.*, 1985). Current anthropogenic warming of the climate could enforce growing physiological restrictions on recruitment and early development, especially through their effects on seed and seedling traits (Walck *et al.*, 2011).

The genus Proteaceae, is a main constituent of many fynbos communities in the species rich CFR with its Mediterranean climate. This type of climate is described by cool, wet winters and hot, dry summers, with fire being a recurring disturbance and driver of ecosystem dynamics (Cowling *et al.*, 2005). There are several cues for seed germination in fire prone Mediterranean-climate ecosystems. These include fire seed release in serotinous taxa with canopy stored seeds (Lamont *et al.*, 1991), heat-induced fracture of water and gas impermeable seeds coats (Jeffrey *et al.*, 1988; Brits *et al.*, 1993), biochemical stimulation of seed embryos (Blommaert, 1972, van de Venter & Esterhuizen, 1988; Musil & De Witt, 1991) by smoke (De Lange & Boucher, 1990; Brown, 1993a; Van Staden, 2000; Brown & Botha, 2004) and gases (e.g. ethylene and ammonia) released through fires (van de Venter & Esterhuizen, 1988), and enhanced diurnal soil temperature amplitudes in immediate post-fire environments (Brits, 1986). The latter, especially relevant where larger increases in diurnal temperature minima than maxima are anticipated with climate warming, the consequent reduced temperature amplitudes accompanying warmer night-time temperatures having potentially negative consequences for seedling recruitment and ecosystem recovery following fire. Seeds can sprout post-fire from soil seeds reserves, like members of the family Cistaceae and many legumes (Thanos, 2000; Hanley & Fenner, 1998) or from canopy seeds banks, like some Conifers (Lamont, 1991).

Species growing in fire-prone areas, display serotiny or canopy seed storage which is a common occurrence (Cowling *et al.*, 1987; Lamont, 1991; Whelan, 1995; Jaleel *et al.*, 2009). Fire resilience is increased through the storing of seeds in the canopy thus protecting them from heat and postponing distribution through the strategy of serotiny (Jaleel *et al.*, 2009; Salvatore *et al.*, 2010). Serotinous cones or fruits release the stored seeds after fire and seeds might benefit from post-fire conditions for germination and seedling formation (Ne'eman *et al.*, 1993; Henig-Sever *et al.*, 2000).

With respect to seedling recruitment, there are several cues in fire prone Mediterranean ecosystems for seed germination. These include fire seed release in serotinous taxa with canopy stored seeds (Lamont *et al.*, 1991), heat-induced fracture of water and gas impermeable seed coats (Jeffrey, Holmes & Rebelo, 1988; Brits *et al.*, 1993), biochemical stimulation of seed embryos (Blommaert, 1972, van de Venter & Esterhuizen, 1988, Musil & De Witt, 1991) by smoke and gases (e.g. ethylene and ammonia) released during fires (Van de Venter & Esterhuizen, 1988; Baskin & Baskin 1998), and increased diurnal soil temperature amplitudes in immediate post-fire environments (Brits, 1986). The latter, especially relevant where larger increases in diurnal temperature minima than maxima are anticipated with climate warming, the consequent reduced temperature amplitudes accompanying warmer night-time temperatures having potentially negative consequences for seedling recruitment and ecosystem recovery following fire. Post-fire seed germination can take place from soil seeds banks such as legumes, Cistaceae (Hanley & Fenner, 1998; Thanos, 2000; Jaleel *et al.*, 2009) or Conifers that germinate from cover seed banks (Lamont, 1991).

A crucial stage following seed germination and seedling establishment in Mediterranean-climate ecosystems is seedling persistence, especially over the hot dry summer period following wintertime seedling recruitment. Consequently, an understanding of the resilience of seedlings of different taxa and life forms to drought and heat stress is required. In Mediterranean ecosystems, germination takes place at moderately low temperatures, between 15 °C and 20 °C (Thompson, 1970; Thanos & Georgiou, 1988; Bell *et al.*, 1985; Luna *et al.*, 2011). Changing climatic conditions and increasing temperature are expected to continue increasing at a fast rate (Solomon *et al.*, 2007; Luna *et al.*, 2011). The consequences of climate change on germination are numerous and far reaching, but little information is available (Fenner & Thompson, 2005; Walck *et al.*, 2011). Several studies were performed on plants in colder areas of the world, and germination was projected to benefit from a warmer climate (Arft *et al.*, 1999; Graae *et al.*, 2008; Milbau *et al.*, 2009). No information is available on the effects of warming on the germination on Mediterranean species. A decrease in germination with warmer temperatures may be expected for plants

growing in these areas (Thanos & Georgiou, 1988; Bell *et al.*, 1995), there are no studies on the germination of plants with different temperature ranges.

In this study, the germination response of three species growing along a moisture gradient to drought and in a greenhouse was examined. The aims of this study were to better understand the impact of changing environmental conditions on germination, and how temperature and moisture affect Proteaceae seed. It is expected that germination of Proteaceae species may be more restricted to higher temperature and lower water availability along the gradient.

## 4.2. Materials and methods

### 4.2.1. Experimental design and statistical analysis

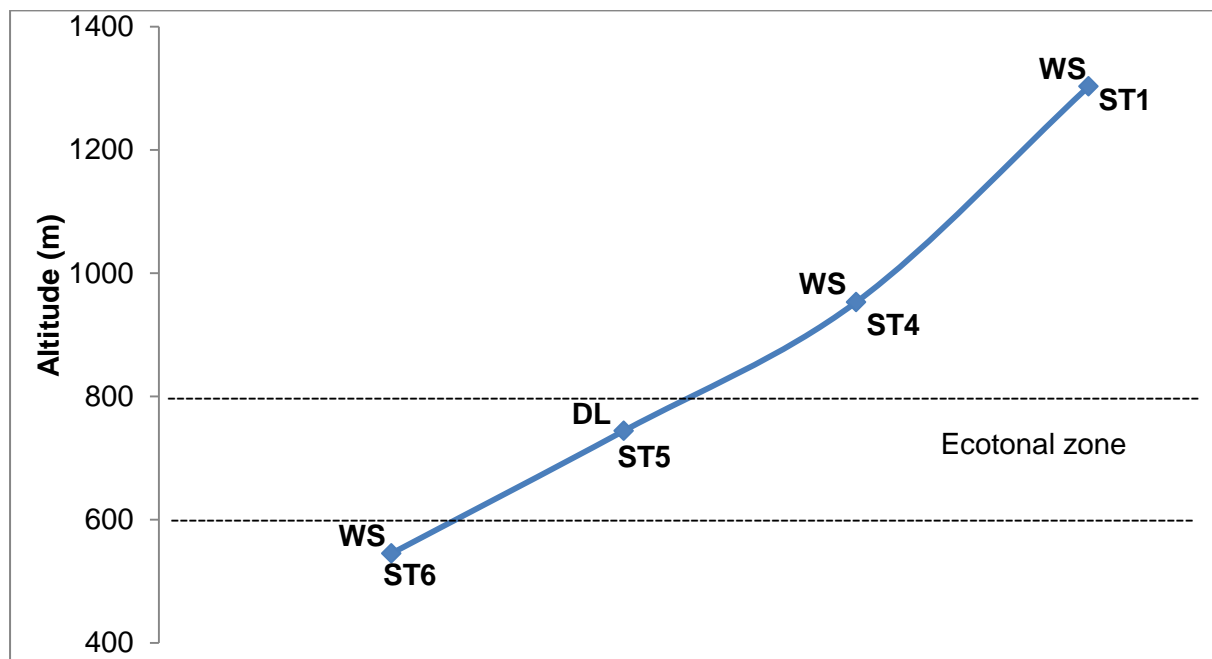


Figure 4.1. The study area, a temperature and moisture gradient on a north-facing mountain slope, showing the location of weather stations (WS), data logger (DL) and experimental plots (ST1-ST6). WS- recording hourly temperature and rainfall and DL- only recorded temperature. (Adapted: L. Agenbag (2006)).

Seeds from three Proteaceae species, *Protea repens* (L.)L., *Protea laurifolia* Thunb. and *Leucadendron lauroolum* (Lam.) Fourc. were collected from different cones at the different sites along the climate gradient and were treated with commercially available smoke primer (Brown & Botha, 2004).

Table 4.1. Location of weather stations along the gradient

	ST-1	ST-2	ST-3	ST-4	ST-5	ST-6
Full weather station	•			•		•
Mini weather station	▪	▪	▪	▪	▪	

The field plots were prepared at four selected altitudes (Figure 1): 1. 1303 m, near the top of the mountain; 2. 953 m, near the middle of the gradient; 3. 744 m, in the ecotonal zone, and 4. 545 m, in the succulent karoo. Station 6 (ST-6) is the hottest site along the gradient. Table 4.1 indicates the location of weather stations at the different stations (sites) along the gradient. This study was conducted between 2011 and 2012.

A vegetation analysis and a phenological study indicated these sites as important thresholds controlling growth and distributions of species on the gradient (Agenbag, 2006). Each plot consisted of six containers, each 0.6 m wide, 0.64 m long and 0.3 m deep. The containers were placed on top of the soil surface and filled with sandstone derived soil collected from the 953 m site, in a randomized design. The bottoms of the containers were left open, but were isolated from the soil below with a layer of coarse gravel and plastic sheeting with regularly spaced holes to facilitate drainage. An outer frame was filled with sand provided insulation around the outer perimeter of the experimental plots. Different age cones (1 to 5 year-old depending on plant age) of broadly and narrowly distributed serotinous Proteaceae species were collected at five sites along the climatic gradient. Seeds (achenes) were extracted from the different aged cones of Proteaceae species located at the different sites along the climate gradient and dried in a forced draft oven at 30 °C. Two hundred seeds per species were collected and 100 seeds were sown per container, to randomly assigned positions in the containers. The seeds were irrigated with one liter of diluted smoke extract solution to promote germination which only occurs once primary dormancy is overcome by

seasonal temperature changes (Ooi, *et al.* 2006) and were left to germinate under natural climate conditions thereafter.

#### *4.2.2. Statistical analysis*

Counts of seedlings emerging above the soil surface were made at weekly intervals. Mean germination percentage was calculated as the average number of seedlings that have emerged over the test period ending soon after completion. An analysis of variance tested for significant effects of plant position along the climate gradient and germination.

#### *4.2.3. Greenhouse germination experiment*

Different age cones (1 to 5 year-old depending on plant age) of broadly and narrowly distributed serotinous Proteaceae species were collected at five sites along the climatic gradient. Seeds (achenes) were extracted from the different aged cones of Proteaceae species located at the different sites along the climate gradient and dried in a forced draft oven at 30 °C. Sixty seeds per species extracted from the different age cones of Proteaceae species located at the different sites along the climate gradient were sown into six germination trays containing sterile soil comprising a mixture of sand, loam and organic material (3:1:1) in a passively ventilated greenhouse. The seeds were irrigated daily with equal volumes of water supplied by an automated irrigation system. A randomized complete block design was adopted. Counts of seedlings emerging above the soil surface were made at 3-day intervals. Mean germination percentage was calculated as the average number of seedlings that have emerged over the test period. An analysis of variance tested for significant effects of plant position along the climate gradient and germination.

### **4.3. Results**

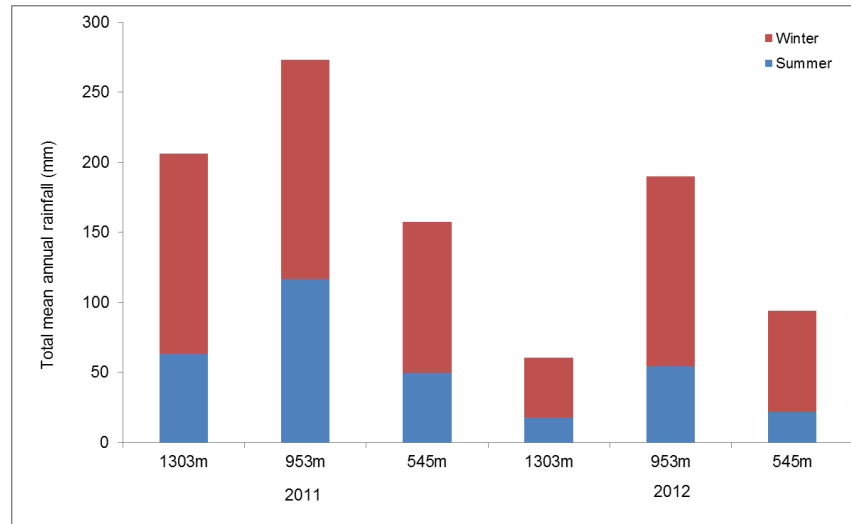
The highest mean total annual rainfall for 2011 was recorded at altitude 953 m (ST 4) and the lowest mean total annual rainfall was recorded at altitude 545 m (ST 5). In 2012 the mean highest total annual rainfall was recorded at altitude 953 m (ST 4) and the lowest mean total annual rainfall was recorded at altitude 1303 m (ST 1). The mean winter total annual rainfall is higher compared to the mean summer total annual rainfall for all three years (Fig. 4.1A). The year 2011 recorded the highest recorded the highest annual total rainfall compared to 2012. The highest mean annual soil moisture was recorded at altitude

1303 m (ST 1) (kPa) and the lowest mean annual soils moisture was recorded at altitude 545 m (ST 5) (kPa) during 2011 (Fig. 4.1B). The lowest mean total annual soil moisture was recorded at 1303 m (ST 1) during 2013 compared to 2011 and 2012 (Fig 4.1B).

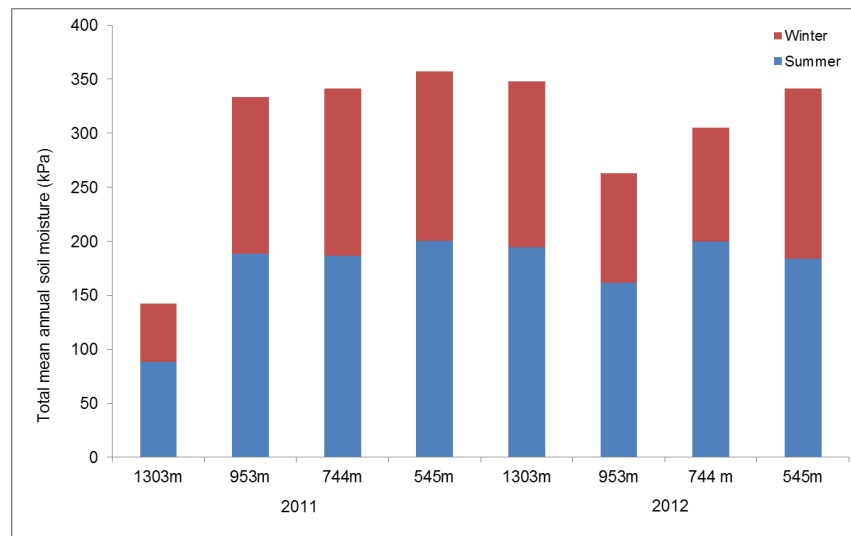
Figure 1C indicated the effect of temperature associated with the different stations (altitudes) at which the experimental plots were placed on germination. The percentage germination at ST 6 (altitude 545 m) was 0% for species *Protea repens*, *Protea laurifolia* and *Leucadendron laureolum*. Station 6 also recorded the highest mean monthly temperature at  $\pm 12.11$  °C. Station 5 (altitude 744 m) indicated a 24% germination rate for *P. repens*, 0.5% for *P. laurifolia* and 0.5% for *L. laureolum* with a mean monthly temperature of  $\pm 10.33$  °C. At altitude 953 m (ST4) *P. repens* showed a germination rate of 20.5%, *P. laurifolia* (0.5%) and *L. laureolum* (0.5%) with a mean monthly temperature of 6.69 °C. Station 1 (altitude 1303 m) *P. repens* indicated a germination rate of 1.5%, *P. laurifolia* (1%) and *L. laureolum* (0.5%) at a mean monthly temperature of 9.27 °C (Fig. 4.1C). Germination occurred at stations 1, 4 and 5 at a mean monthly temperature of 6.7 to 10.4 °C (Fig. 4.1C).

*Leucadendron laureolum* and *Protea laurifolia* germinated in the greenhouse after 74 days (Table 4.2) and *P. repens*, *P. laurifolia* and *L. laureolum* germinated after 227 days in the field (Table 4.2). The total percentage germination for *P. repens* in the greenhouse was 0% for seeds collected at all stations, 16.7% for *P. laurifolia* seeds collected from ST3, 1% for seeds collected from ST2 and 1% for seeds collected at ST1. The total germination % for *L. laureolum* in the greenhouse was 26.7% for seeds collected at ST5. 0% for seeds collected at ST4, 11.7% for seeds collected at ST3, 10% for seeds collected at ST2 and 1.67% for seeds collected at ST1 after 74 days (Table 4.2). Total germination percentages for the three species in the field were: 0% germinated at ST6, 24% at ST5, 20.5% at ST4 and 1.5% at ST1 for *P. repens*; for *P. laurifolia*: 0% germinated at ST6, ST5 and ST1 and 1.5% at ST4 and for *L. laureolum*: 0% germinated at ST6, ST5 and ST1 and 0.5% at ST4 after 227 days (Table 4.2).

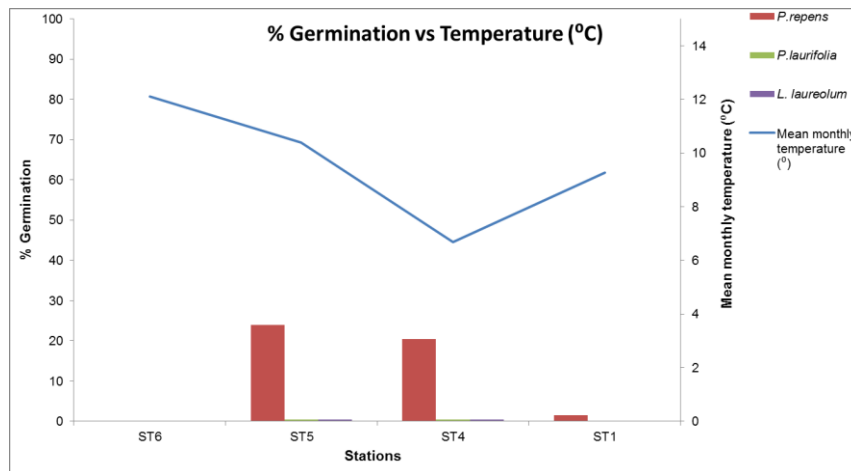
**A**



**B**



**C**



**Figure 4.2. (A)- Total mean annual rainfall (mm) at Jonaskop (2011-2012) , (B) Total mean annual soil moisture (kPa) (2011-2012) at different altitudes and (C) % germination vs temperature (°C) at the different stations**

Table 4.2. Total percentage (%) germination of seeds collected from different stations at Jonaskop (Field and greenhouse experiments)

Species	Total Germination (%)					
	ST-1	ST-2	ST-3	ST-4	ST-5	ST-6
Field (after 227 days)						
<i>L. laureolum</i>	0			1	0	0
<i>P. laurifolia</i>	0			1	0	0
<i>P. repens</i>	1.5			20.5	24	0
Greenhouse (after 74 days)						
<i>L. laureolum</i>	1.67	10	11.7	0	27	
<i>P. laurifolia</i>	1	1	16.7	0	0	
<i>P. repens</i>	0	0	0	0	0	

#### 4.4. Discussion

The constructed climate niches showed that the percentage germination at ST 6 (altitude 545 m) was 0% and is too dry for *Protea repens*, *Protea laurifolia* and *Leucadendron lauroloium*. Station 6 falls outside the climate ranges from under which these species originate and is too hot for the three test species to survive. The germination percentages of the seeds sown at this site might indicate the response the three species under higher temperature and future climate change. Station 5 (altitude 744 m) and ST4 (altitude 953 m) indicated the highest total germination percentage compared to the other stations. Germination percentages of the seeds of the three test species were expected to be high at ST 4 (953 m) and ST1 (1303) sites because of their cooler temperature and higher rainfall/precipitation. The field study was carried out in the year 2012 and because of lower rainfall and soil moisture compared to the previous year (2011). Longer drier and warmer conditions at ST1 and ST4 in 2012 might have influenced germination rates. Station 4 (953 m) and ST5 (744 m) are close to each other and could represent optimum growing conditions for the three species. This could possibly explain the higher germination percentages compared to the other stations. The mean monthly temperature ranged between  $\pm 11$  °C to 13.0 °C at ST4 and ST5 and could be optimum temperature for germination of the three species. According to Probert (2000) temperature is one of the main environmental factors that control germination. Timing of germination through the temperature range over which germination take place is influenced by environmental temperature. Temperature-controlled germination is related to a mechanism that regulates the dormancy status of natural seed populations in numerous species. This type of regulation by temperature allows the seeds to escape severe environmental conditions for seedling establishment (Probert, 2000; Luna *et al.*, 2012). In studies by Thanos and Georghiou (1988) and Bell *et al.* (1995) it was found that Mediterranean environments favour germination at relatively low temperatures, between 15 °C and 20 °C. This finding is in agreement with the results of the present study. This study has shown that Proteaceae species are dependent on temperature conditions.

Several studies have indicated that germination patterns show the seasonal and climatic environments which naturally occur during the process of germination (McDonough, 1977; Young & Evans, 1981; Doescher *et al.*, 1985). In the present study, germination of *P. laurifolia* and *L. lauroloium* happened after 74 days in the greenhouse. *P. repens* did not germinate. Seeds collected from all the stations germinated except for ST4. Only seeds from *P. laurifolia* sown from ST 3 germinated in the greenhouse. Seeds sown from *P. repens* germinated at ST5 and ST4 and *L. lauroloium* germinated at ST 5, ST4 and ST1 and seeds from *P. laurifolia* germinated at ST4 in the field experiment after 227 days. The seeds from the greenhouse experiment germinated faster because of controlled conditions such as cooler temperature and

water availability. Germination of three species in the field needed a longer growing period. Another explanation for the longer germination period in the field experiment could be that the Proteaceae seeds has the ability to avoid germination during the warm and drier season. This could be explained by the mean monthly temperature range between ST4 and ST5 that germination possible for the three species. The year 2012 was warm and dry according to the climatic results found for the experimental period. Therefore the longer germination period. A possible explanation for the shorter growth period in the greenhouse could be that the greenhouse temperature was cooler and water was available. With higher temperatures, fires are also expected to increase (Moreno *et al.*, 2010, Luna *et al.*, 2011) and can adversely affect the survival of Proteaceae in the CFR.

#### **4.5. Conclusion**

The results of the present study suggest that under future predicted higher temperatures due to climate change, Proteaceae in the CFR may be more at risk to extinction. High sensitivity of Proteaceae to external conditions may cause better environmental monitoring by the plant and thus, trigger germination when seedling survival chances are high. The smaller temperature range showed for Proteaceae germination in this study might not be regarded as a disadvantage but a tool of drought avoidance. Higher temperatures and more regular and intense fires could lead to a decrease in Proteaceae in the CFR. In this study, the germination responses were based on seeds that were collected during a single year. Hence, it is unknown whether the germination responses found in this study would have been different if the seeds had been collected during different years with different climatology, especially different temperature conditions.

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## CHAPTER 5

### General discussions and Conclusions

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The main purpose of this study was to determine if reproduction, photosynthesis and growth of selected Proteaceae species from warm, arid sites at low elevations along a moisture gradient will be more resilient to heat and drought stress accompanying warming, than those from cool humid sites at high elevations along the same gradient. This was achieved by comparing the germination of seed of different Proteaceae to elevated temperatures and diminished precipitation approximating climate warming; comparing the resilience of juvenile stages of different Proteaceae genera and ecotypes to drought with respect to photosynthetic capacity, water relations, stem diameter and sap flow. Also, the causes of elevated temperatures and diminished rainfall frequencies on the reproductive development of serotinous Proteaceae species were established in this study.

Enforced seed dormancy was tested by measuring seed germination in 11 Proteaceae species in experimental mesocosms with soil temperature elevated by 1.4 and 3.5 °C above ambient. Significantly diminished mean daily germination and absolute germination at a soil temperature elevation of 3.5 °C were observed in 91% (10) of the species, the exception *L. spissifolium*, but only 64% (7) of the species at a soil temperature of 1.4 °C in experiment 1. Comparison of diurnal soil temperature minima and maxima recorded in the mesocosms during the first month of germination with base and ceiling soil temperatures reported peak Proteaceae germination, clearly indicated that the measured reductions in seedling recruitment were more closely associated with increases in diurnal soil temperature minima and maxima.

Diurnal soil temperature maxima of 18.7 and 20.4 °C recorded in the warming treatments, which were below the ceiling temperatures of between 20 and 24 °C above which Proteaceae germination would be inhibited. These findings supported the hypotheses that the dormancy of Proteaceae seed would be enforced by elevated diurnal temperature minima accompanying climate warming.

In experiment 2, drought resilience was tested in one-year-old seedlings of 16 Proteaceae species in a greenhouse. Measured transpiration rates declined linearly with increasing duration of drought and decreasing soil volumetric content in all 16 Proteaceae test species. Calculated initial transpiration rates at soil field capacity and average transpiration rates were significantly positively correlated among the 16 species, as were the total quantities of water transpired on a drought day basis. Computed drought periods for each Proteaceae species greatly exceeded the average monthly periods without rain during summer in the current distribution of that

species. The most drought-resilient Proteaceae species identified in this study displayed the lowest initial transpiration rates at field capacity, the smallest declines in the tempo of transpiration with decreasing soil moisture content, and the least quantities of moisture transpired. The results obtained in this study leads to the assumption that Proteaceae seedlings are tolerant of summer desiccation periods over a large part of the South African Cape Floristic Region, and that enforced seed dormancy induced by elevated night-time temperatures, is the post-fire recruitment stage among Proteaceae that is most sensitive to climate change.

This study investigated diurnal stem diameter variations of *P. repens* growing along a moisture gradient. The present study revealed that with low dewfall during the summer months at the highest elevation the stem diameter readings were lower compared to the readings for winter with higher dewfall or non-rainfall precipitation. The lowest elevation exhibited lower dewfall over the entire experimental period compared to the dewfall at the highest elevation. This study emphasises the importance of the of the non-rainfall precipitation as a reasonably minor amount of water for dry or semi-dry areas. This important supply adds to the water equilibrium during droughts or low precipitation episodes. The non-rainfall gains would offer added supply of water for Proteaceae during droughts or low rainfall periods. The fitness of *P. repens* as a shrub species within the Fynbos Biome might be changed in the future as a result warming and low rainfall conditions. This study indicated that more long term experimental studies are needed considering the variation in climate conditions and to get more significant results on climate change effects.

Changes in diurnal, monthly sap flow rates, vapour pressure and photosynthetic capacity was measured in *Protea repens* L. along a moisture gradient at five different stations. This species indicated significantly negative correlations between their total daily amplitudes in sap flow and station maximum daily temperature in winter, spring, summer and autumn. This study revealed that significantly higher sap flow rates of *P. repens* occurred at higher elevations and concomitant lower temperatures compared to those at lower elevations and higher temperatures. Under wet conditions especially after good rainfall, sap flow of plants at the highest elevation was inhibited which could be due to soil saturation and inhibition of root metabolism. In the present study the leaves of *P. repens* displayed significantly negatively correlations between their total daily amplitudes in sap flow and photosynthetic effective quantum yield. *P. repens* L. showed characteristics of whole plant water use efficiency which is common to other drought adapted woody plants. In the present study, the test species proved to be highly tolerant of water stress/drought and are well adapted to Mediterranean climatic conditions. This experiment could probably be improved by including more test species and more long term monitoring which could produce more significant results.

The seed germination percentages of three Proteaceae types (*Protea repens*, *Protea laurifolia*, *Leucadendron laureolum*) growing along a moisture gradient and in a greenhouse were measured to better understand the impact of changing environmental conditions on germination. Growth of *P.repens* was also examined in the field.

The results showed the dependence of Proteaceae species on temperature conditions during the process of germination. *Protea laurifolia* and *L. laureolum* took 74 days to germinate in the greenhouse and took 227 days to germinate under field conditions.

Overall, there were differences in the level of responses on the sites along a moisture/temperature/elevation gradient to the effects of warming and drought. Plants in colder, wetter higher elevations were more sensitive to warming and warmer-drier lower elevations were more sensitive to drought and during the different seasons plant processes such as stem diameter and sap flow were more sensitive to warming during the summer than during the winter at lower elevations. This study indicates that more climate change experimental studies and more test species considering the climatic variation so that more accurate predictions regarding temperature responses to climate change can be made. The results of the present study also suggest that under future predicted higher temperatures due to climate change, Proteaceae in the CFR may be more at risk to extinction. Drought conditions and high temperature could lead to unsuccessful germination and possibly local extinctions of Proteaceae. Proteaceae in the CFR could be reduced by elevated temperatures and more recurring and severe fires. The post-fire regeneration stage could be the most vulnerable to the effects of climatic changes. It is suggested that these areas should be monitored long term and after fires for changes in species assemblages.

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