GROWTH CHARACTERISTICS OF THREE *EUCALYPTUS* CLONAL HYBRIDS IN RESPONSE TO DROUGHT STRESS: THE UNDERLYING PHYSIOLOGY

By

ALANA EKSTEEN

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ABSTRACT

Previous research by Drew *et al*. (2009) on a SAPPI dendrometer trial, in northern KwaZulu-Natal, South Africa, yielded growth results for two *Eucalyptus grandis* clones (termed *E. grandis* x *urophylla* (GU) and *E. grandis* x *camaldulensis* (GC)). The GU clone was found to have a greater diameter than the GC clone, and further research has demonstrated that age, not environmental conditions, is the major determinant of tree growth for the GU and GC clone. The clones also showed different patterns of growth response following a rainfall event. In current study, young plants of three *Eucalyptus grandis* clones (two GU clones (GUA and GUW) and one GC clone) were grown in 80 L planting bags for 18 months at UKZN, Westville, South Africa. The experiments for this study entailed subjecting the three clones to three watering regimes. The trial was conducted using a randomised complete block (RCB) with a 3^3 factorial design (i.e. 9 treatments with 12 replicate plants in each treatment). The three watering regimes were monitored daily with a soil moisture probe and were a control (little or no water stress was applied), chronic water stress (mild, long-term, gradual water stress) and acute water stress (rapid, severe, cyclic water stress with periods of recovery from stress by re-watering). Physiological (photosynthesis, plant water relations and hydraulic conductance characteristics) and morphological (height, diameter and total biomass) measurements were performed. Two harvest periods determining K_h and total biomass at 9 and 18 months were undertaken, whereas morphological measurements were taken monthly throughout the trial. Considering that there were differing growth responses of clones in response to rainfall events (observed by Drew *et al*., 2009), the recovery of the plants from water stress was also studied (resistance to water flow in leaves, assimilation rates and stomatal conductance). Further investigation of leaf characteristics was performed to assess different aspects of the water transport system (stomatal density) and improvement of water use efficiency (WUE) in response to water stress by measurement of $\delta^{13}C$ in leaf samples.

The GC clone showed 30% greater height growth than the GU clones. Growth efficiency, root biomass and root:shoot were significantly greater in the GC clone. The GU clones showed significantly greater stem and leaf biomass, primarily due to the 25% greater total leaf area, after 18 months growth. Diameter of the plants subjected to the control, was 8% higher compared with water stress treatments ($p = 0.036$). Water stress significantly reduced tree volume by up to 10% and leaf area by 30%. J_{max} and V_{cmax} were significantly lowered in plants subjected to acute stress at leaf wilting point ($p < 0.001$). After as little as 7 days re-watering however, J_{max} and V_{cmax} were not different from the control.

Plants subjected to chronic water stress showed moderately improved instantaneous WUE (8% increase compared with the control and acute stress). Long-term WUE (by measurement of $\delta^{13}C$ in leaves, was significantly higher in leaves subjected to chronic water stress ($p < 0.0001$). Stomatal density was significantly different among clones, as the GUA clone showed complete stomatal absence on all upper leaf surfaces sampled ($p < 0.001$), although stomatal absence did not occur in leaves of the closely related GUW clone. Assimilation rate, stomatal conductance, K_h and total biomass were significantly positively correlated with one another. Recovery of plants subjected to acute stress differed among the GU and GC clones. A_n, g_s and R_{leaf} (resistance to water flow in leaves) "recovered" (i.e. not significantly different from the control) by day 2 in the GC clone, but only by day 7 in the GU clones. There was hydraulic dysfunction in the GC clone which was suggested to be caused by collapse of the minor veins due to drought stress. The hydraulic dysfunction did not affect mesophyll tissue of the GC clone and thus hydraulic recovery was rapid. Although the GC clone was more drought tolerant (due to significantly greater root biomass), the selection of a GU clone would ensure improved wood productivity when planted commercially. The GUW clone showed enhanced traits of drought tolerance than the GUA clone including 20% less leaf dieback in response to water stress, as well as little to no variability of K_h in response to all watering regimes, and moderately improved WUE. Plants subjected to chronic stress showed long-term and instantaneous improvement in WUE, and greater diameters were maintained than plants subjected to acute stress. Perhaps the most important morphological and physiological parameter identified in the current study was that of leaf area. Leaf area differed significantly among eucalypt clones, in response to water stress and with tree age. Leaf area affected the expression of growth efficiency, hydraulic efficiency, total carbon assimilated and total biomass achieved. For the GU and GC *Eucalyptus* clones in the current study, the primary parameter driving physiological interactions and ultimately determining wood productivity could be considered to be leaf area.

PREFACE

The experimental work described in this thesis was carried out in the School of Biological and Conservation Sciences, University of KwaZulu-Natal, Westville, Durban from May 2009 to December 2011, under the supervision of Prof Norman W Pammenter (UKZN) and Dr Valerie Grzeskowiak (SAPPI).

This study represents original work by the author and has not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others, it is duly acknowledged in the text.

DECLARATION 1 – PLAGIARISM

I, Alana Bridget Eksteen, declare that:

1. The research reported in this thesis, except where otherwise indicated, is my own original research.
2. This thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
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5. This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the References section.

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DECLARATION 2 – PUBLICATIONS

DETAILS OF THE CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis (include publications in preparation, submitted, in press and published).

Publication 1:

"Stomatal characteristics of *Eucalyptus grandis* clonal hybrids in response to water stress."

Completed: Submission to *Southern Forests* in January 2012 is expected.

Publication 2:

"Growth characteristics and co-ordination of plant hydraulic conductance in three *Eucalyptus grandis* hybrids in response to water stress"

Completed (with co-authors for corrections): Submission to *Trees: Structure and Function* in January 2012 is expected.

Publication 3:

"A comparison of water use efficiency in 3 *Eucalyptus grandis* clones in response to water stress: instantaneous versus $\delta^{13}C$ measurement"

In preparation: Submission to *Forest Ecology and Management* in February 2012 is expected.

Publication 4:

"Photosynthetic, stomatal and hydraulic conductance recovery in 3 eucalypt hybrids following drought stress"

In preparation: Submission to *Tree Physiology* in March 2012 is expected.

Signed_____

Please note: The current study was commissioned as a PhD project by SAPPI, in conjunction with Prof Pammenter at UKZN. The details of the study were subject to a confidentiality agreement (Intellectual Property agreement), proposed by SAPPI. The research publications that arose from the results from the current study are only to be submitted for publication after this thesis is handed in for examination, as per the IP agreement.

TABLE OF CONTENTS

LIST OF FIGURES

Page

LIST OF TABLES

Page

LIST OF SYMBOLS

$\delta^{13}C$	Discrimination of ^{12}C and ^{13}C (‰)
$\delta^{15}N$	Discrimination of ^{14}N and ^{15}N (‰)
Γ	CO_2 compensation point (ppm CO_2)
Ψ	Water potential (MPa)
Ψ_p	Pressure potential (MPa)
Ψ_Π	Osmotic potential (MPa)
ΔP	Change in pressure (MPa)
$A:C_i$	Ratio of assimilation to internal carbon dioxide
A_l	Leaf area distal to the segment measured (m^2)
A_n	Assimilation rate (µmol CO_2 m^{-2} s^{-1})
ANOVA	Analysis of Variance
A_{sw}	Cross-sectional area of conductive sapwood (m^2)
C_a	CO_2 concentration at ambient conditions (ppm)
C_i	CO_2 concentration internally within the leaf (µmol CO_2)
E_n	Evapotranspiration rate (mol CO_2 m^{-2} s^{-1})
EF	Evaporative flux
F	Flow (kg s^{-1})
g_s	Stomatal conductance (mol CO_2 m^{-2} s^{-1})
GC	*Eucalyptus grandis* x *camaldulensis*
GC 438	*Eucalyptus grandis* x *camaldulensis* clonal name
GDP	Gross domestic product
GE	Growth Efficiency (cm^3 wood m^{-2} leaf area $year^{-1}$)
GU	*Eucalyptus grandis* x *urophylla*
GUA 380	*Eucalyptus grandis* x *urophylla* clonal name
GUW 1700	*Eucalyptus grandis* x *urophylla* clonal name
HCl	Hydrochloric acid (M)
HPFM	High Pressure Flow Meter
IRGA	Infra-red gas analyser

J_{max}	Maximum assimilation rate at saturating CO_2 (µmol CO_2 m^{-2} s^{-1})
k	Conductance (kg s^{-1} MPa^{-1})
K	Conductivity (kg s^{-1} MPa^{-1} m^{-1})
K_h	Hydraulic conductance normalised by leaf area (kg s^{-1} MPa^{-1} m^{-2})
K_{leaf}	Leaf hydraulic conductance normalised by leaf area (kg s^{-1} MPa^{-1} m^{-2})
K_{root}	Root hydraulic conductance normalised by leaf area (kg s^{-1} MPa^{-1} m^{-2})
K_s	Specific hydraulic conductivity (kg s^{-1} MPa^{-1} m^{-1})
K_{stem}	Stem hydraulic conductance normalised by leaf area (kg s^{-1} MPa^{-1} m^{-2})
$K_{total\ leaf}$	Total leaf hydraulic conductance (kg s^{-1} MPa^{-1} m^{-2})
L	Litre
LAR	Leaf area ratio
LSC	Leaf specific conductivity (kg s^{-1} MPa^{-1} m^{-2})
PAR	Photosynthetically active radiation (µmol quanta m^{-2} s^{-1})
PPFD	Photosynthetic photon flux density (µmol quanta m^{-2} s^{-1})
PWR	Plant water relations
PWUE	Photosynthetic water use efficiency (µmol CO_2 per mol water)
RCB	Randomised complete block
r	Resistance (MPa s kg^{-1})
R	Resisitivity (MPa s m kg^{-1})
$R_{extravascular}$	Hydraulic resistance of the extravascular tissue (MPa s m^2 kg^{-1})
R_h	Hydraulic resistance of the extravascular tissue normalised by leaf area (MPa s m^2 kg^{-1})
R_{lamina}	Hydraulic resistance of the lamina normalised by leaf area (MPa s m^2 kg^{-1})
R_{leaf}	Leaf hydraulic resistance normalised by leaf area (MPa s m^2 kg^{-1})
R_{leaves}	Hydraulic resistance of the leaves normalised by leaf area (MPa s m^2 kg^{-1})
$R_{petiole}$	Hydraulic resistance of the petiole normalised by leaf area (MPa s m^2 kg^{-1})
R_{root}	Hydraulic resistance of the roots normalised by leaf area (MPa s m^2 kg^{-1})
R_{shoot}	Hydraulic resistance of the whole shoot normalised by leaf area (MPa s m^2 kg^{-1})
R_{stems}	Hydraulic resistance of the stems normalised by leaf area (MPa s m^2 kg^{-1})

$R_{venation}$	Hydraulic resistance of the ventaion normalised by leaf area (MPa s m^2 kg^{-1})
RWC	Relative water content (%)
SLA	Specific leaf area (m^2 g^{-1})
SPAC	Soil-plant-atmosphere continuum
UKZN	University of KwaZulu-Natal
V_{cmax}	Initial slope of the A:C_i curve, carboxylation efficiency
WUE	Water use efficiency

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"For where your treasure is, there your heart will be also." Matthew 6:21

1. INTRODUCTION

1.1.1 *Eucalyptus*

Eucalyptus is a diverse genus of flowering trees and a member of the family Myrtaceae. Indigenous to Australia, there are more than 800 known species of *Eucalyptus* worldwide. *Eucalyptus* species occur in a wide range of habitats and are the most widely grown commercial hardwood in world (Turnbull, 2000). First described by Charles L'Hertier de Brutelle, the genus *Eucalyptus* boasts the tallest flowering trees in the world (*E. rengnens*) found in Tasmania (Brooker and Kleinig, 2006). Eucalypts are grown for a number of commercial purposes including pulp wood production, wood production, ornamentals, as well as extracted oil from the leaves. Primarily, eucalypts are produced commercially for the provision of medium and low density short-fibred pulp for paper (Turnbull, 2000). Globally, short-fibred pulp production is used for the manufacture of paper for printing, copying, writing and tissue papers and for this reason no other sector in world forestry has expanded as rapidly as the use of *Eucalyptus* (Turnbull, 2000). Because of short rotation periods consequent upon acceptable growth rates in suboptimum environments, *Eucalyptus* plantations can be found in 70 countries with more than 15 million hectares planted worldwide (Morris, 2008). Currently, the world's largest producer of *Eucalyptus* is Brazil and other major producers include Chile, Morocco, Spain and South Africa. The most commonly planted species of *Eucalyptus* in these countries is *Eucalyptus grandis*.

1.1.2 *Eucalyptus* in South Africa

In South Africa, the first planting of alien trees occurred in 1875 and presently, forestry accounts for 1.2% of the GDP (van der Zel, 1995; Boreham and Pallett, 2009). Forestry plantations are located in the high rainfall eastern and southern regions of the country and cover 1.1% of total land area of South Africa (DWAF, 2004). Between 35 – 40% of the 1.5 million hectares of afforested land in southern Africa are *Eucalyptus* plantations (DWAF 2005; Morris, 2008; Boreham and Pallet, 2009). South African forestry is highly reliant on the fast-growing, low-cost wood that *Eucalyptus* provides in order to remain globally competitive.

Eucalyptus plantations in the subtropical environment that is found in southern Africa, produce large quantities of biomass in stands of short rotation that are no longer than seven years. South Africa is considered a semi-arid country (area weighted mean annual rainfall < 500mm), and the land area available for forestry is extremely limited (DWAF, 2005; Dyer, 2007). This limitation is driven by competition for water, and the vast majority of forestry plantations are found in less than 20% of the country that receive greater than 800 mm of annual rainfall (Dye and Versfeld, 2007). South African legislation has further limited the available land area for forestry plantations because land potential suitable for afforestation is now preferable allocated to conservation and agriculture (Dyer, 2007; Jacobson *et al.*, 2008). The increase in the accommodation of predominantly wetland conservation areas in the future may cause current forestry area to decrease further despite the rise in demand for forest products. Land-use for afforestation still remains desirable from an economic standpoint, and the forestry industry in southern Africa directly and indirectly employs up to 500 000 people (Dyer, 2007). The forestry industry, therefore, can be considered not only from an economic perspective but from the social and environmental aspect as well.

1.1.3 *Eucalyptus* and the southern African environment

The production of sufficient quantities of raw woody material has been the focal point of the global forest industry for many years, with less importance being placed upon the quality of the wood. More recently, significant research has been performed on the properties of the wood fibres in terms of pulp and paper production. In South Africa and abroad, current forestry research has focused on growing trees at suitable sites with the right climate to ensure optimal wood anatomical attributes for the final product (Downs *et al*, 1999). These resources include environmental conditions *e.g*. rainfall, temperature, soil type *etc*. It is therefore possible that the production of fibres with desirable properties can be produced by growing specific tree species under the right conditions. The growth and development of trees is dependent upon the degree to which photosynthesis can take place, which in turn is affected by the prevailing environmental conditions at the site (Farrar, 1999).

Eucalyptus plantations are often found on sandy soils that have a low nutrient availability and poor water retention capacity (Laclau *et al.*, 2003). Potentially *Eucalyptus* productivity is very high but these high rates of productivity are not often achieved because of environmental constraints (Whitehead and Beadle, 2004). The availability of water, nutrients and suitable temperatures are the most important environmental factors affecting *Eucalyptus* growth (Beadle and Turnbull, 1992; White *et al.*, 2009). When assessing the literature in terms of water relations studies, it can be shown that *Eucalyptus* adapts to severe drought in terms of stress avoidance and/or tolerance (White *et al.*, 2000; White *et al.*, 2009). Although the southern African environment is suboptimal for achieving maximum growth potential of *Eucalyptus* species, acceptable growth rates can be achieved in environments that experience frost, drought or low nutrient availability (Beadle and Sands, 2004).

At any one site there are a large number of environmental variables that constitute the overall environmental conditions. Identifying which variable is the most important in terms of growth is often complex. The primary environmental variable limiting growth and determining plant distribution in South Africa is the availability of water. Water availability is the one environmental factor that has the greatest effect on tree stand growth (Dye, 1996). In South Africa, the areas of forestry for commercial purposes are in the higher rainfall regions of the country, with adequate temperatures and suitable soils for good growth (Dye, 2000). It has been assumed that differences in tree growth rates are primarily because of the diverse availability of water in tree stands (Turner *et al*, 2000). When soil water is unobtainable for uptake due to drought, each cell within the tree is limited in terms of water availability. The lack of water within the cells directly affects the tree in terms of both growth and development. The availability of water to trees is not determined only by the extent to which rainfall occurs. The balance between water loss and absorption allows for the conduction of the sap within the xylem column and ultimately the transport of water to the leaf canopy (Zimmerman, 1983). Gaining insight into the relationship between how water taken up is lost by the tree, and how this determines growth, is imperative for determining the correct species for a water-limited tree stand (White *et al.*, 1999). In environments where water is scarce, trees have evolved numerous morphological and biochemical mechanisms in order to survive (Merchant *et al*., 2007).

The primary environmental concern with regards to forestry in southern Africa is that *Eucalyptus* plantations affect water yield from natural river or stream catchments (Whitehead and Beadle, 2004; van Dijk and Keenan, 2007). As a semi-arid country, South Africa is already under pressure from low water resource availability and afforestation of *Eucalyptus* has the potential to decrease surface water generation and ground-water recharge (Dye and Versfeld, 2007). Forestry plantations are found in the areas of the country where up to 50% of the country's mean annual streamflow occurs (Scott *et al*, 1999). Afforestation has been shown, however debatable, to reduce streamflow by 3% and this has a significant effect on South African water resources (Dye and Versfeld, 2007). The extent of the impact of *Eucalyptus* afforestation is highly controversial and is ultimately dependent on the region, species, environment and land management practices used (Dye, 2000; Almeida *et al.*, 2007). Research into understanding the ecology of the plantation area and site-species matching are crucial for improving productivity in limited land-use and low rainfall areas (Louw and Scholes, 2002).

Technology-driven improvement of *Eucalyptus* productivity i.e. tree improvement programmes and more robust clonal hybrids, has allowed Brazil to emerge as the world's largest industrial *Eucalyptus* wood producer (Goncalves *et al.*, 2004). Originally, due to the diverse range of habitats in South Africa, *Eucalyptus grandis* was most commonly planted species for industrial forestry purposes (Denison and Kietzka, 1993). Gradually, in the warmer regions of South Africa, e.g. Zululand/Northen KwaZulu-Natal, *E.grandis* is being replaced with clonal hybrids, *E. grandis* x *E. urophylla* or *E. grandis* x *E. camaldulensis* (Morris, 2008). These clones are better alternatives for subtropical areas and have the benefits of faster growth and improved disease tolerance (Denison and Kietzka, 1993). In cooler regions e.g. KwaZulu-Natal midlands, *E. nitens* is planted because it has superior tolerance to frost (Clarke *et al.*, 1997).

1.1.4 *Eucalyptus* and climate change

Climate change is predicted to affect southern African forests in terms of temperature and precipitation. Mean annual temperatures in the eastern escarpment may rise between 2 – 6 ºC and mean annual rainfall is presumed to be on the decline (Dyer, 2008). Under conditions of climate change, optimal commercial forestry areas may shift (Warbuton and Schultze, 2008). The most significant factor to which forest species are most sensitive is that of rainfall.

Tree breeding programmes have already begun to breed and deploy robust, drought-tolerant *Eucalyptus* clonal hybrids in an attempt to maintain productivity under suboptimal conditions (Morris, 2008). Therefore investigation of the physiological and morphological mechanisms that accommodate drought-tolerance in *E. grandis* clonal hybrids is essential. The study of ecophysiological characteristics of *E.grandis* hybrids in response to differing water-stress regimes is vital for understanding how *Eucalyptus* trees will react to the weather experienced at a given ecological site. Stomatal, photosynthetic and hydraulic response to changes in water supply of *E. grandis* hybrids will elucidate how productivity is affected by periodic water-stress.

1.2 Plant water relations

1.2.1 Cohesion-Tension Mechanism

The soil-plant-atmosphere continuum (SPAC) describes the movement of water through the plant driven by the process of transpiration. Transpiration is an unavoidable consequence imposed by photosynthesis, whereby plants lose water through their stomata during the processes of absorbing and fixing carbon dioxide from the atmosphere. The translocation of an uninterrupted water column from the roots to the leaves is facilitated by the adhesive and cohesive properties of water molecules and the xylem cell walls. Water potential gradients from the roots to the leaves become increasingly negative and the hydraulic architecture of the plant has a direct effect on the xylem water potential gradient.

There are three theories explaining the ascent of sap in plants, but the most commonly accepted one is that of the cohesion-tension theory proposed by Dixon and Joly in 1894. The Cohesion-tension theory is based on the principle that water ascends the plant under tension, i.e. negative xylem pressure (Tyree and Dixon, 1983). The driving force for this negative pressure is created by the surface tension at the evaporative surface of the leaf which results in a decrease of the water potential (Ψ) of the mesophyll cell walls (Tyree and Zimmerman, 2002).

The lowering of the water potential is a primary result of the lowering of the pressure potential (Ψ_p). Pressure potential is one of the main components driving water potential, with other being that of solute potential ($\Psi_л$). The relationship can be described as follows:

$$\Psi = \Psi_p + \Psi_л$$

Van der Honert (1948) proposed an Ohm's Law analogy of the soil-plant-atmosphere continuum, stating that the driving force of the ascent of sap within any given plant is the constant lowering of the pressure potential in the direction of sap flow. There is however a point at which the water in the xylem column is subjected to pressures that are too negative (tensions are too great) to sustain. When this occurs, the xylem column breaks and the breaking of the continuous water column is known as a cavitation (Tyree and Zimmerman, 2002).

1.2.2 Xylem cavitation

Water in the xylem is said to be in a metastable state at a pressure below atmospheric pressure (Zimmerman, 1983). Under extreme tension, the continuous column of water can break and disturb water supply to the leaves (Tyree and Ewers, 1991). The dissolved gases found in the xylem come out of solution to form a micro-void. Due to the high tension in the xylem, the void can enlarge exponentially and completely fill the xylem conduit. This process is referred to as xylem cavitation (Tyree and Sperry, 1989). When the void forms a sufficient radius to completely fill the xylem conduit, the conduit is considered to be dysfunctional and unable to conduct water flow, and is known as an embolism (Tyree and Ewers, 1991).

Xylem cavitation can be induced by drought, freezing, pathogen and mechanical damage (Zimmerman, 1983; Tyree and Sperry, 1989; Sperry and Pockman, 1993). Embolised conduits can be refilled with water and continue functioning normally (Lambers *et al.*, 1998). The refilling of embolised conduits can occur under positive pressure experienced at night or during an occurrence of rainfall. The expansion of embolised air to adjoining xylem conduits has to be prevented and conduits are connected by means of a pit chamber (Tyree and Zimmerman, 2002). The porosity of the pit membrane determines the extent to which embolisms may spread from one conduit to another (Tyree and Sperry, 1989).

"Safe" pit membrane pores are regarded as those pores of a narrow diameter that essentially prevent the majority of embolisms from spreading to other conduits. Species-specific differences in vulnerability to cavitation are controlled by a strong selective pressure on the genetics of the pit membrane pores (Tyree *et al.*, 1995). The size of the pit membrane pores consequently forces a trade-off between the safety and ultimately the efficiency of sap flow of the conduit (Sperry and Sullivan, 1992). The risk of cavitation is inescapable, even for slightly water-stressed plants.

In many environments, water is the primary limiting factor affecting photosynthetic CO_2 fixation (Kramer and Boyer, 1995). Xylem embolisms cause xylem conductivity to decrease and hence water potential gradients to be greater, which can result in the closure of stomata. While stomatal closure beneficially decreases the amount of water lost by the plant, it also reduces the amount of carbon fixed via photosynthesis (Becker *et al*., 2000). A reduction in shoot growth rate, especially leaf growth, can be seen in even mildly water-stressed plants in response to xylem embolism formation, because xylem embolism has caused stomatal closure (Schultz *et al.*, 1988). Cavitation is the most deleterious cause of productivity loss of an agricultural environment, when drought stress is experienced (Lo Gullo and Salleo, 1993). Drought-adapted species will consequently possess smaller pit membrane pores to allow for a higher degree of resistance to cavitation (Lambers *et al.*, 1998). Changes in the dimensions of xylem conduits, from continued embolism formation in response to drought, have been shown to reduce whole-plant hydraulic conductance (White *et al*., 1999).

1.2.3 Plant Hydraulic Conductance

Plant hydraulic characteristics can be described in terms of conductivity or conductance. Conductivity is the flow rate through a plant per unit pressure gradient, K (kg s^{-1} MPa^{-1} m^{-1}). Conductance does not take into account the length of a conducting system and is simply the flow rate per unit pressure drop, k (kg s^{-1} MPa^{-1}). The inverse of conductivity is resistivity and the inverse of conductance is resistance. Resistances are assumed to be additive in series (Tyree and Zimmerman, 2002).

Hydraulic conductivity can be expressed as follows:

$K_h = F * L / \Delta P$ kg m s^{-1} MPa

Where F = flow rate (kg s^{-1}); L = length (m); and ΔP = pressure gradient (MPa).

Hydraulic conductivity can also be expressed in terms of area of conductive sapwood, Specific Hydraulic conductivity (K_s). $K_s$is a measure of the efficiency of the stems or branches or twigs to conduct water:

$K_s = K_h / A_{sw}$ kg s^{-1} m^{-1} MPa^{-1}

Where A_{sw} = cross-sectional area of the conductive sapwood (m^2).

Leaf-specific conductivity (LSC) is a measure of the hydraulic sufficiency of a plant to supply water to the leaves at the end of the branch segment:

$K_l = K_h / A_l$ kg s^{-1} m^{-1} MPa^{-1}

Where A_l = leaf area distal to segment measured (m^2) (Zimmerman, 1978).

Measurement of the hydraulic conductance parameters expressed above show that the trends observed will differ depending on species, growth conditions and the growth form within a species (Ewers *et al*., 1991; Cochard *et al*., 1997). The segmented growth form of the plant dictates that the hydraulic construction of a plant can be divided into components that determine overall whole-plant hydraulic conductance. Whole-plant hydraulic conductance can be apportioned into the roots, stems and leaves, and the leaves can be further sub-divided into petiolar, vascular and extravascular conductivity. Perennial plants conserve a distinct drop in conductance at the petiolar insertions of the leaf, and this allows plants to sacrifice their leaves during winter or periods of drought stress (Tyree and Zimmerman, 2002).

There are a number of techniques that have been used to measure plant hydraulic characteristics. A low-pressure hydraulic conductivity apparatus was developed by Sperry *et al*. (1988). In a number of studies, measurements of plant hydraulic conductance were initially focused on branches of whole shoots (Tyree *et al*., 1991; Cochard *et al*., 1992; van der Willigen and Pammenter, 1998; Jaquish and Ewers, 2001). A different technique, which attached hydrostatic couplings to saplings, was then used to measure whole root conductivity (Brodribb and Hill, 2000). The evaporative flux (EF) method involved the measurement of leaf to soil water potential gradients and the measurement of steady-state evaporative flux densities from total canopy leaves (Tsuda and Tyree, 1997; Tsuda and Tyree, 2000; Brodribb and Hill, 2000). Hubbard *et al*. (1999) showed that whole plant hydraulic conductance could be measured from sap flux densities through the trunk, in conjunction with leaf water potential and leaf gas exchange. An alternative method to the low-pressure flow system is that of the high-pressure flow meter (HPFM) developed by Tyree *et al*. (1995). The HPFM measures whole shoot hydraulic conductance by filling all the leaf air spaces with water under pressure (Tyree *et al*., 1995). The results acquired using an HPFM have been found to be consistent with that of the low-pressure flow system in a comparative study (Sperry *et al*., 1988; Zotz *et al*., 1998). It is also possible to measure the hydraulic conductance of the roots, and localize the resistances to water flow in the whole plant with the use of the HPFM (Tyree *et al*., 1995).

Root, stem and leaf hydraulic resistance can be measured separately and assessed to identify which plant component presents the greatest proportion of resistance to water flow. Vascular and non-vascular pathways of resistance to water flow have been shown to exist in whole plant hydraulic measurements, but the relationship of these pathways is unclear (Tyree *et al*., 1995).

1.2.4 Plant hydraulic conductance and drought stress

The water balance of plants results as a consequence of the intricately controlled hydraulic conductance of all plant organs in response to stimuli from the environment (Maurel and Chrispeels, 2001; Nardini *et al*., 2005). Environmental variation can affect the distribution of hydraulic resistance in plants.

Plants have the capability of acclimating in response to different environmental variables, a phenomenon referred to as phenotypic plasticity (Maherali *et al*., 2004). Physiological acclimation of the plant to low water availability is required when water is a limited environmental variable. Acclimation to drought encompasses a number of physiological and morphological mechanisms which permit either a drought tolerance or drought-delay strategy (Tyree *et al*., 2001). Physiological and morphological traits shown by plants that employ the drought-delay strategy include deeper roots, stomatal closure and leaf area loss (Mencuccini and Grace, 1996). These physiological traits increase access to water resources when drought stress is mild, rather than severe. Drought-tolerance comprises traits that allow for water transport to continue at more negative pressures, vulnerability to xylem cavitation is lowered (but only in post-stress xylem) and cells are permitted to exist at lower water potential values (Tyree *et al*., 2001). Hydraulic conductance can be related to drought-tolerance or drought-delay strategy, and the former strategy ensures that as the severity of the drought stress increases, the hydraulic conductance of the roots reduces more slowly than that of the shoots. As leaf area is lost, the below-ground resources per unit leaf area increase, and the reduction in supply (due a reduction in demand, from leaf loss) ensures continued hydraulic supply (Costa E Silver *et al*., 2004; Nardini *et al*., 2005). A reduction in hydraulic conductance in response to drought stress has been documented for a number of species, including *Eucalyptus globulus*, *Pondorosa* pine, *Licania platypus* and *Banksia* species (Maherali *et al*., 2002; Tyree *et al*., 2001; Costa E Silva *et al*., 2004 and Canham *et al*., 2009).

Drake and Franks (2003) found that stem and leaf hydraulic conductivity were significantly reduced during the dry season in five tropical species. Similarly, *Pondorosa* pine exhibited a considerable reduction in leaf specific conductivity when exposed to periodic drought stress (Maherali *et al*., 2002).However, Maherali *et al*. (2002) also showed that whole-plant hydraulic conductivity of *Pondorosa* pine was not significantly lower in drought stressed *versus* well-watered plants. The lack of hydraulic response was considered to be caused by the fact that well-watered plants were larger and hence had a higher hydraulic conductance. These findings suggest that hydraulic conductance can be correlated with plant size and it is further complicated by environmental stress.

Leaf specific conductivity (K_l) has been shown to be lower for drought-adapted species (through leaf loss), thereby increasing plant survival during periodic or prolonged drought stress and safeguarding the maintenance of a favourable water balance (Nardini and Tyree, 1999). Lower K_l is also associated with differing vulnerability to cavitation for similar drought-adapted species and between differing genotypes of the same plant species (Tyree and Ewers, 1991; van der Willigen and Pammenter, 1998). The reduction in LSC is therefore associated with a less gradual decrease in root conductivity, as reflected in terms of increased below-ground resources. The allocation of biomass and slower reduction of hydraulic conductance in roots extends plant survival during periods of severe or mild drought stress. The hydraulic changes in response to water stress are fundamental determinants of plant performance during drought.

1.2.5 Leaf Hydraulic Characteristics

The leaf anatomy of angiosperms, gymnosperms and ferns determines different strategies regarding how water is transported in a leaf. Angiosperms exploit a highly branched reticulate system of leaky veins, which allow for water to be transported to the evaporation sites of the leaf (Zwieniecki *et al*., 2002). The hydraulic conductance of an individual leaf (K_{leaf}) is a measure of how efficiently water is being transported through the leaf, and is usually normalized by leaf area. Concurrent with whole-plant hydraulic conductance, R_{leaf} is the inverse of K_{leaf}, and the resistances within the leaf can be partitioned as resistances are additive in series (Sack and Holbrook, 2006). In correlation with measurement of R_{leaf}, many studies have investigated the relationship between hydraulic resistance and/or conductance with that of stomatal conductance and gaseous exchange (Kuppers, 1984; Aasamaa *et al*., 2001; Sack *et al*., 2004; Santiago *et al*., 2004). There appears to be a strong co-ordination across species between K_{leaf}, stomatal pore area, maximum stomatal conductance and photosynthetic capacity (Aasamaa *et al*., 2001; Sack *et al*., 2003; 2004). Leaf dehydration causes a concomitant decline in K_{leaf}; ψ_{leaf}; and stomatal closure, which leads to decreases in photosynthetic rate and ultimately lower growth rates. The extent to which K_{leaf} affects stomatal conductance in response to dehydration differs among species. Investigation into the correlation of K_{leaf}/ R_{leaf} with stomatal conductance and gaseous exchange is not well known for *Eucalyptus* species, and the mechanisms of recovery from drought stress in individual leaves, has not been established yet.

The conductance of water through the leaf lamina is determined by the vascular and non-vascular pathways of transpiring water (Yang and Tyree, 1994). The petiole is the site at which the conductance of water commences through the leaf, following which water flows through the xylem vein orders (vascular tissue) in either a series or parallel pathway (Sack *et al*., 2003). Water then crosses the bundle sheath and flows apoplastically and symplastically in the mesophyll (extravascular tissue) and then into the airspaces of the stomatal chamber and is evaporated into the atmosphere (Sack *et al*., 2003).

Resistance to water flow in the leaves (R_{leaf}) can differ by up to 65-fold among different plant species and contributes an average of 30% of R_{plant} (Sack *et al*., 2003). Environmental variables, for example temperature, irradiance and water supply, change R_{leaf}, and R_{leaf} can also change with leaf age (Sack and Hoolbrook, 2006). R_{leaf} has been measured to be between 25-90% of the total whole-shoot hydraulic resistance (Nardini, 2001; Sack *et al*., 2003). The fact that R_{leaf} is high, relative to the rest of the plant, demonstrates that the hydraulic resistance of the leaves has a disproportionate hydraulic influence and the leaves can be considered a 'hydraulic bottleneck' in the water conductance pathway.

R_{leaf} can be partitioned into specific leaf components, although most of the mechanisms pertaining to leaf hydraulic resistance are not well understood (Sack *et al*., 2004). Sack *et al*. (2003) expressed R_{leaf} as:

$$R_{leaf} = R_{petiole} + R_{venation} + R_{extravascular}$$

The components that comprise R_{leaf} have been measured by means of specialized vein-cutting or freezing techniques as described by Sack *et al*. (2003; 2004) and Nardini *et al*. (2005), respectively.

Controversy has surrounded studies concerning leaf hydraulic resistance, as the methodology used to measure R_{leaf} has differed substantially. Early studies by Tyree and Cheung (1977) found that most of the hydraulic resistance in the leaf resides outside the vascular pathway (i.e. in the extravascular / mesophyll tissue). This pattern of hydraulic resistance partitioning was confirmed by experiments that targeted the removal of all the resistance that was associated with the extravascular / mesophyll membranes (Tyree *et al*., 2001; Salleo *et al*., 2003). Extravascular resistance to water flow is variable, dependent on the species and can account for up to 90% of R_{leaf} in studies shown by Yang and Tyree (1994); Cochard *et al*. (2004) and Salleo *et al*. (2003). In contrast to these studies, Sack *et al.* (2003; 2004; 2006) showed that the majority of the resistance to water flow in the leaves of *Acer saccharum* and *Querus rubra* was located in the vascular / venation water-flow pathway. Zwieniecki *et al*. (2002) and Sack *et al*. (2004) reported that $R_{venation}$ contributed 69-74% of R_{leaf}.

Despite differences in R_{leaf} partitioning into $R_{venation}$ and $R_{extravascular}$, the ratio between them is dynamic. $R_{venation}$ is said to increase during water stress due to xylem embolism, whereas $R_{extravascular}$ can change diurnally according to circadian rhythms (Sack *et al*., 2002; Nardini *et al*., 2005). Cochard *et al*. (2005) suggested that there are disadvantages to either $R_{venation} > R_{extravascular}$ or vice versa partitioning of R_{leaf}. If the proportional allocation to $R_{extravascular}$ is higher than $R_{venation}$, then the xylem hydraulic efficiency of the venation would be lowered. If the majority of R_{leaf} was located in the extravascular tissue, then the integrity of the xylem could possibly be maintained in response to drought stress. Thus, the hydraulic conductance pathway will be preserved until recovery from the drought stress period. An increase in R_{leaf} would impose a decrease in leaf gaseous exchange, thereby causing a decrease in relative growth rate.

Given that changes in leaf resistance affect plant water balance, it is not surprising that short and long-term changes of leaf resistance have been measured. Nardini *et al*. (2005) and Sack *et al.* (2003), have tested the response of leaf resistance to environmental and developmental factors, to assess how plants adapt to the changing environment. Changes in R_{leaf} partitioning in response to differing irradiance levels have been the primary focus of study. R_{leaf} of a number of sun species has been found to be 15-67% lower than that of measured shade leaves of the same species (Sack *et al*., 2003).

When assessing R_{leaf} in response to drought stress, an increase in $R_{petiole}$ can be observed, primarily due to xylem cavitation at low leaf water potentials (Hacke and Sauter, 1996; Hacke *et al.*, 2001; Linton and Nobel, 2001; Cochard *et al*., 2002; 2004). Drought stress may also possibly cause an increase in $R_{extravascular}$, although the mechanisms explaining this remain to be investigated. Even though increases in R_{leaf} and $R_{petiole}$ have been well documented when exposed to periodic drought stress, more interesting to note is the rapid and complete recovery from drought stress during rehydration from re-watering (Linton and Nobel, 2001; Lo Gullo *et al*., 2003). The underlying processes causing such a short-term increase in K_{leaf} (decrease in R_{leaf}) include elastic xylem recovery, reverse embolism from root pressure and possibly active ion-pumping or transient pressures (Bucci *et al*., 2003; Trifilo *et al*., 2003; Cochard *et al*., 2004; Brodribb and Holbrook, 2005).

1.3 Photosynthetic Characteristics

1.3.1 Photosynthetic capacity and stomatal conductance

The process of photosynthesis is vital for all plant growth, and can continue even when environmental conditions are less than optimal. Photosynthesis is a co-ordinated response of all the physiological processes within the plant, and is affected by any environmental stress/es imposed on the plant (Chaves, 1991; Freeden *et al*., 1991). Transpiration and the inevitable loss of water through the stomata leads to a decrease in leaf relative water content (RWC). When root water supply does not match leaf water loss, the decline in RWC will directly and indirectly affect photosynthesis (Lambers *et al*., 1998). Drought stress causes the stomata to close in order to prevent cavitation and desiccation, and the supply of CO_2 through the stomata declines (Wong *et al*., 1985). Kirschbaum *et al*. (1987) found that water stress facilitated stomatal closure and photoinhibition in *Eucalyptus pauciflora*. Decreasing CO_2 supply stimulates photosynthetic down-regulation and this process (of down-regulation) is seen as one of the earliest effects of soil-drying (Lawlor, 2002; Correira *et al*., 2006).

In *Eucalyptus globulus*, water-stressed plants exhibited lower stomatal conductance and lower rates of photosynthesis, in comparison with plants that were well-watered (Pereira *et al*., 1993). Photosynthetic capacity in response to drought stress depends on the severity, speed and duration with which soil drying occurs (Rouhi *et al*., 2007). Drought stress can affect photosynthetic capacity not only by means of stomatal closure, but also via non-stomatal factors *e.g*. decreased carboxylation efficiency in the mesophyll of the leaf (Ramanjulu *et al*., 1998).

Physiologically, photosynthesis is affected by stomatal and non-stomatal factors, however whole-plant reductions in photosynthetic capacity are usually observed in response to loss in leaf area (Rouhi *et al*., 2007). Drought stress stimulates leaf shedding in many plant species as a mechanism to reduce water loss through transpiration. Loss in leaf area reduces canopy net assimilation rate, and a reduction in photosynthetic rate per unit leaf area as well as stomatal conductance are characteristics of drought-tolerant species (Rouhi *et al*., 2007). The correlation of assimilation rate and stomatal conductance has been observed in many studies, and lower photosynthetic capacity is associated with lower stomatal conductance (primarily due to a reduction in transpiration rate) (Franks, 2005).

1.3.2 Co-ordination of photosynthetic capacity and hydraulic conductance

Xylem hydraulic characteristics have been shown to influence plant form and function, and environmental variables affect hydraulic conductance, which in turn can be constrained by xylem characteristics (Brodribb and Field, 2000; Macinnis-Ng *et al*., 2004). The same principle can be applied to photosynthesis and hydraulic conductance, where for any given allocation of carbon to a leaf, the photosynthetic potential of the leaf is constrained by the hydraulic conductance of the system (Franks, 2005). Several studies have shown that differences in hydraulic conductance could affect photosynthesis because gaseous exchange was affected. Sober (1997) showed that the hydraulic conductance of water-stressed *Phaseolus vulgaris* plants was positively correlated with stomatal conductance and photosynthetic rate. Experiments on drought-stressed mature Scots pine revealed an increased hydraulic resistance associated with stomatal closure and a reduced growth rate associated with low assimilation rates (Irvine *et al*., 1998).

Measurement of leaf photosynthetic capacity and stem hydraulic supply per leaf area displayed a close significant relationship in rainforest conifers and angiosperms (Brodribb and Field, 2000). Leaf specific hydraulic conductivity of *Eucalyptus grandis* was however found not to be influenced by changing fertilization regimes which were associated with higher photosynthetic capacity (Clearwater and Meinzer, 2001). The relationship between photosynthesis and hydraulic conductance is seen to be an indirect one, linked by the correlation of both parameters with stomatal conductance. Ultimately, the hydraulic architecture would be reflected in physiological and anatomical traits of leaf photosynthesis and evidence has shown that plant hydraulic characteristics play a fundamental role in limiting stomatal conductance and gaseous exchange (Brodribb and Field, 2000; Brodribb and Jordan, 2008).There are no studies specifically reporting measurements pertaining to the physiological processes relating water relations (stomatal conductance, transpiration and hydraulic conductance) with photosynthetic capacity of *Eucalyptus grandis*.

1.4 Biomass growth and partitioning in response to environment

There are three main environmental variables that influence plant growth: light, nutrient availability and water supply. Resulting growth is dependent not only on inherent carbon assimilation rates of leaves but also the relative sizes of plant organs (allocation) and morphology (Aphalo, 2010).The distribution and partitioning of carbon to plant organs (root, stems and leaves) will change in response to a change in environment, e.g. when light is limited, it has been shown that a greater allocation of carbon resources are made to the leaves of the plant (to maximize light capture). However, plants that are limited by nutrient and / or water supply will allocate more resources to the roots, therefore increasing root surface area and acquiring an increasing amount of water or nutrient supply (Gardiner, 1991, Hess and de Kroon, 2009). Under water deficits, investment of more biomass to roots facilitates an increase in water or nutrient absorption, and less biomass to leaves to reduce transpiration surface and efficient stomatal closure (Gindaba *et al*., 2005).

The allocation of resources to plant organs in response to water supply varies considerably between plant species and often depends on the way in which the water stress period was applied (short-term (acute) or gradual (chronic) drought stress) (Pereira and Chaves, 1993; McDonald and Davies, 1996; Osorio *et al*., 1998). Acclimation to moderate water stress involves changes in plant structure that include change in biomass allocation in order to enhance the plant's ability to avoid dehydration from water stress. The diversion of resources to plant parts that require it most is considered a necessary growth mechanism, and the response of changing resource allocation will prolong survival of environmental stress.

Plants have a functional balance between root and shoot activity, in which below-ground resources that are acquired will be in approximate balance with the above-ground resources acquired by the roots i.e. root:shoot (Garnier, 1991). Root to shoot ratio is usually seen to increase when water is a limiting environmental factor, primarily because of a reduction in shoot growth (Sharp and Davies, 1979; Steinberg *et al*., 1990). However, in many studies, *Eucalyptus* species do not exhibit an increase in root:shoot in response to water stress (Pereira and Kozlowski, 1976; Pereira and Pallardy, 1989; Farrell *et al*., 1996; Osorio *et al*., 1998). Aphalo (2010) reported that water deficits do not affect biomass allocation to root in *Eucalyptus camaldulensis* and *E. globulus*. *E.globulus* and *E.camaldulensis* do not display an "optimized strategy" for root growth (i.e. there is no increase in biomass allocation to roots when water is limiting). This suggests that root:shoot ratio is primarily controlled by genetic factors in *Eucalyptus* species (Osorio *et al*., 1998).

Conversely, when allocating biomass to leaves, a proportionately larger photoassimilate allocate to photosynthetic organs will increase relative growth rate (RGR) (Aphalo, 2010). An increase in RGR is dependent on the morphology of the photosynthetic organs through increases in specific leaf area (SLA) and the effect of leaf area on leaf area ratio (LAR). In a number of ecological transect studies, SLA is shown to decrease in *Eucalyptus* species in response to increasing aridity (Schultze *et al*., 1998; Searson *et al*., 2004). Mokotedi (2010) showed that drought-stressed *Eucalyptus nitens* x *nitens* plants displayed a reduction in total leaf area but maximized photosynthetic capacity of the remaining leaves to maintain a growth rate comparable with that of non-stressed plants.

1.5 Aims and Objectives of this study

Northern KwaZulu-Natal houses a Sappi dendrometer trial growing two different clones of *Eucalyptus grandis* species – *grandis* x *urophylla* (GU) and *grandis* x *camuldulensis* (GC). This trial has to date provided invaluable tree diameter data measured on a quarter-hourly basis and has been analysed for any correlations with the weather data that has been collected at the same site. Previous research has shown that the diameter and height of both GU and GC clones are higher at higher rainfall areas (Drew and Pammenter, 2006; Drew *et al*., 2009) and that GU clone was also found to have a greater diameter than GC (Drew and Pammenter, 2006; Drew *et al*., 2009). Work at their site has demonstrated that age, rather than environmental conditions, is the major determinant of tree growth for both the GU and GC clone (unpublished data, 2007). The dendrometer data, which is very powerful in terms of the radial resolution of the data set, has the disadvantage of having low vertical and physiological data resolution.

Other physiological aspects of the diameter data set were also observed. The first of these was the lack of seasonal response to weather in terms of the tree growth. There appears to be no suppression or increase in growth rate during winter or summer months respectively. Secondly, the clones showed a different pattern of response to short-term weather changes that occur at the dendrometer trial site. The trees of the GU clone produced short bursts of growth following periods of rainfall, and then periods of slow or little growth subsequent to the period of rapid growth. The trees of the GC clone showed steady growth that did not appear to be influenced by rainfall events in contrast to that of GU (Drew *et al*, 2009).

In the current study young plants of three eucalypt clones were grown in large (80 L) planting bags for 18 months and subjected to different watering regimes. This permitted study of the growth and underlying physiological responses to these short-term manipulations of water availability. Given the differing growth response to rainfall events observed by Drew *et al.* (2009), the recovery of the plants from stress was also studied.

Few studies have been carried out on the morphological and physiological response of drought stress on *Eucalyptus* clonal hybrids in South Africa. Manoharan (2002) investigated the response of *Eucalyptus* clonal hybrids to drought stress by measuring the hydraulic conductance and architecture of branches. Mokotedi (2007) measured the hydraulic conductance of roots of micro / macro-propagated *Eucalyptus grandis* x *nitens*. Fewer still have related photosynthetic capacity with the resistance of water flow in the leaf. Sack *et al*. (2003; 2004; 2006) determined not only the ideal technical method to measure resistance to water flow in the leaf (R_{leaf}) but also measured R_{leaf} in response to environmental variables such as light and water supply. Co-ordination of R_{leaf} with assimilation rate has been determined indirectly with both parameters correlating with stomatal conductance. The experiments for the current study consisted of growing three *Eucalyptus* clonal hybrids in pots and subjecting them to three watering regimes (control - high water; chronic - gradual water stress and acute - rapid water stress). Physiological and morphological measurements were taken monthly throughout the growth trial period. Harvests were performed at 9 and 18 months in order to determine total growth allocation of biomass to plant organs as well as hydraulic resistance to water flow. The relationship between R_{leaf} and assimilation rate was examined in response to drought stress and subsequent recovery on release from the stress (re-watering).

The specific objectives of the study were to:

a) Measure the impact of watering regime on the morphology (height and diameter) of three *Eucalyptus* clonal hybrids.

b) Evaluate the effects of water stress and clonal hybrid on hydraulic characteristics and biomass partitioning at the juvenile (9 months) and early adult (18 months) stages.

c) Determine the influence that drought stress and consequent drought stress recovery after re-watering has on resistance to water flow in the leaf, stomatal conductance and instantaneous photosynthetic rate.

d) Assess whether the physiological characteristics (water relations, photosynthetic capacity and hydraulic conductance) of the three *Eucalyptus* clonal hybrids differ with tree age, water availability, and among clones.

2. MATERIALS AND METHODS

2.1.1 Experimental design

The experiment was designed as a Randomized Complete Block with three clones GU A380, GU W1700 and GC 438 and three drought stress treatments (Fig. 2.1). The treatments were a control (no water stress imposed), chronic drought stress (watered often in small amounts) and acute drought stress (watered rarely in large amounts). The chronic drought stress treatments were watered everyday but only up to 20% of the water required to prevent any drought stress, whereas the acute drought stress treatments were allowed to dry close to complete dryness and the soil was re-watered for a period of recovery before being completely dehydrated again. As an RCB design there were 9 treatments, and each treatment had 12 replicates. A total of 108 saplings were planted for experimental purposes, as well as 40 guard row plants to reduce edge effects. Saplings were planted at the end of May 2009, and monthly height and diameter data were measured. At the age of five months (November 2009), once the saplings were established, the water stress treatments were initiated.

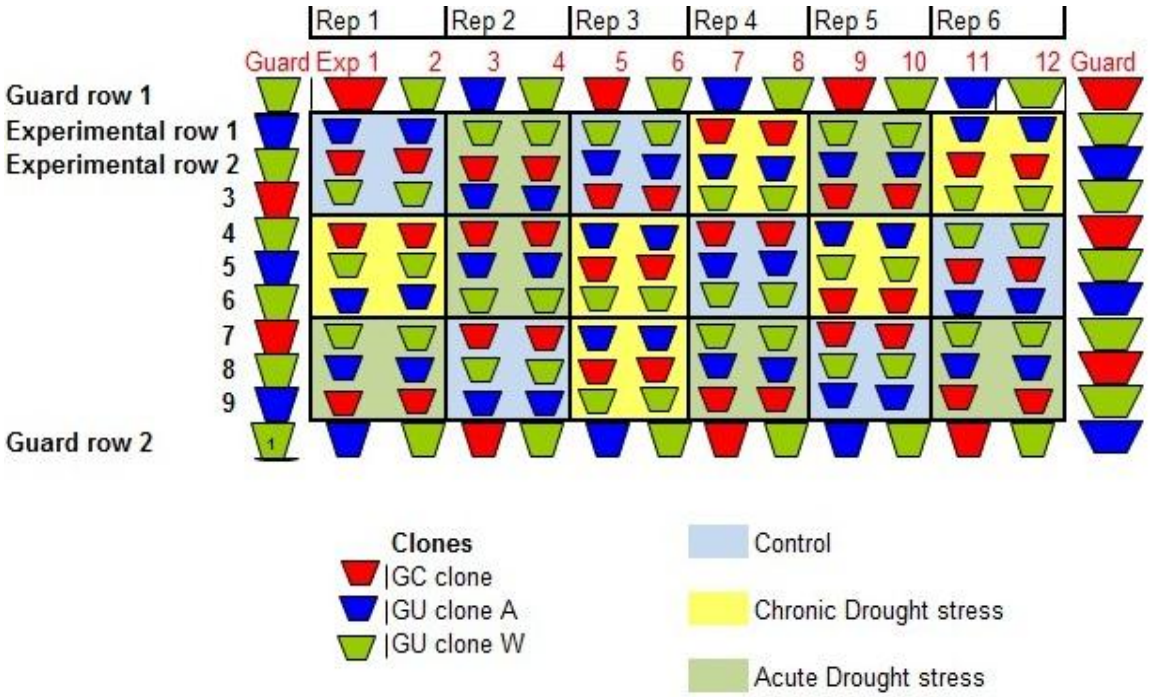


Figure 2. 1: Experimental design (Randomised Complete Block) of the *Eucalyptus* trial planted at UKZN, Westville campus.

2.1.2 Experimental Site

Figures 2.2 to 2.5 show the growth of the *Eucalyptus* trees at UKZN, Westville campus, from planting in May 2009 (Fig. 2.2) until September 2010 (Fig. 2.5). Prior to June 2010 (Fig. 2.4), 45 trees were harvested and removed from the trial after nine months of growth.

Figure 2.2: One week after planting (01 June 2009)

Figure 2.3: Six months after planting (02 December 2009)

Figure 2.4:12 months after planting (01 June 2010)

Figure 2.5:15 months after planting (01 September 2010)

2.1.3 Water treatments

Field capacity of the soil was measured so that the amount of water necessary for each of the three treatments could be calculated. The water content of the soil was measured with a Theta HH2 (Delta-T Devices, Cambridge) Soil Moisture Probe (Fig. 2.6), and the field capacity was found to be 0.15 m^3 m^{-3} or 15% by volume soil water content. The soil water content at field capacity is considered to be relatively low as the soil was primarily river sand-based (known locally as Umgeni River sand). The control water treatments were watered daily to field capacity (i.e. they were not water stressed). The Chronic water stress treatment was watered daily to 2% soil moisture content (approximately 0.5 Litre per day). The Acute water stress treatment was drought stressed cyclically, where the trees were stressed to the point of wilting (<0.1% soil water content) and then re-watered to field capacity. The trees were then watered daily to field capacity for a recovery period of one to two weeks before being drought stressed again. During the first 12 months of growth, the acute drought stress treatment was imposed six times.

Figure 2.6: Theta HH2 Soil Moisture Probe (Delta-T Devices).

2.2 Methods: Growth and Physiology Measurements

Figures 2.7a – g illustrate the growth and physiological measurements performed for the 18-month period of the growth trial. Growth and physiology measurements were divided into two types of measurements: non-destructive and destructive measurements. Non-destructive measurements comprised detailed monthly measurement of tree height and diameter. Photosynthetic characteristics and plant water relations parameters were measured seasonally (at 6, 9, 12 and 18 months tree growth). Destructive measurements such as biomass harvest and whole-plant hydraulic conductance were measured after 9 and 18 months growth. Individual leaves were destructively harvested for measurement of R_{leaf} (hydraulic resistance of individual leaves) at 12 months and at 18 months, individual leaves were harvested for measurement of stomatal characteristics and $\delta^{13}C$.

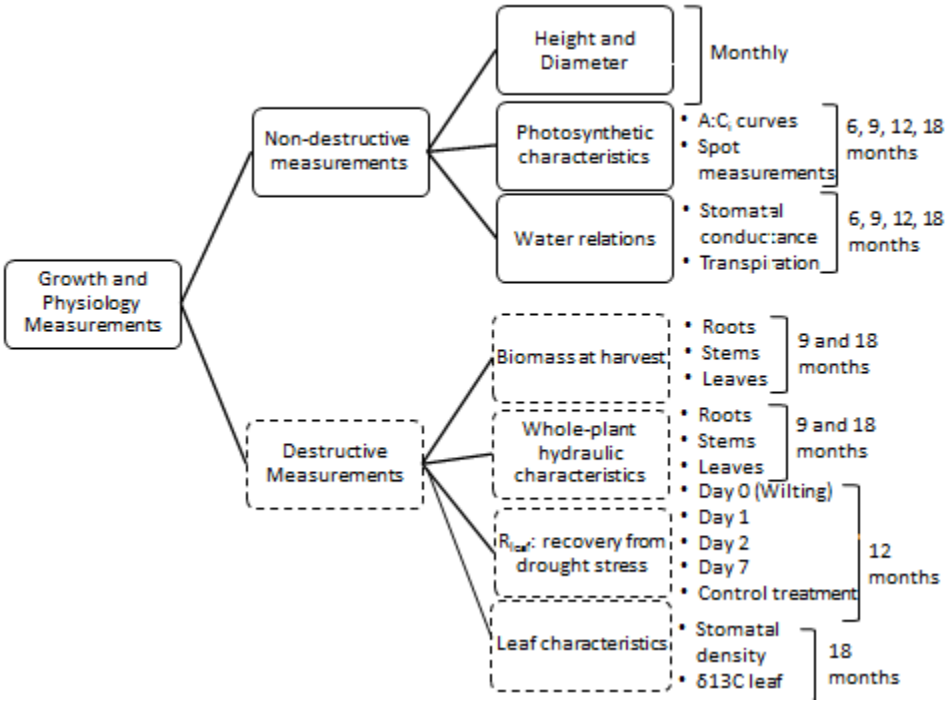


Figure 2.7a: Flow diagram illustrating the growth and physiology measurements performed on standing trees (non-destructive measurements) and destructive measurements performed at harvest one (nine months growth) and harvest two (18 months growth).

2.2 Non-destructive measurements

2.2.1 Morphological measurements: Tree height and diameter

Height and diameter measurements were performed monthly and continuously (from one week after planting for each replicate in each treatment) throughout the trial. The height of the trees was measured with a 5m tape measure and recorded as close as possible to the nearest centimeter. The diameter of the stems was measured 10cm above soil level with of a digital pair of calipers (in mm) and measurements were recorded at two decimal places of a millimeter (Fig. 2.8).

Figure 2.8: Digital caliper measuring tree diameter in mm (to two decimal places).

2.2.2 Photosynthetic Measurements

Photosynthetic measurements are needed for understanding and comparing biomass accumulation (productivity) and can be used as a short-term physiological response tool. Gas exchange of CO_2 and H_2O by leaves represent the basis for the design of photosynthesis meters. CO_2 and H_2O share the same biochemical pathway and sample leaves can be isolated in a closed chamber of a known area and measurements of carbon assimilation, stomatal conductance and transpiration can be recorded. Figure 2.7b shows that photosynthetic measurements can be divided into two different categories.

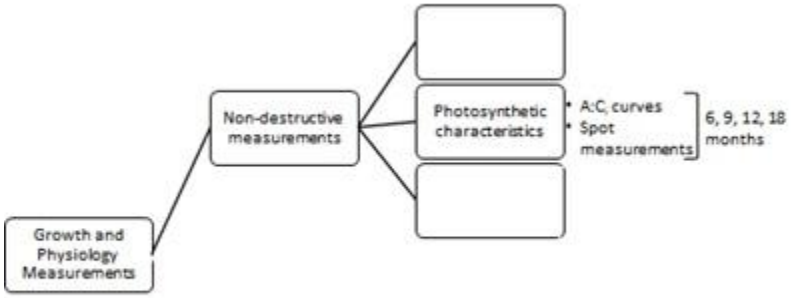


Figure 2.7b: Growth and physiology measurements showing measurements pertaining to photosynthetic characteristics.

Photosynthetic measurements may be performed on leaves of standing trees in two different ways. The first method of measuring photosynthesis involves the measurement of photosynthetic potential in the form of A:C_i curves (A: maximum assimilation rate; C_i: intercellular CO_2 concentration). A:C_i curves measure the response of photosynthetic potential to increasing intercellular CO_2 concentrations at constant light intensity. The plant response to change in CO_2 concentration provides information on the biochemical and stomatal limitations to photosynthesis. Measurement of A:C_i curves can facilitate investigation of short-term physiological response to water stress and physiological traits i.e. stomata closure that infer drought tolerance.

The down-regulation of photosynthesis in terms of photosynthetic potential is one of the earliest effects of soil drying due to water stress. When the drought stress was initially imposed, the photosynthetic potential was measured for each treatment. The measurement of photosynthetic potential was performed using a LiCor 6400 (Li-Cor, Lincoln, Nebraska, USA) photosynthesis measuring system. Photosynthetic potential was measured by changing the CO_2 concentration, while keeping the incident light, humidity and CO_2 flow rate constant. The photosynthetic rate, recorded at the corresponding change in CO_2 concentration, was used to construct an A:C_i curve, otherwise known as a CO_2 response curve.

A:Ci curves were measured at 9 and 18 months tree growth (in order to correspond with trees that were to be harvested for total biomass accumulation). Light intensity in the closed LED chamber was 1500 µmol quanta m^{-2} s^{-1} and leaf temperature was controlled at 26 °C. Chamber CO_2 was initiated at 400 µmol CO_2 m^{-2} s^{-1}, and then CO_2 were pre-set at 400, 300, 200, 100, 50, 400, 600, 800, 1000, 1200 µmol CO_2 m^{-2} s^{-1}. Measurements were made on 6 cm^2 sections of intact eucalypt leaves of the same leaf age. Leaves were positioned in the 0.25 dm^3 cuvette and the 3rd uppermost leaves (usually the youngest fully expanded leaf) were studied. No plant was sampled more than once on a particular day and no leaf was sampled more than once. Five replicates from each clone and each water treatment were recorded at both 9 and 18 months tree growth (n = 45 per harvest).

The photosynthetic rates acquired from CO_2 measurements were fitted to an exponential saturation curve (Fig. 2.9). This was done by non-linear regression of the data to the equation 1:

$y = a (1- \exp(b-c*C_i))$ for CO_2 response

The values of the parameters a, b and c were obtained for each individual curve. These parameters were then used to calculate photosynthetic variables.
For A:C_i curves:

a = J_{max} (maximum rate of electron transport at saturating CO_2);

b/c = CO_2 compensation point
(point at which CO_2 concentration initiates photosynthesis);

$a*c*e^b$ = initial slope of the curve
(the carboxylation coefficient – measure of rubisco activity)

$a (1-e^b)$ = photorespiration rate.

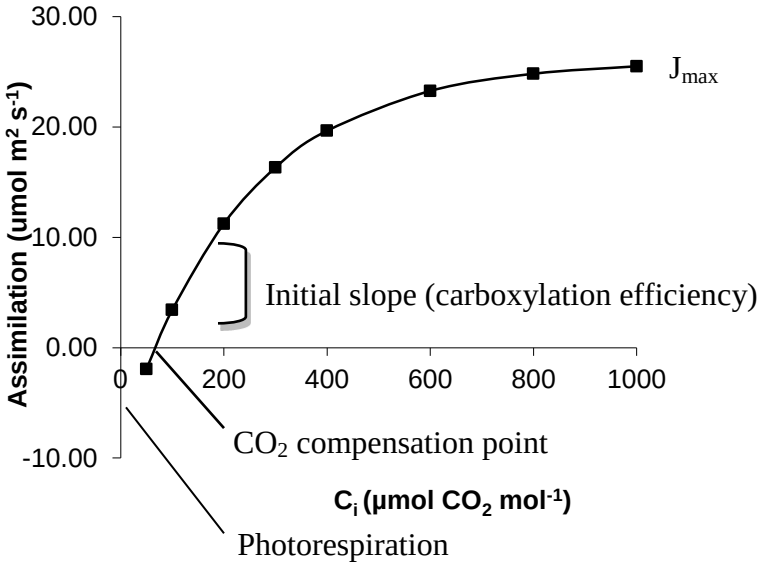


Figure 2.9: A:C_i curve showing the point at which J_{max}, CO_2 compensation point, initial slope and photorespiration occur.

Photosynthesis was also measured in terms of instantaneous photosynthetic rate, which is the actual assimilation rate of a specific leaf under ambient conditions (or otherwise referred to as a "snapshot"). "Snapshots" or spot measurements provide rapid point measurements of assimilation, transpiration and stomatal conductance and can be used as an indicator of plant health. The leaf chamber was fitted with a sun/sky attachment that allowed leaves to photosynthesise using natural light, while maintaining ambient CO_2 concentration (400 µmol CO_2 mol^{-1}). Leaf temperature, in the chamber, was set at 26°C and VPD and relative humidity were controlled. The assimilation rate values that were recorded under these conditions were considered to be actual photosynthetic rates. Spot measurements were performed on five replicates of each treatment at 6, 12 and 18 months tree growth in order to establish how photosynthesis differs seasonally.

Photosynthetic spot measurements were also recorded during drought stress cycles (at stress and recovery period) at the same time that leaf hydraulic characteristics were performed (at 12 months tree growth).

2.2.3 Water relations measurements

Plant water relations measurements monitor H_2O exchange of a leaf, and can be studied in conjunction with photosynthesis. Plant water relations include measurement of rate of passage of CO_2 exiting or H_2O entering the stomata on a leaf (stomatal conductance) and the emission of water lost to the environment which is essential in the process of photosynthesis (transpiration). Measurement of stomatal conductance and transpiration provide an indication of plant health status and stomatal closure can be detected in plants that are drought-stressed.

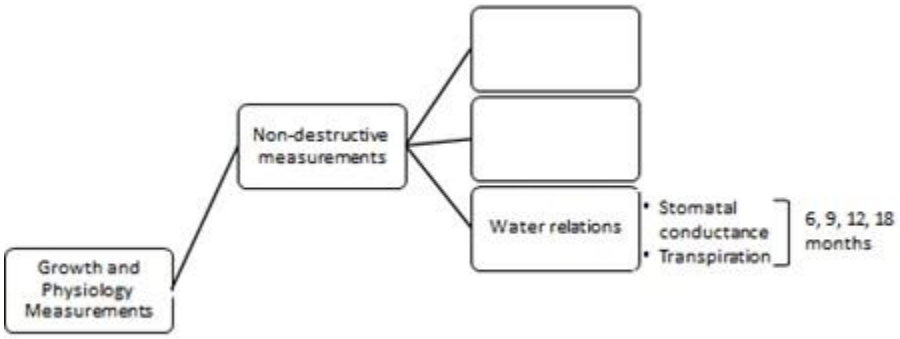


Figure 2.7c: Growth and physiology measurements showing measurements pertaining to water relations.

Transpiration (E) and stomatal conductance (g_s) were measured concurrently with photosynthetic capacity (A:C_i curves) and spot / survey measurements with use of the LiCor 6400 (LiCor, Lincoln, Nebraska). g_s and E were measured between 0800 and 1200 h and chamber [CO_2] was kept at 370-400 µmol CO_2 m^{-2} s^{-1}. Five replicates of g_s and E were measured in the control, chronic and acute (at the point of wilting) stress treatments. Following wilting point of the leaves in the acute stress treatment, g_s and E were measured on plants that had been re-watered to field capacity.

Measurement of photosynthetic rate and transpiration can be used to calculate instantaneous water-use efficiency, which is an indicator of the amount of carbon assimilate acquired per unit water lost from transpiration.

Photosynthetic leaf water-use efficiency (PWUE) was calculated as:

$$PWUE = \frac{A\ (\mu mol\ CO_2\ m^{-2}\ s^{-1})}{E\ (mmol\ H_2O\ m^{-2}\ s^{-1})}$$

2.3 Destructive Measurements

2.3.1 Biomass at harvest

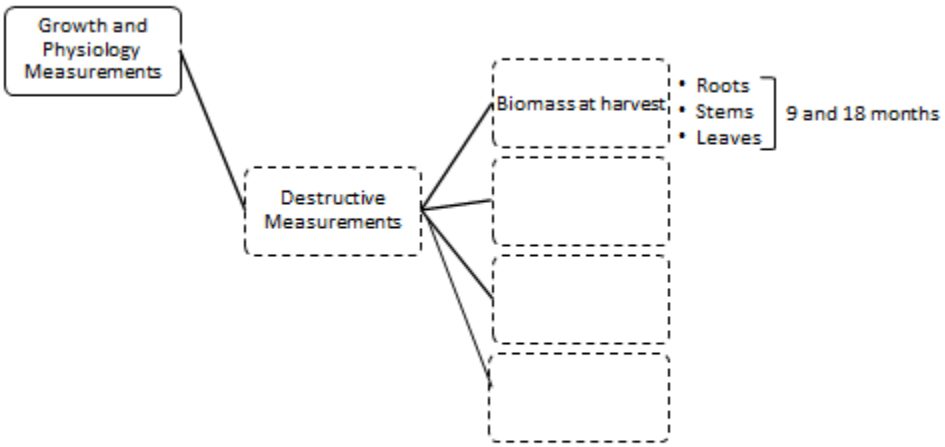


Figure 2.7d: Growth and physiology measurements showing measurements pertaining to destructive harvesting of trees after 9 and 18 months growth.

After nine months of growth, harvest one was performed in March and April 2010 on five out of nine replicates for each treatment (Fig. 2.10 shows the experimental design with the selected trees removed indicated in black). A total of 45 plants were harvested, and the soil from each tree was removed from the experimental site.

After 18 months growth, harvest two was performed in November and December 2010 on 4-5 replicates of each treatment. A total of 39 plants were harvested, and the remaining plants were discarded. At both harvest periods, the leaves, stems and roots from each plant were dried separately in a drying oven at 80ºC for 48 hours. The final dry weights were measured to the milligram level and the biomass ratios for each treatment were calculated. Allocation of biomass to plant components (roots, stems and leaves) could be expressed as a percentage when divided by total biomass (kg).

The accumulated leaf area of the leaves of each harvested plant was measured using the CI-202 leaf area meter (CID Inc, Carnas, USA).

Specific leaf area (SLA) was calculated as follows:

$$SLA = \frac{\text{Leaf area } (m^2)}{\text{Dry mass of leaves (kg)}}$$

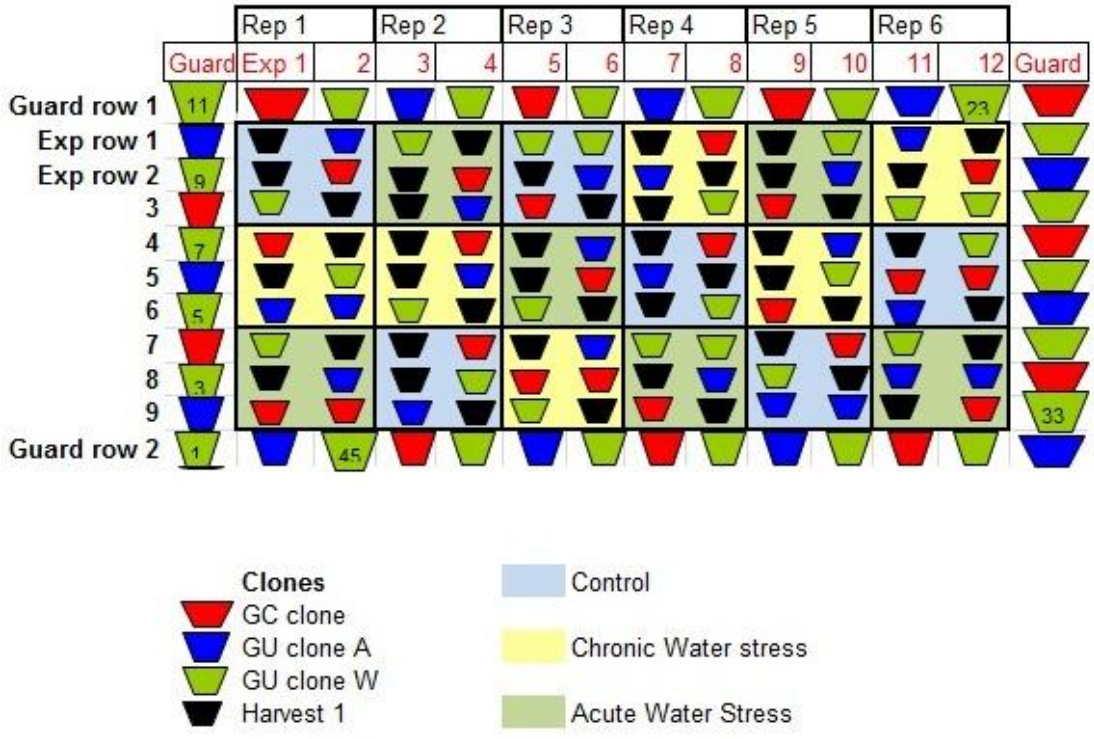


Figure 2.10: Experimental design showing (in black) the trees that were harvested for harvest 1 of the experimental trial.

2.3.2 Whole Plant Hydraulic measurement

Hydraulic measurements provide a method to perform quantitative root and stem hydraulic analysis without digging up roots from the soil. The major hydraulic conductance measurements can be performed in order to study root and shoot hydraulics of trees through seasons and with age. Although, it is not necessary to dig up the roots, the measurements are destructive and were performed on roots, stems and leaves of eucalypt trees after 9 and 18 months tree growth (Fig. 2.7e).

Figure 2.7e: Growth and physiology measurements showing whole-plant hydraulic characteristics measurements pertaining to destructive harvesting of trees after 9 and 18 months growth.

Whole plant hydraulic studies, which include the measurement of the hydraulic conductance of shoots, stems and roots, were conducted using a High Pressure Flow Meter (HPFM) Tyree *et al*. 1995. For these measurements, values were reported in terms of hydraulic resistance (MPa m^2 s kg^{-1}). Five plants from each treatment were selected for whole shoot hydraulic measurements, and subsequently harvested for total leaf area and dry weight measurements at 9 and 18 months tree growth (n = 45 per harvest).

Each whole-shoot was transported from the growing area to the laboratory, in order to keep other variables e.g. temperature, humidity and light intensity as constant as possible when performing measurements on whole shoots and stems.

Samples were initially cut about 10 centimeters above the shoot base and the shoot was placed in a 0.1 M HCl solution to hydrate. The basal portion of the stem attached to the roots was attached to one end of the HPFM compression fitting, after which it was cut with a sharp razor blade to ensure that the surface was even and that water could be perfused through it (Fig 2.11). Then the compression tubing was inserted into the other end of the fitting, which was attached to the root and filled with distilled water. Pressurized water was then forced through the root in order to measure flow rate, using the transient measurement mode of the HPFM. The flow rate was converted to hydraulic conductance by means of a line-fitting linear regression.

K_{root} was calculated as $1/R_{root}$.

Figure 2.11: The removed root of a eucalypt tree after root resistance had been measured using the HPFM.

The hydrated eucalypt clonal hybrid shoot was measured by the quasi-steady state method of the HPFM. The compression fitting was attached to the cleanly cut end of the shoot and water was then forced through the tubing under pressure. The hydraulic conductance of the shoot ($K_{shoot} = 1/R_{shoot}$) was recorded once the shoot had filled up, which can be seen when water droplets form on the under-surface of the leaves and flow rate was constant (Fig. 2.12). The conductance had to have been stable for a minimum of 2 minutes before a reading was taken. Following this, the leaves of the plant were removed from each branch until only the branches and main stem were left. Once the hydraulic conductance had stabilised again and remained at the same value for more than 2 minutes, the value was recorded and considered the hydraulic conductance of the stem i.e. $K_{stem} = 1/R_{stem}$ (Fig. 2.13).

The total leaf hydraulic conductance could be calculated only by using the resistances of the components of the whole shoot. Therefore the total leaf hydraulic resistance is the difference between shoot resistance and stem resistance:

$R_{total\ leaf} = R_{shoot} - R_{stem}$

$K_{total\ leaf} = 1 / R_{total\ leaf}$.

Figure 2.12: Water droplets on the undersurface of the leaves of *Eucalyptus* before resistance to water flow of the shoot was recorded with the HPFM.

Figure 2.13: Measurement of resistance to water flow with a HPFM in the stem of *Eucalyptus* (i.e. all leaves removed).

2.3.3 Leaf Hydraulic Characteristics

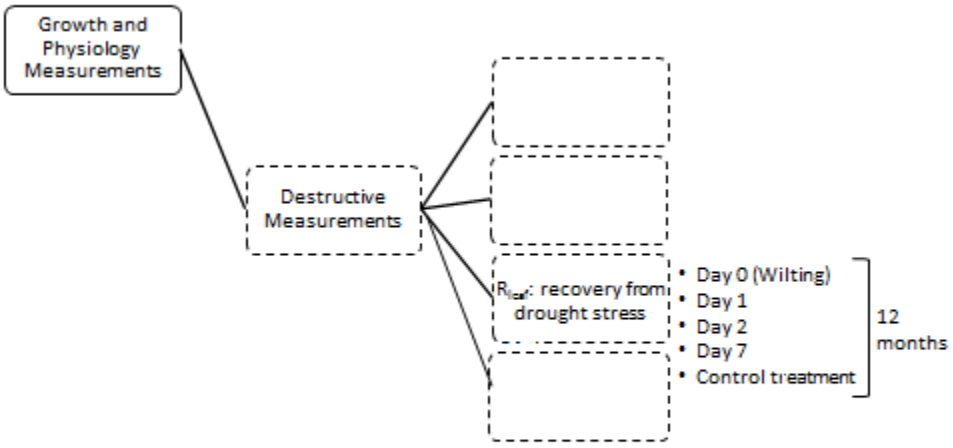


Figure 2.7f: Growth and physiology measurements showing leaf hydraulic characteristics measurements pertaining to recovery from drought stress after 12 months growth.

Leaf hydraulic characteristic measurements were made on leaves from the standing trees remaining after the first harvest. The leaves represent a significant portion of the resistance to water flow in the trees grown in the trial (up to 38% of the total hydraulic resistance). Investigation into the components of the leaf that contribute to the resistance to water flow was carried out using the HPFM. The method followed by Sack *et al* (2003) was used to isolate the three components that contribute to leaf hydraulic resistance. The theory assumes that the components of leaf hydraulic resistance are additive in series. For the purpose of this study, looking specifically at leaf hydraulic properties, leaf hydraulic resistance normalized by leaf area (MPa s m^2 kg^{-1}) was used as the unit of measure therefore:

$R_{leaf} = R_{petiole} + R_{venation} + R_{extravascular}$

The first leaf hydraulic measurement made after measuring R_{leaf}, involved severing the connection of the lamina to the petiole (Fig. 2.14). This gave the portion of resistance allocated to the petiole ($R_{petiole}$). R_{lamina} (MPa kg^{-1} s m^2) was then calculated by subtracting the petiolar resistance from R_{leaf}. This was expressed by the following equation: $R_{lamina} = R_{leaf} - R_{petiole}$.

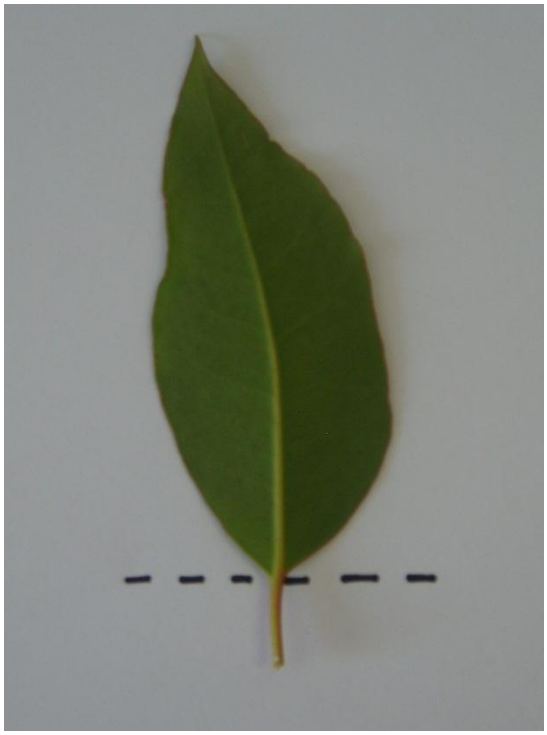


Figure 2.14: Leaf point at which the petiole is excised during measurement of $R_{petiole}$.

The remaining partitioning of leaf hydraulic resistances were performed using one of two different vein-cutting techniques as described in Sack *et al*, 2004. In order to determine the resistance of the extravascular tissue, the resistance downstream of the minor veins had to be removed. The minor veins were therefore cut (1.5 – 2 mm in length) at random locations over the entire lamina (with a scalpel) (Fig. 2.15). Extreme care was taken not to cut any major veins and between 120-150 cuts were applied to each leaf (until the resistance did not decline any further). The resistance measured after cutting minor veins was regarded as the $R_{venation}$ (the resistance of the venation of the leaf). The resistance of the leaf venation was therefore: $R_{venation} = R_{leaf} - R_{extravascular}$.

Figure 2.15: Minor vein cuts on the *Eucalyptus* leaf used in the measurement of $R_{venation}$.

The hydraulic resistance to water flow in the extravascular tissue is the portion of hydraulic resistance that is found in the mesophyll tissue, where the process of photosynthesis takes place in the chloroplasts. Correlating the hydraulic resistance to water flow in the mesophyll with that of instantaneous assimilation rate provides valuable insight into how drought stress ultimately affects growth rate. Hydraulic resistance to water flow was measured in selected leaves of each treatment of each clone. After 12 months tree growth (during winter), a minimum of 9 leaves per water treatment (control, chronic and acute stressed / recovery treatments) and eucalypt clone were subjected to the "vein cutting" technique described above.

The acute water stress treatment was measured when at wilting point (i.e. completely drought stressed), and then again when the trees were recovering at day 1, 2 and 7 after re-watering. The acute treatment leaves in "recovery" were then compared to the chronic and control treatment. The instantaneous assimilation rate and stomatal conductance were also measured for the control, chronic and acute stress treatment on day 1, 2 and 7 of water stress recovery in order to investigate the relationship between R_{leaf}, A_n, g_s and the recovery response.

2.4 Destructive measurements: Leaf Characteristics

A side investigation was performed in order to measure interesting leaf characteristics that were not originally part of the current study. Stomatal density (the number of stomata on the upper and lower leaf surfaces, per mm^2), stomatal size (in mm) and $\delta\ ^{13}C$ (carbon isotope discrimination of leaf dry matter) were determined after 18 months tree growth (Fig. 2.7f).

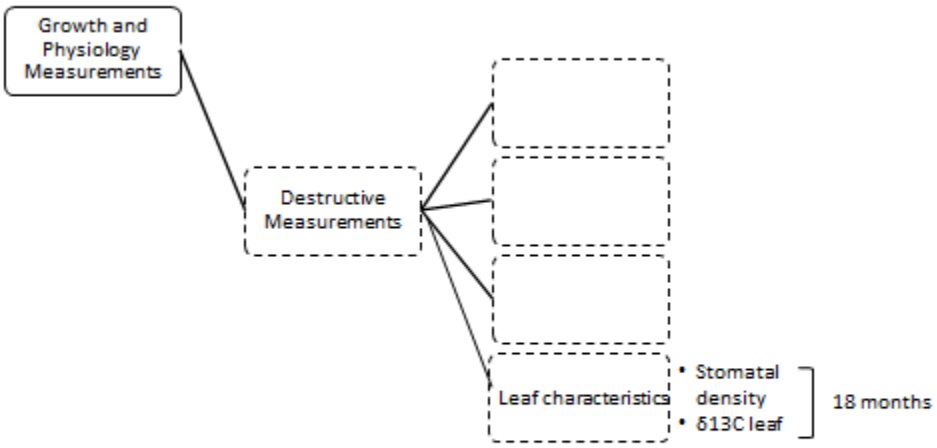


Figure 2.7f: Growth and physiology measurements showing leaf characteristics measurements pertaining to recovery from drought stress after 18 months growth.

2.4.1 Leaf stomatal density

After 18 months growth (during the second harvest) leaves were selected from each treatment for the determination of stomatal size and density. A total of 6 leaves from each eucalypt clone subjected to each water treatment (9 treatments) were used for the determination of stomatal density. Stomatal density was determined by using cellulose acetate film to produce a 'replica' of the leaf surface. A strip of cellulose acetate film was placed on a leaf surface which had a drop of acetone on it.

After approximately 60 seconds, the dried acetate strip was peeled off and mounted onto a glass slide with a drop of acetone to enable the replica to remain flat on the slide. Slides were viewed under a Nikon Biophot light microscope using 20x objective magnification. The images were used to calculate stomatal density (per mm^2) as well as average stomatal size per treatment. Replica's of both the abaxial and adaxial surfaces were produced, making sure that the replica was taken parallel to the leaf midrib, on either side of the leaf midrib.

2.4.2 Leaf $\delta\ ^{13}C$ measurements

Post-harvest at 18 months tree growth, one gram of dried leaf from replicates of each treatment (each eucalypt clone subjected to each watering treatment) were sent for determination of $\delta\ ^{13}C$ (n = 45). The samples were sent to the Light Stable Isotope Unit at the University of Cape Town where $\delta\ ^{13}C$ was measured using mass spectrophotometry and run against a standard set of samples.

2.5 Statistical Analysis

All the statistical analyses for this study were conducted using SPSS (version 18). Differences were considered significant at the $p < 0.05$ level. The photosynthetic CO_2 and light response data were initially analysed using a non-linear regression to obtain the values of the parameters of the line-fitting equation from each photosynthetic measurement.

The derived parameters used to calculate J_{max}, CO_2 light compensation point, initial slope and photorespiration were tested for significance between treatments and clones by means of a 2-way ANOVA or Univariate Analysis of Variance. The assumptions of these tests (for both light and CO_2 response measurements) require the normality of the residuals of the ANOVA and the residuals to have equal variance. The normality of the residuals were analysed by means of a non-parametric K-S test, and the equality of variance of the residuals was analysed by Levene's test of equality. If either of these assumptions were violated, the data in question were log transformed and checked again. Subsequent to the ANOVA, a post-hoc Tukey test was performed, which allowed the data for each treatment to be compared with each of other seven treatments.

The measured values of leaf hydraulic resistances (intact leaf lamina, petiole, and lamina with minor veins cut) were subjected to a 2-way ANOVA with the same procedure followed by the assumptions of the test. The components of the leaf hydraulic resistance reported as proportions of R_{leaf} ($R_{petiole}$, $R_{extravascular\ tissue}$, $R_{venation}$) were first $\sqrt{\arcsin}$ transformed and then also subjected to a 2-way ANOVA to compare significant differences between treatments. The whole shoot hydraulic resistance measurements ($R_{total\ leaf}$, R_{stem} and R_{shoot}), biomass measurements (dry weights of leaves, stems and roots), accumulated leaf area, stem diameter and biomass allocation were all also subjected to a 2-way ANOVA and its related assumptions.

Finally a Pearson correlation was used to investigate the relatedness of certain variables measured in this study, the principle one being A_{max} and K_h (whole plant hydraulic conductance normalized by total leaf area). The normality of the variables considered for the Pearson correlation was tested using a K-S test to ensure the correlation was correct. The values generated from the correlations were summarized and correlation graphs were shown only for those variables that had a significant correlation, positive or negative.

3. *Eucalyptus* Growth and Physiology in response to 18 months drought stress

Growth and physiology of *Eucalyptus* clonal hybrids were measured continuously for 18 months. Non-destructive morphological (height and diameter) and physiological (photosynthesis and plant water relations measurements) were performed at the time intervals indicated in Figure 3.1. Destructive morphological (biomass) and physiological (hydraulic characteristics) measurements could be performed only at the two harvests at 9 and 18 months (Fig. 3.1). Leaf characteristics (including R_{leaf}) will be discussed separately in Chapter 4, as these measurements were not part of the original experiment, but were interesting adjunct investigations. The results that have been presented in this chapter were analysed by a 2-way ANOVA, where the main effects of "clone", and "water treatment" and "clone*water treatment" interaction were assessed. As it was difficult to clearly present the results for nine different treatments, the results have been shown in terms of clone and water treatment only. In the case of a significant interaction, those results (for all nine treatments) were then presented below the results of clone and water treatment.

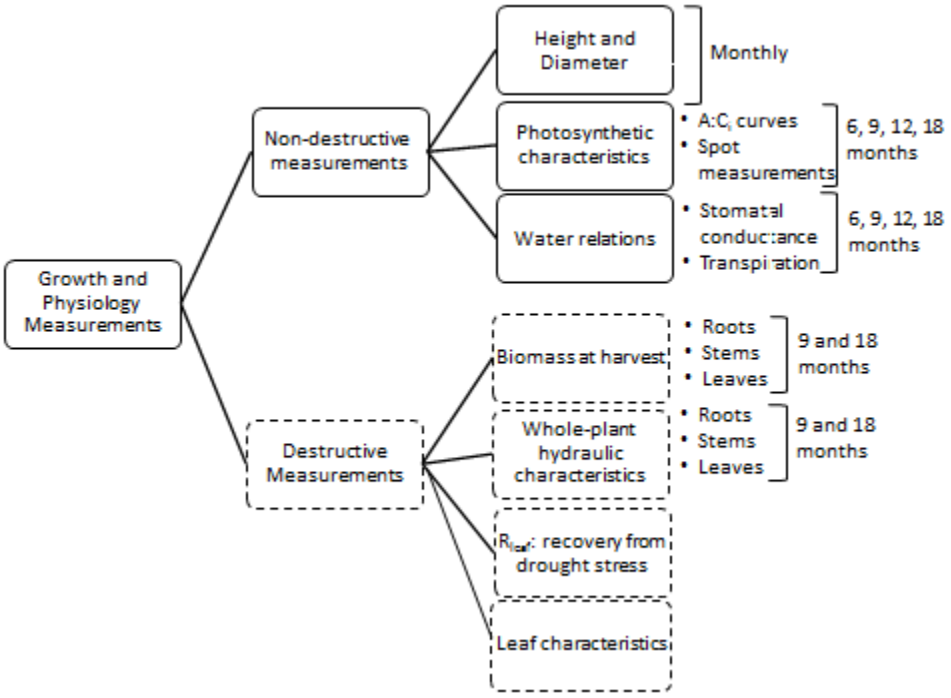


Figure 3.1: Flow diagram illustrating the growth and physiology measurements performed on standing trees (non-destructive measurements) and destructive measurements performed at harvest 1 (nine months growth) and harvest 2 (18 months growth).

3.1 Non-destructive morphological measurements: Height and Diameter

GC eucalypt clones were significantly taller than GUA and GUW after 18 months growth (mean = 2.5 m; $p < 0.0001$; Fig. 3.2; 3.3 (a)). Figure 3.1 shows that the GC clones overtook the GU clones, in terms of height growth, after 70 days. The height of the GUA and GUW clones did not differ by more than 5% (mean = 2.07 m and 1.96 m respectively), but GC clones are up to 30% taller than GU. After approximately 200 days growth, height of the GC clones in the control treatment surpassed the drought stress treatments (Fig. 3.2). The control treatments had the greatest height in all three clones, but the differences were not significant (Figure 3.3 (b); $p = 0.127$). Drought stress imposition did not significantly affect the height growth of GUA and GUW clones. Drought stress did reduce the growth in terms of height of GC, where the most severe drought stress (acute) showed the least growth in height (Fig. 3.1). Variability of height growth within treatments increased with time, as displayed in Figure 3.2. These results show that height growth was primarily determined by the clonal hybrid (GC > GU) rather than the imposition of chronic or acute drought stress.

For the first 190 days (winter and spring seasons; juvenile phase), growth rates were less than 0.002 m day^{-1}. After 220 days growth, at the start of the summer season, growth rates of the *Eucalyptus* clones increased to between ± 0.007 – 0.010 m day^{-1} for all three clones. The final growth rate measurements (during summer at the early adult phase) showed an average of 0.003 m day^{-1} in all treatments. GC trees had a significantly higher mean growth rate per day (mm height growth) in comparison with GUA and GUW (Fig. 3.4 (a); $p = 0.002$). Mean growth rate (in height per day) of GUA and GUW were almost identical (GR = 3.20 and 3.14 mm day^{-1}, respectively). The control treatment had a greater growth rate than the chronic and acute treatments, although this difference was not significant (< 10% difference between treatments; Fig. 3.4 (b)).

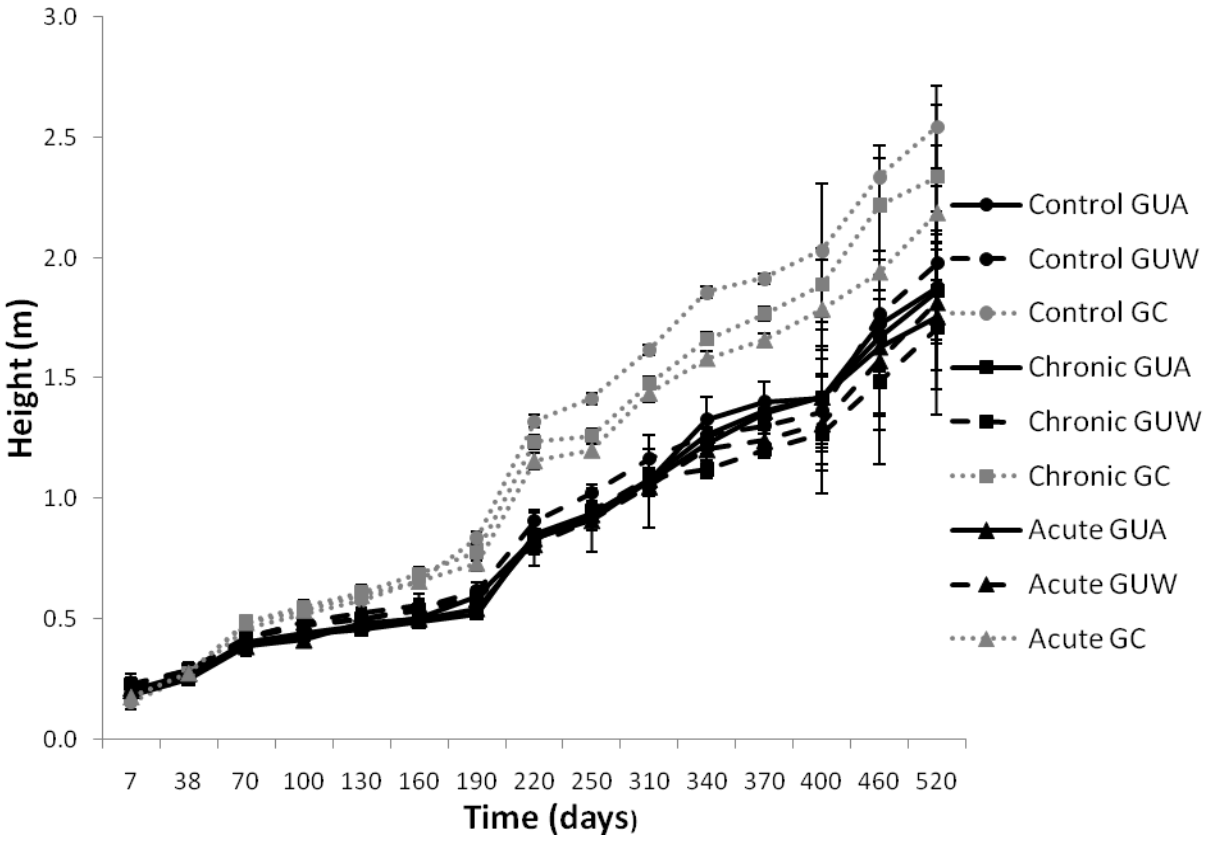


Figure 3.2: Mean height of plants of three *Eucalyptus* clones grown in response to drought stress for 18 months (n=7 / 12; bars represent ± 1.0 standard deviation).

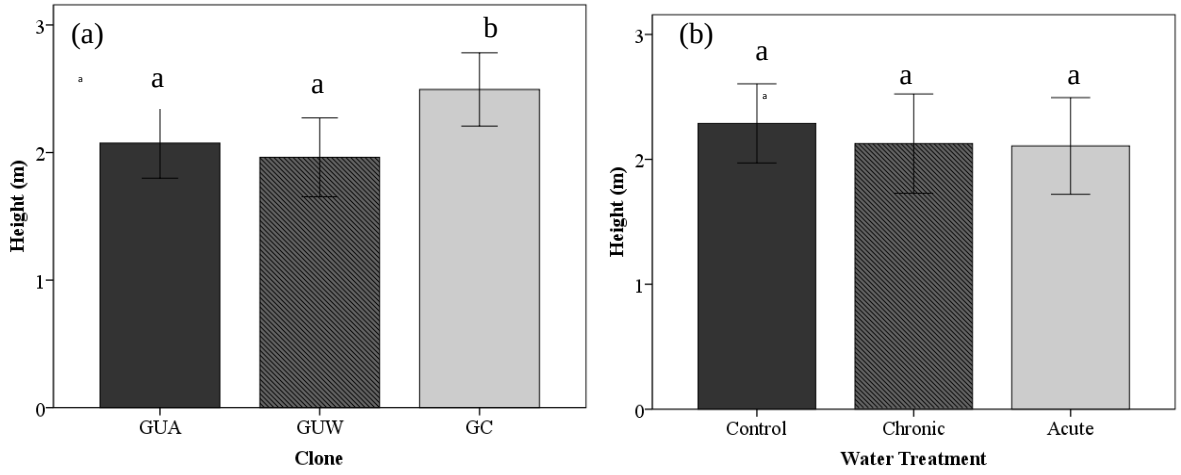


Figure 3.3: Mean height of plants of *Eucalyptus* (a) clones and (b) response to drought stress treatment after 520 days growth (n = 7; bars represent ± 1.0 standard deviation ; different letters denote significance difference).

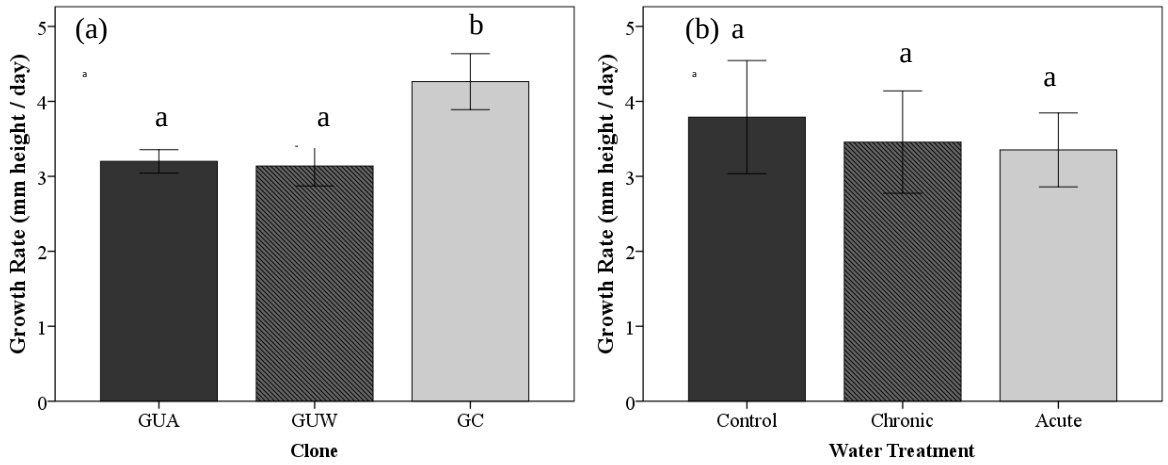


Figure 3.4: Mean growth rate (mm height day^{-1}) of plants of *Eucalyptus* (a) clones and (b) response to drought stress treatment after 520 days growth (n = 7; bars represent ± 1.0 standard deviation; different letters denote significance difference).

GUW trees have a greater diameter than GUA and GC, although it was never more than 10% greater (Fig. 3.5; 3.6 (a)). Figure 3.5 shows that there was no distinct difference among clones or in terms of diameter, in contrast to height (Figure 3.2). There was no significant difference in diameter between eucalypt clones after 520 days ($p = 0.890$). The diameter of *Eucalyptus* trees was significantly greater in the control treatment (mean = 40.5 mm; $p < 0.0001$; Fig. 3.6 (b)). Diameter growth was shown to decrease with the imposition of drought stress, and Figure 3.6 (b) shows that the smallest tree diameter was measured in the acute drought stress treatment (mean = 33.2 mm). Variability of diameter growth increased with time, but variability within treatments was considerably less than that of height growth (Fig. 3.5). Diameter appeared to be influenced primarily by water stress (Control > Chronic > Acute). The acute water stress treatment (drought stress watering cycles of complete wilting and then recovery for a period of time) had a more negative impact on eucalypt diameter than the chronic water treatment (watered daily to 2% soil water content) by decreasing diameter by up to 8% in comparison with the control.

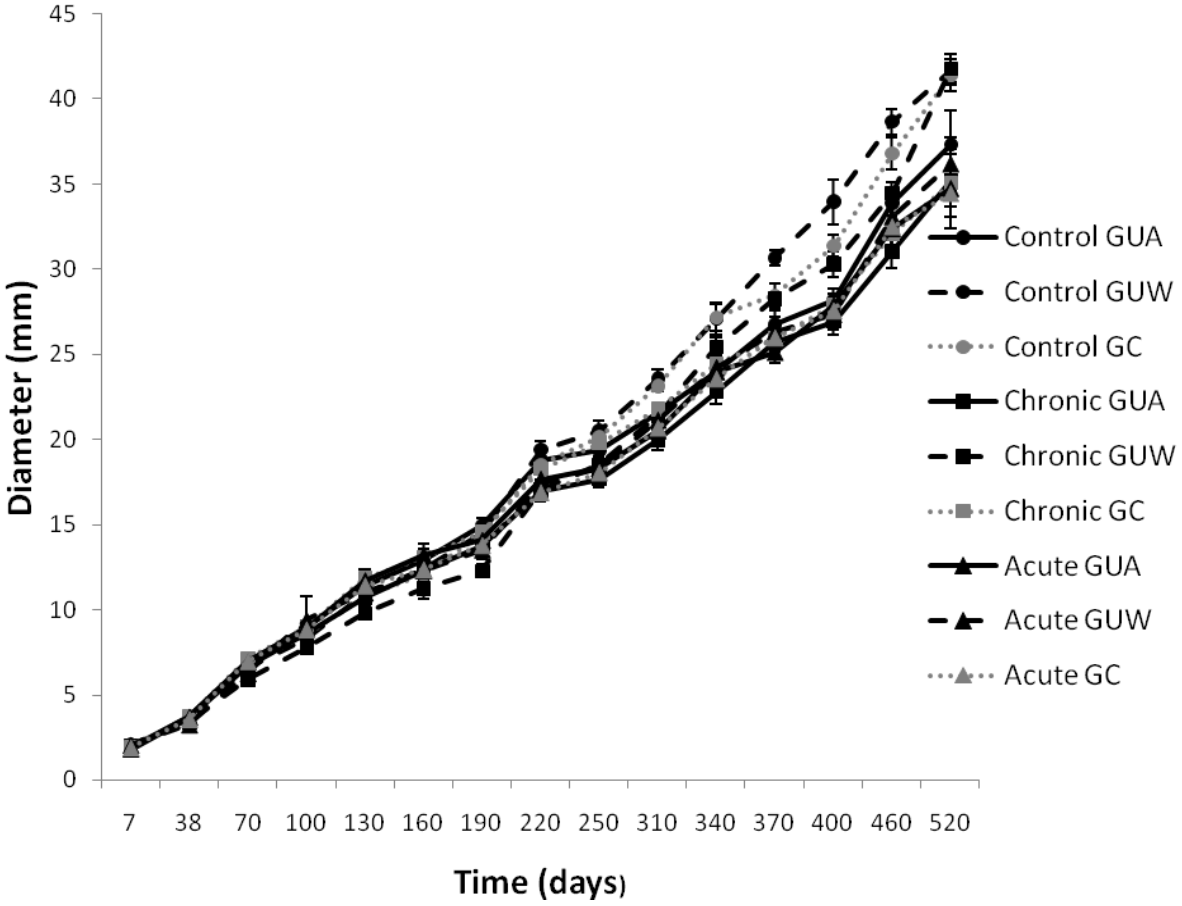


Figure 3.5: Mean monthly diameter of plants of three *Eucalyptus* clones grown in response to drought stress for 18 months (n=7 / 12; bars represent ± 1.0 standard deviation).

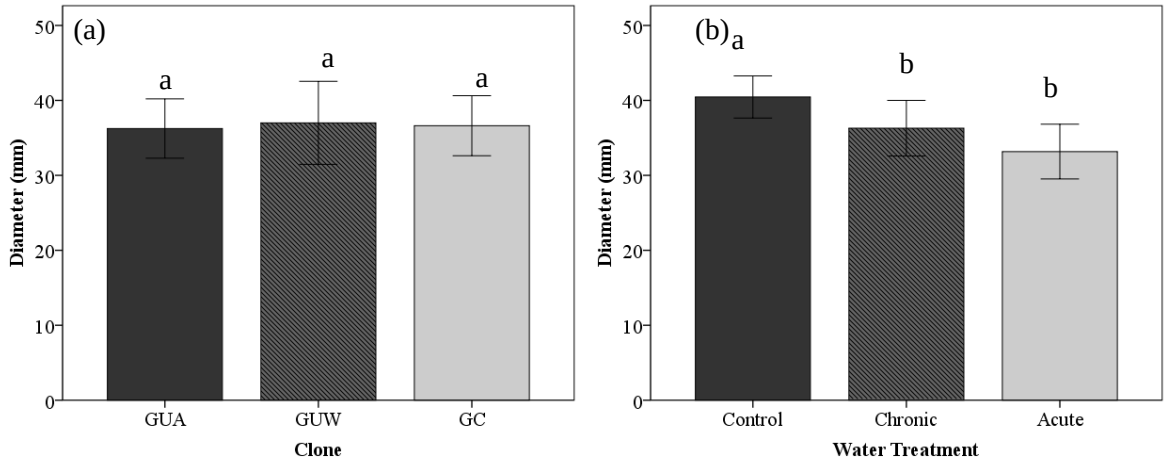


Figure 3.6: Mean diameter of plants of *Eucalyptus* (a) clones and (b) response to drought stress treatment after 520 days growth (n = 7; bars represent ± 1.0 standard deviation; different letters denote significant difference).

Growth rate, in terms of wood area (mm^2 / day) was greatest in GUW *Eucalyptus* clones (Fig. 3.7 (a); p = 0.005). Figure 3.7 (b) shows that the control treatment had the highest growth rate, of wood area per day, and was significantly greater than the acute treatment (p = 0.001). When growth was expressed as a function of diameter*height, the GC clones grew significantly more than GUA and GUW (Fig. 3.8 (a); p = 0.002). This was prodominantly because GC was significantly taller than the other clones. As was evident in growth rate (height or area per day), growth as diameter*height was greatest in the control treatment (Fig. 3.8 (b); p = 0.003). The acute stress treatment had the lowest growth, thereby showing that that periodic drought stress cycles affected growth more negatively than a chronic (constant, low water availability) water treatment.

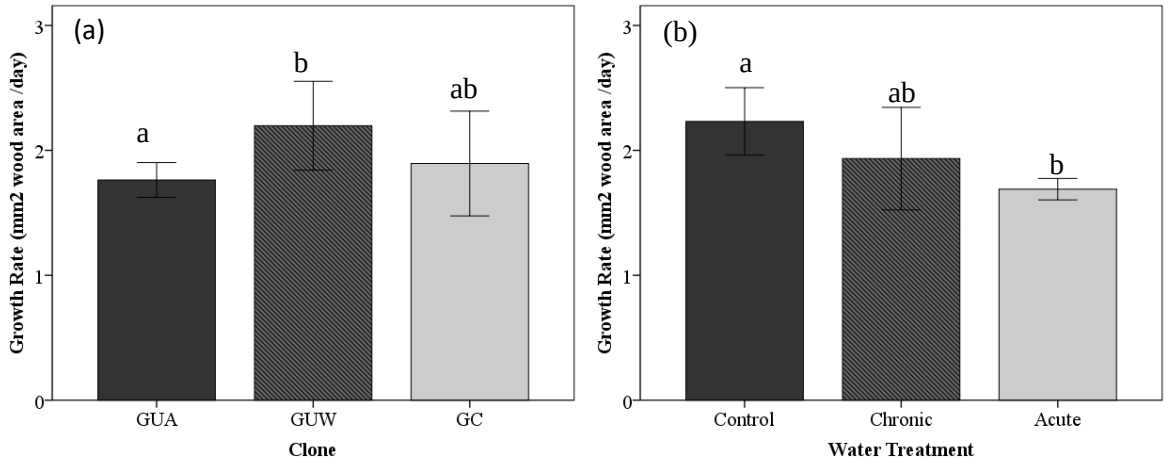


Figure 3.7: Mean growth rate (mm^2 diameter day^{-1}) of plants of *Eucalyptus* (a) clones and (b) response to drought stress treatment after 520 days growth (n = 7; bars represent ± 1.0standard deviation; different letters denote significant difference).

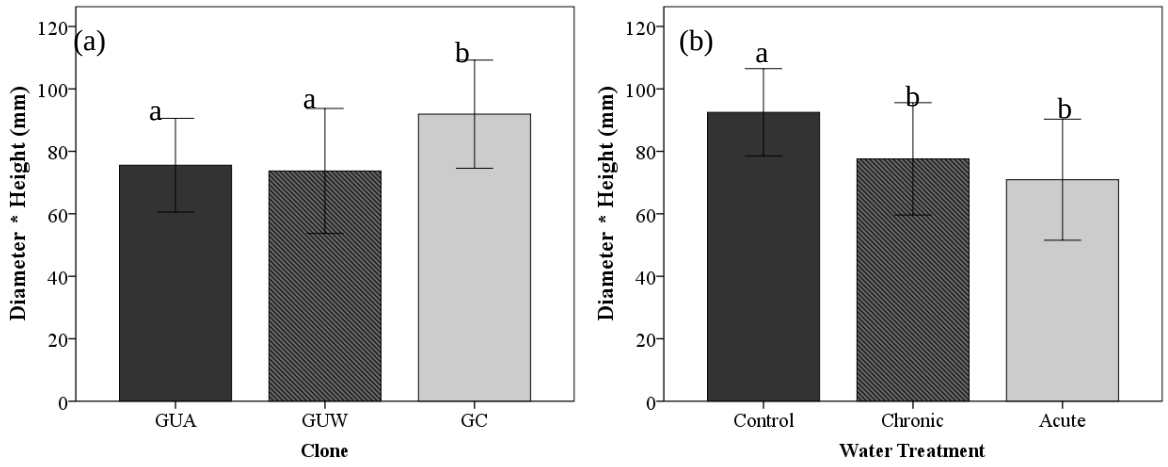


Figure 3.8: Mean diameter*height of *Eucalyptus* (a) clones and (b) in response to drought stress treatment after 520 days growth (n = 7; bars represent standard deviation ± 1.0; different letters denote statistical significance).

Eucalypt tree volume was calculated with reference to Bredenkamp, 1982 (vander Willigen and Pammenter, 1997). Diameter and height were used to determine volume (m^3) in an euqation specific for eucalyptus volume growth. Volume (m^3) was significantly greater in GC clones ($3.46 \pm 0.16\ 10^{-3}\ m^3$) compared with GUA clones ($2.71 \pm 0.17\ 10^{-3}\ m^3$; $p = 0.019$; Fig. 3.9(a)). Figure 3.9 (b) shows that eucalypt tree volume was signifcantly greater in the control treatment compared with chronic and acute treatments ($p = 0.039$).

Growth efficiency was calculated as the amount of wood volume per total leaf area (m^2) per year (vander Willigen and Pammenter, 1997). The GC clone had a significantly higher growth efficiency than GU clones (GC = $5.1\ 10^{-3}\ m^3/m^2/yr$; $p = 0.022$; Fig. 3.10 (a)). GC clones produce more wood volume by a smaller leaf area than GU clones. There was no signifcant difference between water treatments in terms of growth efficiency, however the acute treatment was slightly higher, primarily because trees treated with acute water stress had significantly less total leaf area i.e. see also Fig. 3. 28 ($p = 0.175$; Fig. 3.10 (b)).

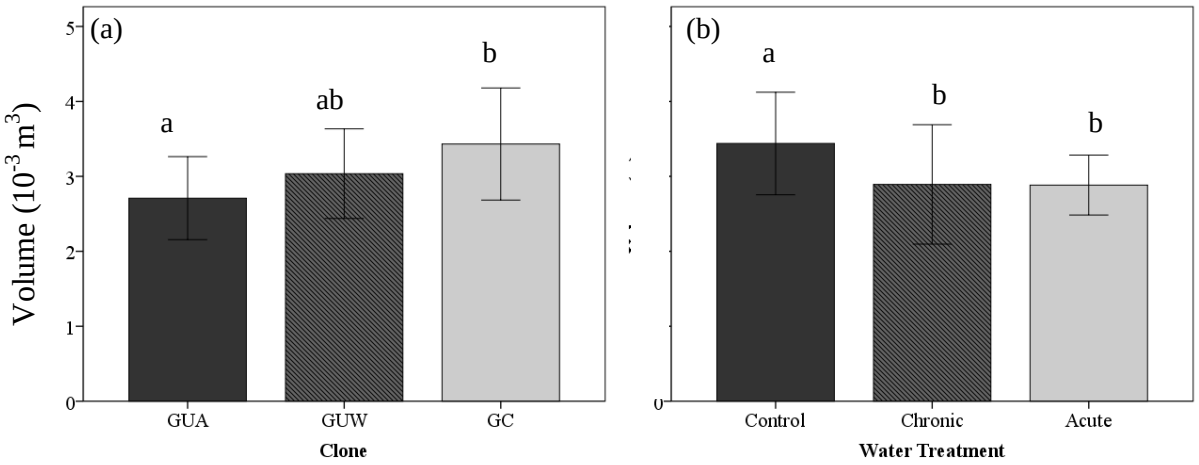


Figure 3.9: Mean volume (m^3) of plants of *Eucalyptus* (a) clones and (b) in response to drought stress treatment after 520 days growth (n = 7; bars represent ± 1.0 standard deviation; different letters denote significant difference).

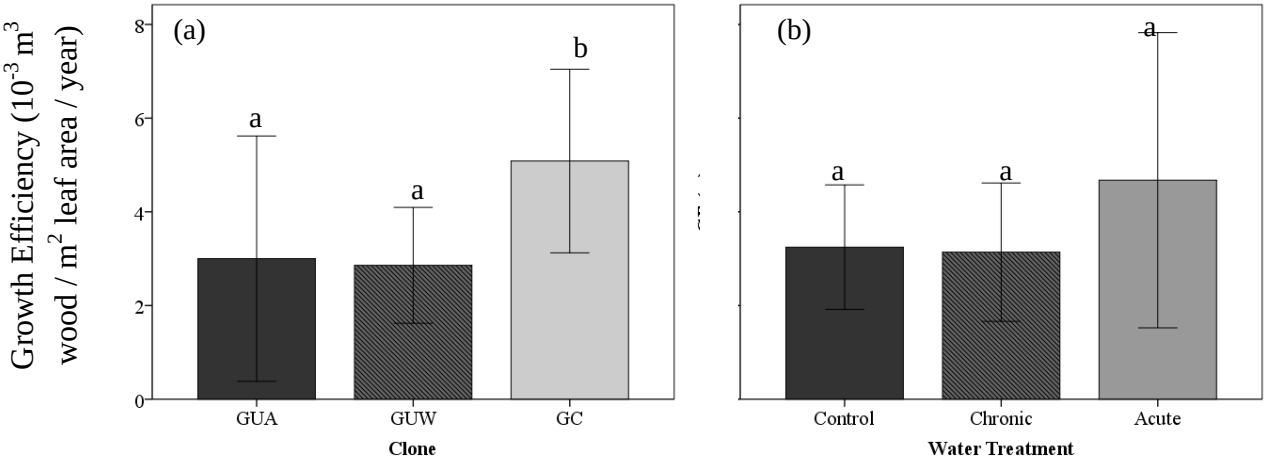


Figure 3.10: Mean growth efficiency (m^3 wood / m^2 leaf area / year) of *Eucalyptus* (a) clones and (b) in response to drought stress treatment after 520 days growth (n = 7; bars represent ± 1.0 standard deviation; different letters denote significant difference).

3.2 Non-destructive physiological measurements: Photosynthetic characteristics

3.2.1 A:$C_i$curves (Photosynthetic Potential)

Maximum photosynthetic potential (assessed as net CO_2 assimilation rate at saturating C_i) of the trees subject to the control water treatment (after 6 months growth) was highest for the GUA clone (33.7 µmol CO_2 m^{-2} s^{-1}) and GUW (29.3 µmol CO_2 m^{-2} s^{-1}) (Fig. 3.11 (a) and (b)). Trees of the GC clone showed the highest photosynthetic potential for the chronic drought stress treatment (Fig. 3.11 (c)). There was a photosynthetic reduction of approximately 33% in the stress treatments of the GUA clone, in comparison with the control. The GC clone had the greatest potential to withstand drought stress, and displayed the highest photosynthetic rate when experiencing chronic stress treatments (mean = 34.8 µmol CO_2 m^{-2} s^{-1}). The GUW clone had the least variable photosynthetic potential between drought stress treatments because the maximum photosynthetic rate of the control, chronic and acute recovery treatments were within a 5 µmol CO_2 m^{-2} s^{-1} range i.e. photosynthesis was not reduced by more than 20% in the stress treatments compared with the control (Fig. 3.11 (b)). This infers that the GUW clone is less affected in terms of assimilation rate (and hence possibly growth rate) when experiencing either chronic drought stress or during recovery from an acute drought stress period.

During the acute drought stress cycle, when trees were being deprived of water, the photosynthetic rate was 75% lower than the control (at leaf wilting point and soil moisture content of < 0.001 m3 m-3). After *Eucalyptus* trees were re-watered for two weeks, photosynthetic potential was then re-measured (i.e. acute stress recovery). More detailed day by day photosynthetic recovery was measured in conjunction with R_{leaf} in Chapter 4. In all three *Eucalyptus* clones, the photosynthetic rate measured for the acute water stress recovery treatment was not significantly different from the control (Fig. 3.11 (a) – (c)).

It was not possible to accurately reproduce A:C_i curves with many replicates for *Eucalyptus* trees after 18 months growth. Technical difficulties were experienced when the LiCor 6400 was used, primarily due to extreme weather conditions where daily temperatures and relative humidity reached 42ºC and 95% respectively. It was however possible to calculate individual parameters such as J_{max}, CO_2 compensation point, photorespiration and carboxylation efficiency and compare with the values obtained after 6 months growth.

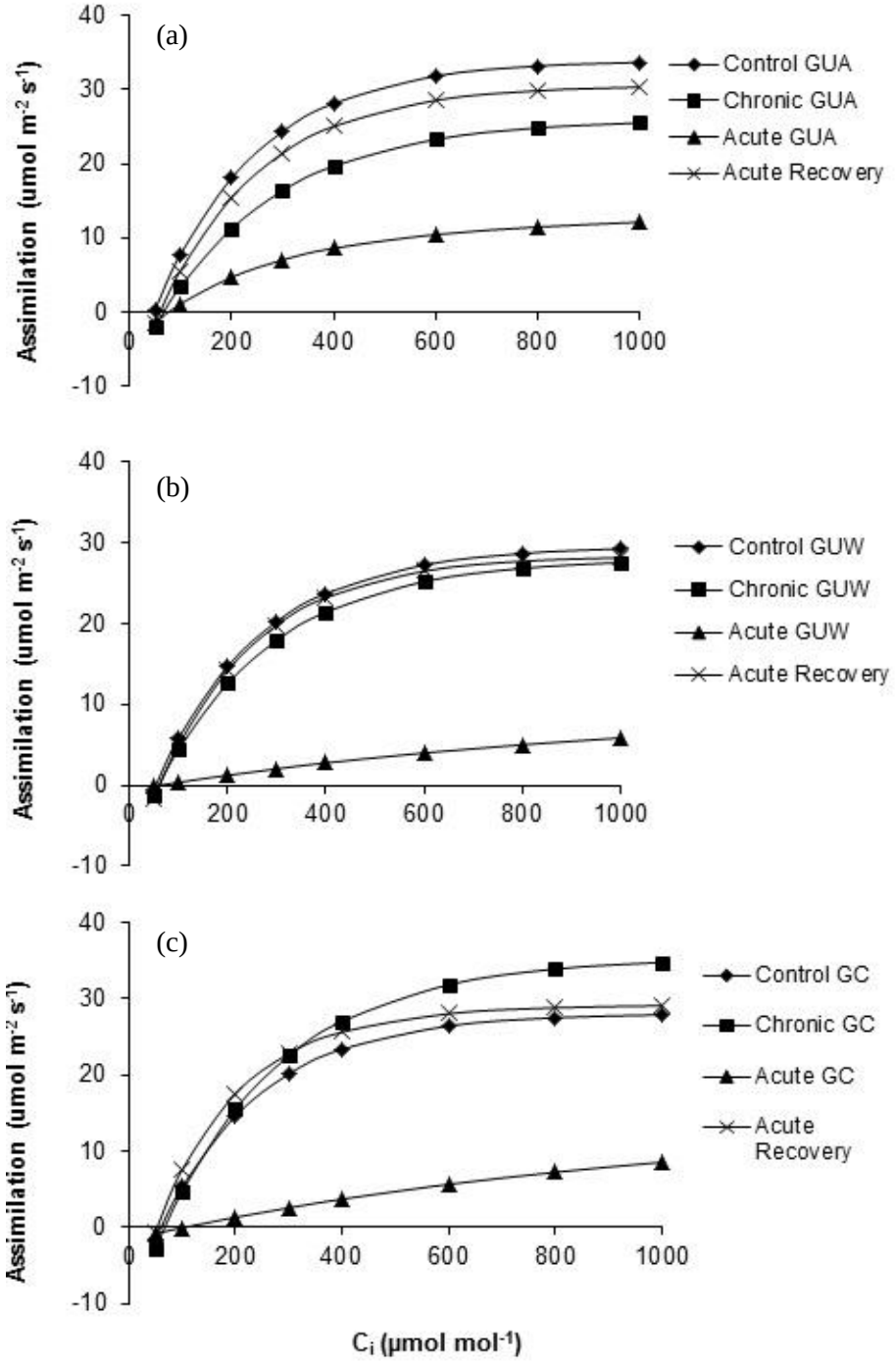


Figure 3.11: Mean photosynthetic potential (A:C_i curves) of three *Eucalyptus* clones (a) GUA, (b) GUW and (c) GC in response to drought stress and subsequent drought stress recovery (after 2 weeks of re-watering) (n = 5 per treatment, standard deviation not included because it distracts from the curve shape).

Figure 3.12 (a) and (b) show that J_{max} (maximum photosynthetic rate in response to change in CO_2 concentration) was not different between *Eucalyptus* clones at six or 18 months ($p = 0.061$ and $p = 0.761$ respectively). Maximum photosynthetic rates were 10% (± 2 -3 µmol CO_2 m^{-2} s^{-1}) lower after 18 months compared with 6 month old trees (Fig. 3.12 (a) and (b)). The decrease in J_{max} could suggest a down-regulation of photosynthetic activity with tree age.

J_{max} was highest in the control treatment (mean = 30.6 µmol CO_2 m^{-2} s^{-1}) but was not significantly different from the chronic stress treatment (mean = 29.3 µmol CO_2 m^{-2} s^{-1}) after 6 months growth (Fig. 3.13 (a)). The acute stress treatment was 60% less than the control (mean = 12.8 µmol CO_2 m^{-2} s^{-1}) and was significantly different from the other three water treatments measured ($p < 0.0001$). After re-watering *Eucalyptus* trees subject to acute stress treatment, J_{max} was re-measured and was not different from the control or chronic stress treatment (mean = 29.3 µmol CO_2 m^{-2} s^{-1}). Figure 3.13 (b) shows that the J_{max} measured after 18 months growth was completely different from that at six months. J_{max} was highest in the acute recovery stress treatment (mean = 27. 3 µmol CO_2 m^{-2} s^{-1}; $p < 0.0001$). Due to equipment difficulties, it was not possible to measure J_{max} (or any other photosynthetic parameters) during the imposition of acute drought stress. J_{max} was lowest, but different from the GUW clone (mean = 23.9 µmol CO_2 m^{-2} s^{-1}), in GUA *Eucalyptus* clones (mean = 18.3 µmol CO_2 m^{-2} s^{-1}). Similar to *Eucalyptus* clonal response to age, J_{max} decreases in water treatments from 6 – 18 months.

After six months growth, J_{max} showed a significant clone*water treatment interaction ($p = 0.001$). Figure 3.14 shows that J_{max} decreased significantly at the point of wilting (in all eucalypt clones) when exposed to acute drought treatment. Although J_{max} was relatively lower (< 5 µmol CO_2 m^{-2} s^{-1}) when recovering from acute drought stress, the difference was not significant from the control for any of the clonal hybrids (Fig. 3.14). Water stress therefore affects maximum photosynthetic rate in a limited capacity as long as there was a period of recovery, which was facilitated by re-watering *Eucalyptus* trees.

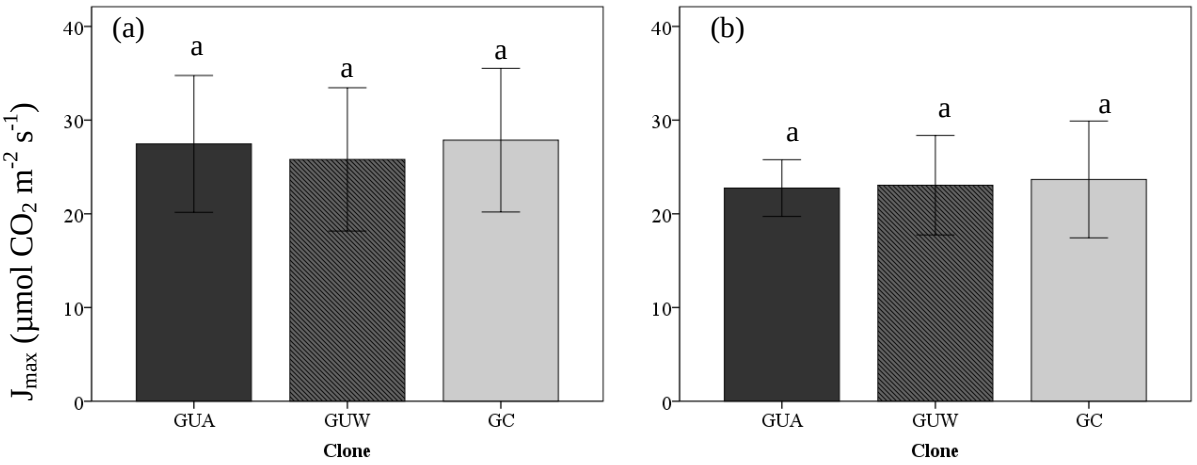


Figure 3.12: Mean maximum photosynthetic rate (J_{max}) of clones when *Eucalyptus* trees were (a) 6 months and (b) 18 months old (n = 15 per clone; different letters denote significant difference).

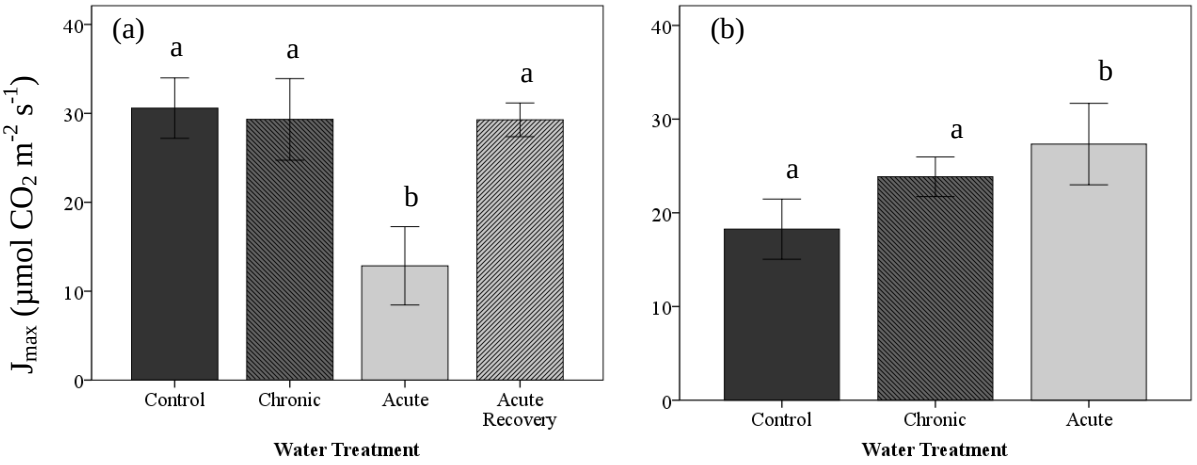


Figure 3.13: Mean maximum photosynthetic rate (J_{max}) of water treatments when *Eucalyptus* trees were (a) 6 months and (b) 18 months old (n = 15 per water treatment; different letters denote significant difference).

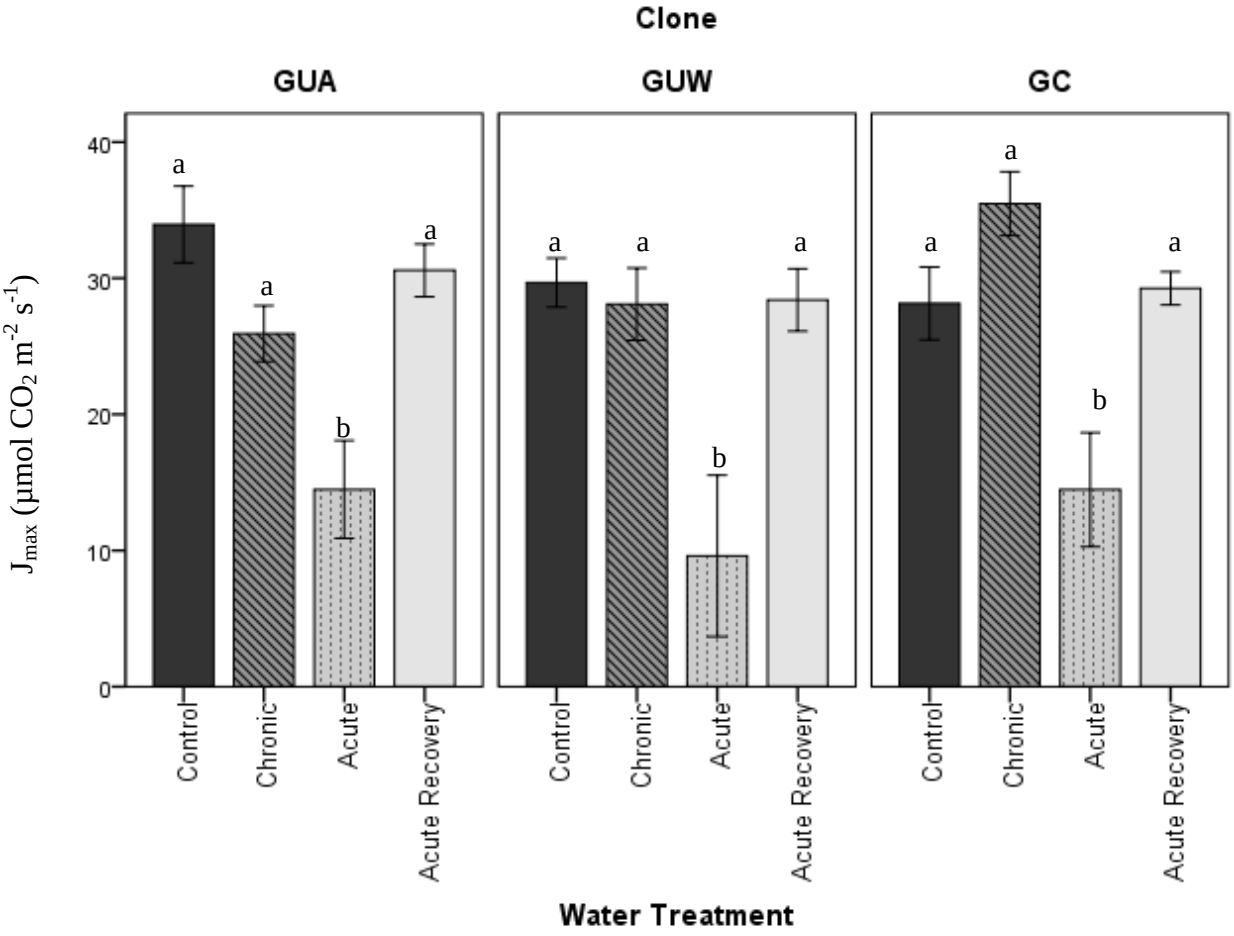


Figure 3.14: Mean maximum photosynthetic rate (J_{max}) of clone*water treatments of eucalypt plants when *Eucalyptus* trees were 6 months old (n = 5 per treatment; different letters denote significant difference) amongst treatments within a clone.

The CO_2 compensation point (Γ) illustrates the CO_2 concentration at which net photosynthesis becomes positive (Fig. 3.15 and 3.16). Neither clone nor water treatment had any impact on the CO_2 compensation point of *Eucalyptus* trees after 6 and 18 months growth. CO_2 compensation point was greater after 18 months growth, but the variability within water treatments and clones was also greater (Fig. 3.15 (b) and 3.16 (b)).

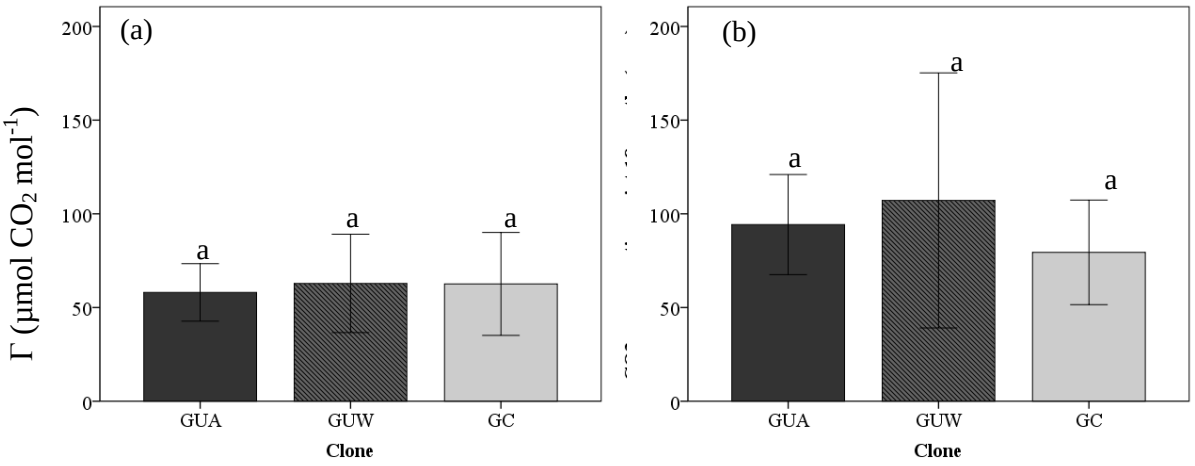


Figure 3.15: Mean CO_2 compensation point (Γ) of clones mol^{-1} when *Eucalyptus* trees were (a) 6 months and (b) 18 months old (n = 15 per clone; different letters denote significant difference).

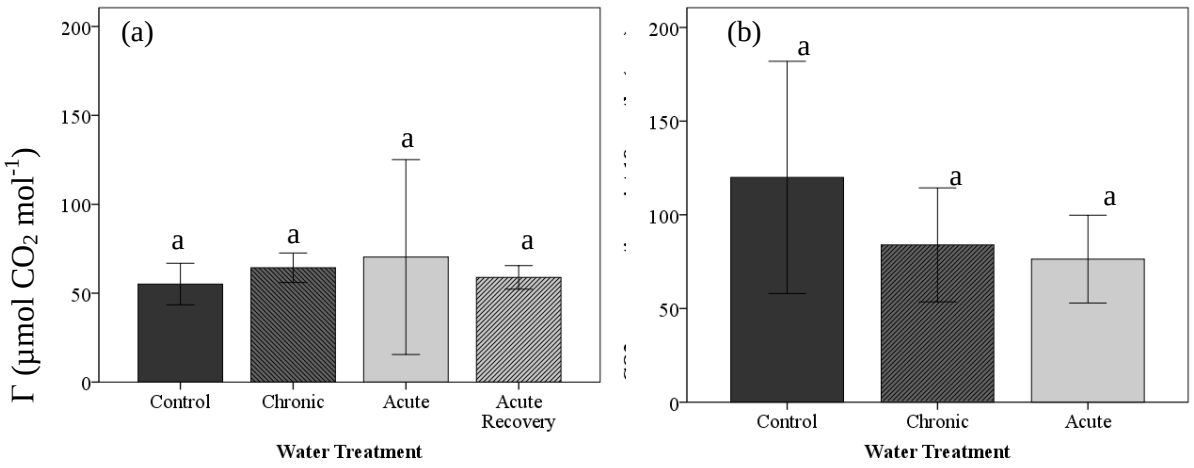


Figure 3.16: Mean CO_2 compensation point (Γ) of water treatments measured at when *Eucalyptus* trees were (a) 6 months and (b) 18 months old (n = 15 per water treatment; different letters denote significant difference).

Photorespiration, the rate at which CO_2 is produced instead of consumed, can be viewed as a protective measure when stress reduces CO_2 fixation. Figure 3.17 shows that photorespiration of the GUW clones was higher (less photorespiration) than GUA and GC, and the difference was significant ($p = 0.034$). GC had the highest photorespiration rate at both 6 and 18 months (mean = -9.5 and -7.75 µmol CO_2 m^{-2} s^{-1} respectively). Photorespiration was significantly less in the acute stress treatments (at wilting point) (mean = -2.2 µmol CO_2 m^{-2} s^{-1}; $p < 0.0001$; Fig. 3.18 (a)). After 18 months growth, photorespiration was less than the control in the acute and chronic stress treatment ($p = 0.068$; Fig. 3. 18 (b)).

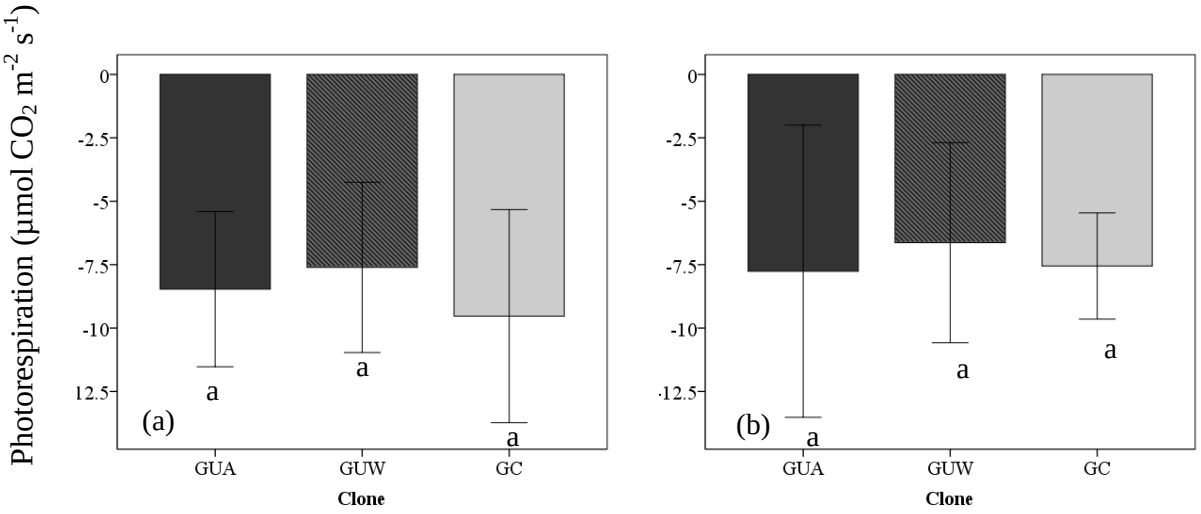


Figure 3.17: Mean photorespiration of clones when *Eucalyptus* trees were (a) 6 months and (b) 18 months old (n = 15 per clone; different letters denote significant difference).

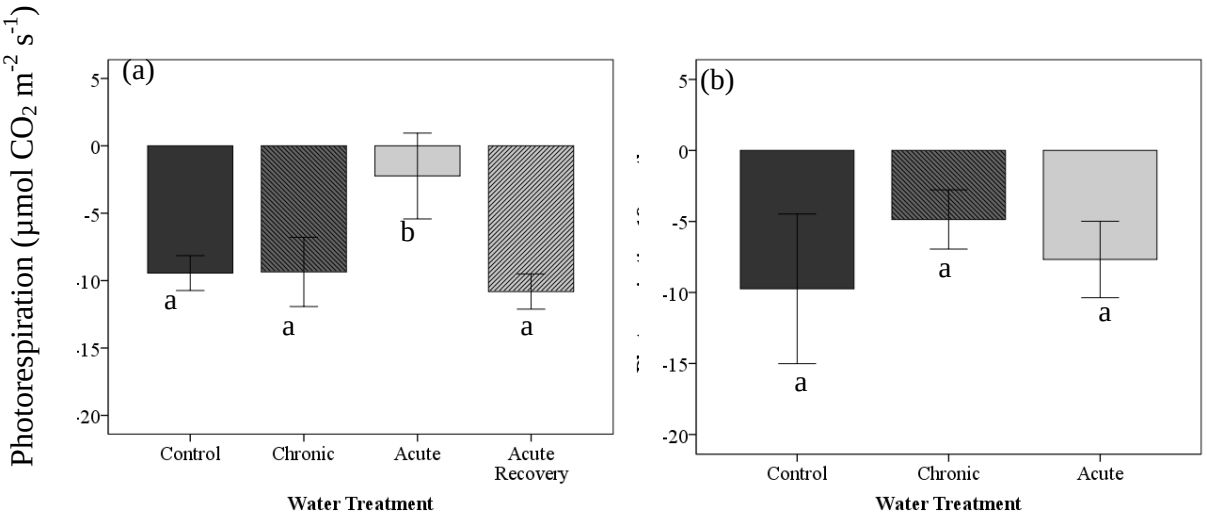


Figure 3.18: Mean photorespiration of water treatments when *Eucalyptus* trees were (a) 6 months and (b) 18 months old (n = 15 per water treatment; different letters denote significant difference).

Carboxylation efficiency (Vc_{max}, which is the initial slope of an A:C_i curve) was greatest in the GC clone after 6 and 18 months growth ($p = 0.063$ and $p = 0.358$ respectively; Fig. 3.19 (a) and (b)). The difference between clones was not statistically significant, possibly due to high variability within each clone. As shown in Figure 3.11 (a) and (b) in terms of J_{max}, Vc_{max} was lower after 18 months growth in comparison with 6 months growth (Fig. 3.19 (a) and (b)). Vc_{max} of eucalypt leaves could also possibly down-regulate with age, and perhaps have an influence on net photosynthetic rate.

Figure 3.20 (a) illustrates that Vc_{max} was significantly reduced only in the acute stress treatment (at wilting point) although the variability measured is extremely high (mean = 0.050 ± 0.100; $p < 0.0001$). After 18 months growth, Vc_{max} is lowest in the chronic treatment (mean = 0.068; $p = 0.071$) and concurrent with the date for the clones, there appears to be a marked reduction in Vc_{max} with age in all water treatments (Fig. 3.19 (b)).

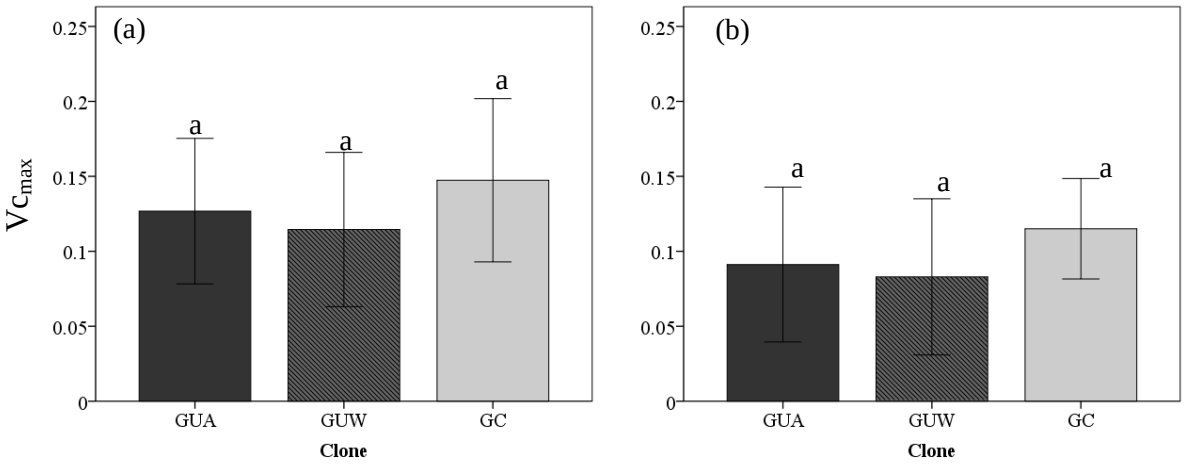


Figure 3.19: Mean carboxylation efficiency (Vc_{max}) of clones when *Eucalyptus* trees were (a) 6 months and (b) 18 months old (n = 15 per clone; different letters denote significant difference).

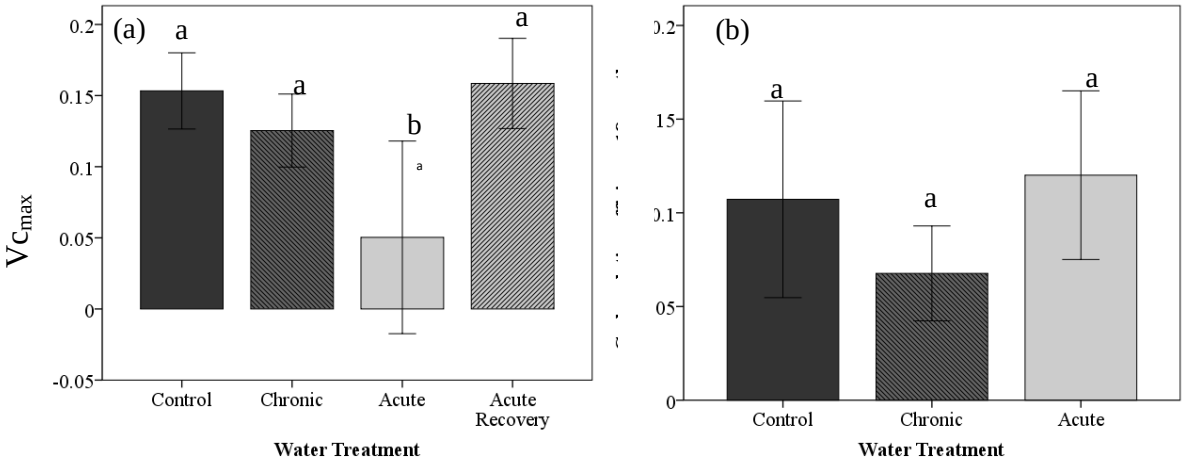


Figure 3.20: Mean carboxylation efficiency (Vc_{max}) of water treatments when *Eucalyptus* trees were (a) 6 months and (b) 18 months old (n = 15 per water treatment; different letters denote significant difference).

3.2.2 Actual Photosynthetic Rates (Spot Measurements)

Photosynthetic spot measurements (actual photosynthetic rate, using the sun / sky LiCor chamber top) were measured on *Eucalyptus* leaves at 6, 12 and 18 months. Spot measurements were therefore performed in spring, winter and summer (6, 12 and 18 months respectively) in order to determine seasonal photosynthetic change, although tree age then did become a contributing factor. Measurements performed on the acute stress *Eucalyptus* trees were done on leaves at least one week after recovering from a water stress cycle event. Accurate spot measurements could not be executed on eucalypt leaves when at their wilting point (end of the drought stress cycle) as the data were unreliable. This occurred because stomata were closed at the wilting point and internal C_i values were measured as negative numbers. To continuously compare the acute stress treatment it was decided that acute recovery photosynthetic rates could be contrasted against the control and chronic treatments.

Mean assimilation rate (A_n) was significantly lower in the GUW clones at 6 and 12 months, compared with the GC clone ($p = 0.05$; $p = 0.005$; Table 3.1). After 18 months tree growth, A_n did not differ between all three *Eucalyptus* clones ($p = 0.217$). During the winter measurements (at 12 months growth), A_n declined in all three eucalypt clones by up to 50% (Table 3.1). A_n was seen to increase again during summer (18 months) but with the exception of GUW, assimilation rate had decreased in comparison with juvenile *Eucalyptus* tree assimilation rates. The decline of photosynthetic rate, with increasing tree age, was also evident when photosynthetic potential (J_{max}) was measured.

A_n did not change by more than 0.5% in the control treatment over 18 months and 3 different seasons (Table 3.1). The chronic and acute (recovery) stress treatment both showed a decline in A_n during winter. Although A_n increased at 18 months, it was approximately 25% less than A_n at juvenile tree growth (6 months). The only difference between water treatments was shown to be in the chronic treatment, which was significantly less at 12 months, in comparison with the control and acute recovery treatments ($p = 0.0001$; Table 3.1). After 12 and 18 months, the control treatment had the greatest actual assimilation rate.

Table 3.1: Mean actual photosynthetic rate (A_n, µmol CO_2 m^{-2} s^{-1}) of *Eucalyptus* clonal hybrids in response to water stress (p values derived from a two-way analysis of variance; different letters denote significance; clone*water treatment interactions are reported only if significant)

Parameter	Treatment / Clone	Mean ± SE 6 months	Mean ± SE 12 months	Mean ± SE 18 months
A_n	GUA	14.15 ± 1.08[a]	6.48± 0.56[a]	10.56± 0.73[a]
	GUW	11.44± 1.08[b]	6.91± 0.60[a]	12.23± 0.73[a]
	GC	15.15± 1.02[a]	9.22± 0.6[b]	12.28± 0.67[a]
	p-value	**0.05**	**0.005**	**0.217**
	Control	12.47± 1.02[a]	12.71± 0.78[a]	12.57± 0.73[a]
	Chronic	14.22± 1.08[a]	8.82± 0.74[b]	10.59± 0.73[a]
	Acute (Recovery)	13.95± 1.08[a]	9.33± 0.74[b]	11.86± 0.69[a]
	p-value	**0.421**	**0.0001**	**0.202**

3.3 Non-destructive measurements: Plant Water Relations

3.3.1 Stomatal Conductance

Stomatal conductance (g_s) was greatest in GC clones at 6, 12 and 18 months (Table 3.2). Table 3.2 shows that GC and GUA were significantly greater than GUW at six months growth only (p = 0.001). Stomatal conductance was highest during winter (at 12 months growth) for all three *Eucalyptus* clones. After six months growth, $g_s$was lowest in the control treatment but at 12 and 18 months, g_s was highest in the control (Table 3.2). For all three water treatments, $g_s$was highest during winter. Transpiration (E_n) was greatest, for all three *Eucalyptus* clones, after six months growth (table 3.2). E_n was lowest during winter (in contrast to gs) for *Eucalyptus* clones, and GC was higher than GUA and GUW, although never significantly so. Table 3.2 shows that E_n was greatest at six months growth for all three water treatments. At 12 and 18 months the chronic water treatment was significantly lower than the control and acute (recovery) treatments (p = 0.0001; p = 0.024). E_n showed a similar trend, when compared with A_n, where E_n decreased

during winter (12 months) and was still reduced after 18 months tree growth. Evapotranspiration could also possibly show down-regulation with age, as evident in A_n.

Table 3.2: Mean actual stomatal conductance (g_s, mmol mol^{-1}) and transpiration rate (E, mmol m^{-2} s^{-1}) of *Eucalyptus* clones in response to drought stress (p values derived from a two-way analysis of variance; different letters denote significance; clone*water treatment interactions are reported only if significant)

Parameter	**Treatment**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**
		6 months	**12 months**	**18 months**
g_s	GUA	0.206 ± 0.02[a]	0.351 ± 0.21[a]	0.341 ± 0.09[a]
	GUW	0.166 ± 0.01[a]	0.421 ± 0.22[a]	0.254 ± 0.09[a]
	GC	0.256± 0.02[b]	0.759 ± 0.23[a]	0.423 ± 0.08[a]
	p-value	**0.001**	**0.378**	**0.449**
	Control	0.190± 0.02[a]	1.590± 0.29[a]	0.429 ± 0.09[a]
	Chronic	0.201± 0.02[ab]	0.341± 0.28[b]	0.266 ± 0.09[a]
	Acute (Recovery)	0.237± 0.01[b]	0.490± 0.28[b]	0.334 ± 0.08[a]
	p-value	**0.071**	**0.003**	**0.490**
E_n	GUA	4.67 ± 0.61[a]	2.36± 0.21[a]	3.90± 0.40[a]
	GUW	4.12±0.61[a]	2.48± 0.22[a]	3.60± 0.40[a]
	GC	5.94± 0.58[a]	2.73± 0.23[a]	4.12± 0.38[a]
	p-value	**0.105**	**0.475**	**0.701**
	Control	5.36± 0.58[a]	4.21± 0.29[a]	4.62± 0.40[a]
	Chronic	4.22± 0.61[a]	2.54± 0.27[b]	2.82± 0.40[b]
	Acute (Recovery)	5.14± 0.61[a]	3.27± 0.27[ab]	4.22± 0.38[a]
	p-value	**0.372**	**0.0001**	**0.024**

Water use efficiency (WUE, the amount of CO_2 fixed per unit water transpired) was marginally higher in GUW *Eucalyptus* clones, but none of the mean WUEs were significantly different from one another (Fig. 3.21). GUA showed a decrease in WUE after 18-months growth, while WUE of GC clones increased after 18-months growth.

The chronic water treatment had the greatest WUE, compared with the control and acute (recovery) treatments (Fig. 3.22). High variability within treatments led to lack of significance between any water treatments. The control and chronic treatments increased WUE with age (6 – 18 months), although acute recovery treatments decreased (Fig. 3.22).

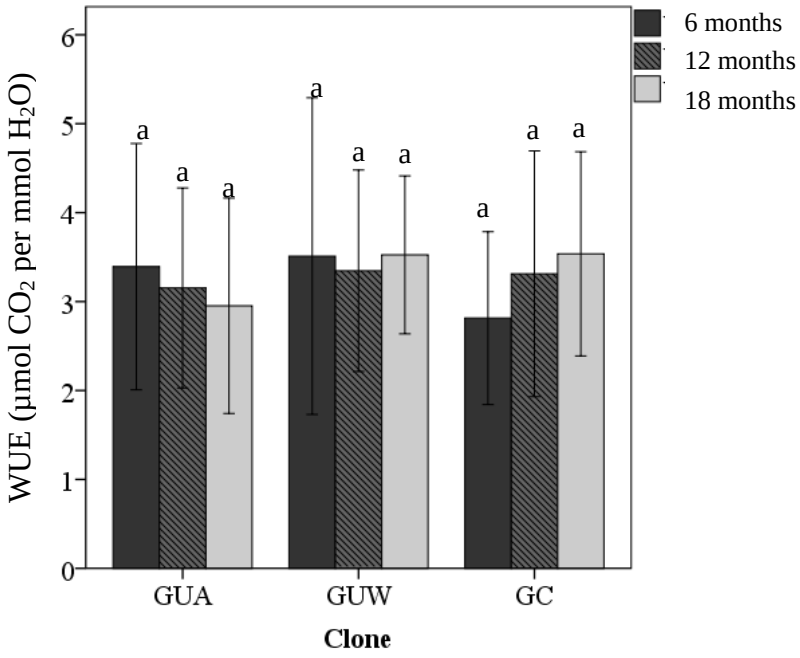


Figure 3.21:Mean Water Use Efficiency (WUE, CO_2 fixed per unit water transpired) measured at 6, 12 and 18 months of *Eucalyptus* clones grown under to water stress (different letters denote significant difference).

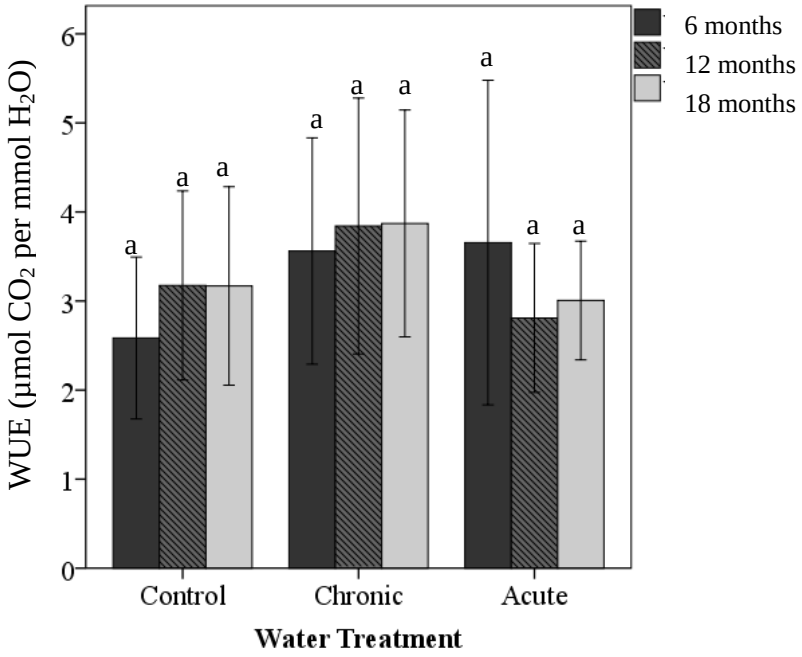


Figure 3.22: Mean Water Use Efficiency (WUE, asssimilate per unit water transpired) measured at 6, 12 and 18 months of *Eucalyptus* in response to drought stress (different letters denote significant difference).

Stomatal conductance (g_s) and assimilation rate (A_n) were correlated with each other to determine the relationship between the two. Figure 3.23 shows that A_n and g_s were linearly and positively correlated for the three water treatments at 6 months old. The acute (recovery) treatment had the strongest linear relationship ($R^2 = 0.551$) and the overall relationship between A_n and g_s was significant and positive ($p = 0.014$). As g_s increased, A_n increased linearly. Figure 3.24 illustrates that there was a positive, linear relationship between A_n and g_s ($R^2 = 0.288$). After 18 months tree growth, A_n was positively and significantly correlated with g_s when expressing the relationship in terms of water stress treatment ($p = 0.025$; Figure 3.25).

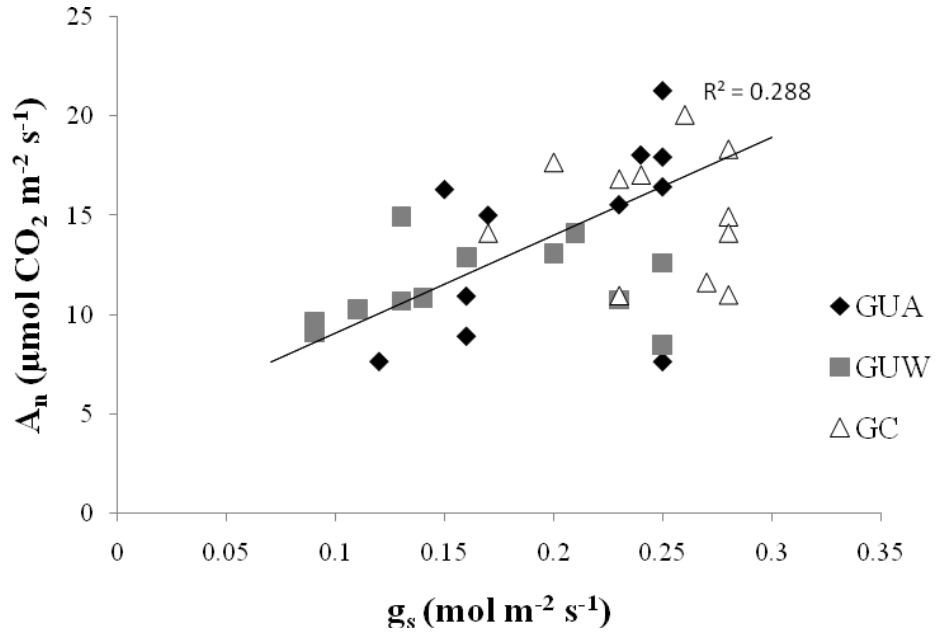


Figure 3.23: Correlation between stomatal conductance (g_s) and assimilation rate (A_n) of *Eucalyptus* clonal hybrids (GUA, GUW and GC) at six months old.

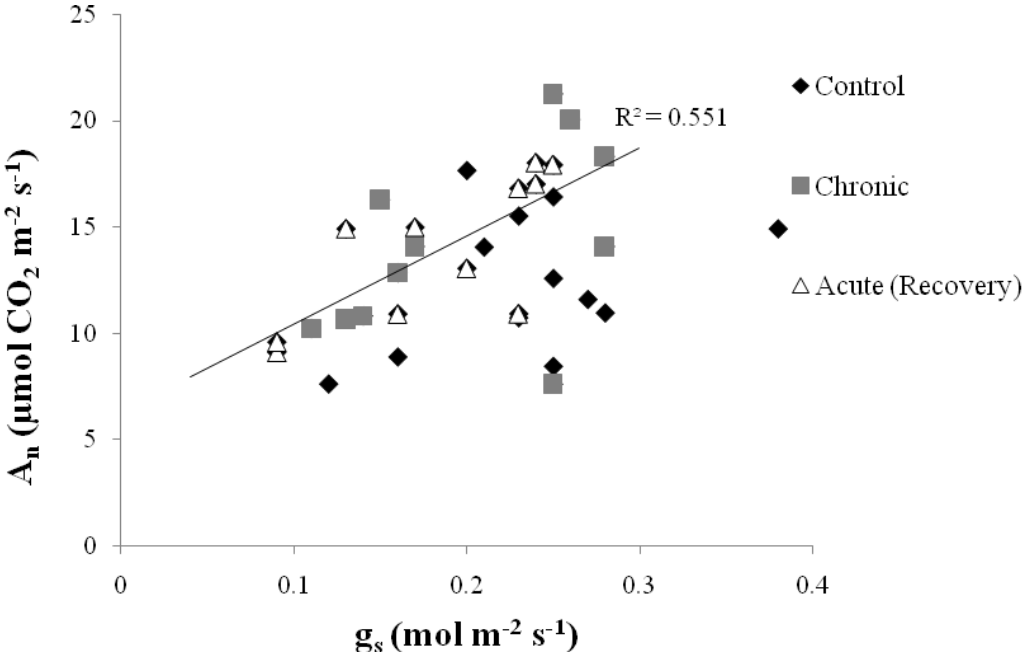


Figure 3.24: Correlation between stomatal conductance (g_s) and assimilation rate (A_n) of water treatments (control, chronic and acute stress) imposed on *Eucalyptus* trees at six months old.

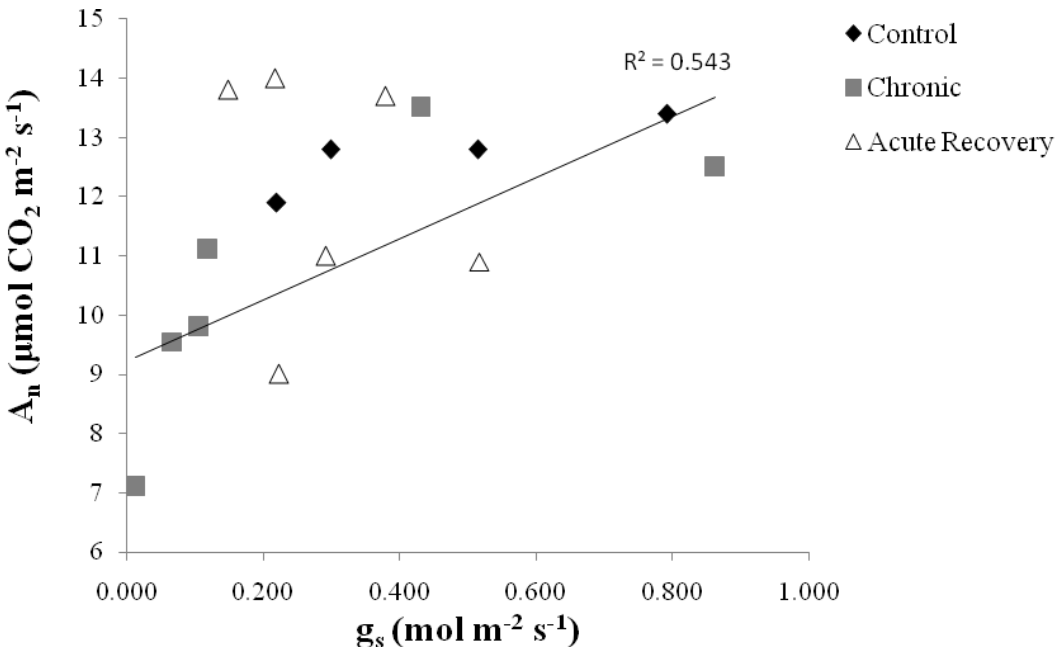


Figure 3.25: Correlation between stomatal conductance (g_s) and assimilation rate (A_n) of water treatments (control, chronic and acute stress) imposed on *Eucalyptus* trees at 18 months tree growth.

3.4 Destructive measurements: Biomass at harvest (9 and 18 months)

Total biomass after nine months growth was significantly greater in the GC clone (Fig. 3.26 (a); p = 0.02). After 18 months growth however, there was no statistically significant difference between clones although GUA had the greatest total biomass (mean = 1.7 kg; Fig. 3.27 (a); p = 0.061). The GUW clone had the lowest total biomass (mean = 1.3 kg) and the variability within clones was relatively high. Figure 3.26 (b) showed that total biomass did not differ between water treatments after nine months growth (p = 0.493). Total biomass was shown to decrease in water treatments (control > chronic > acute) after 18 months growth, but the differences in total biomass were not found to be statistically significant (p = 0.073).

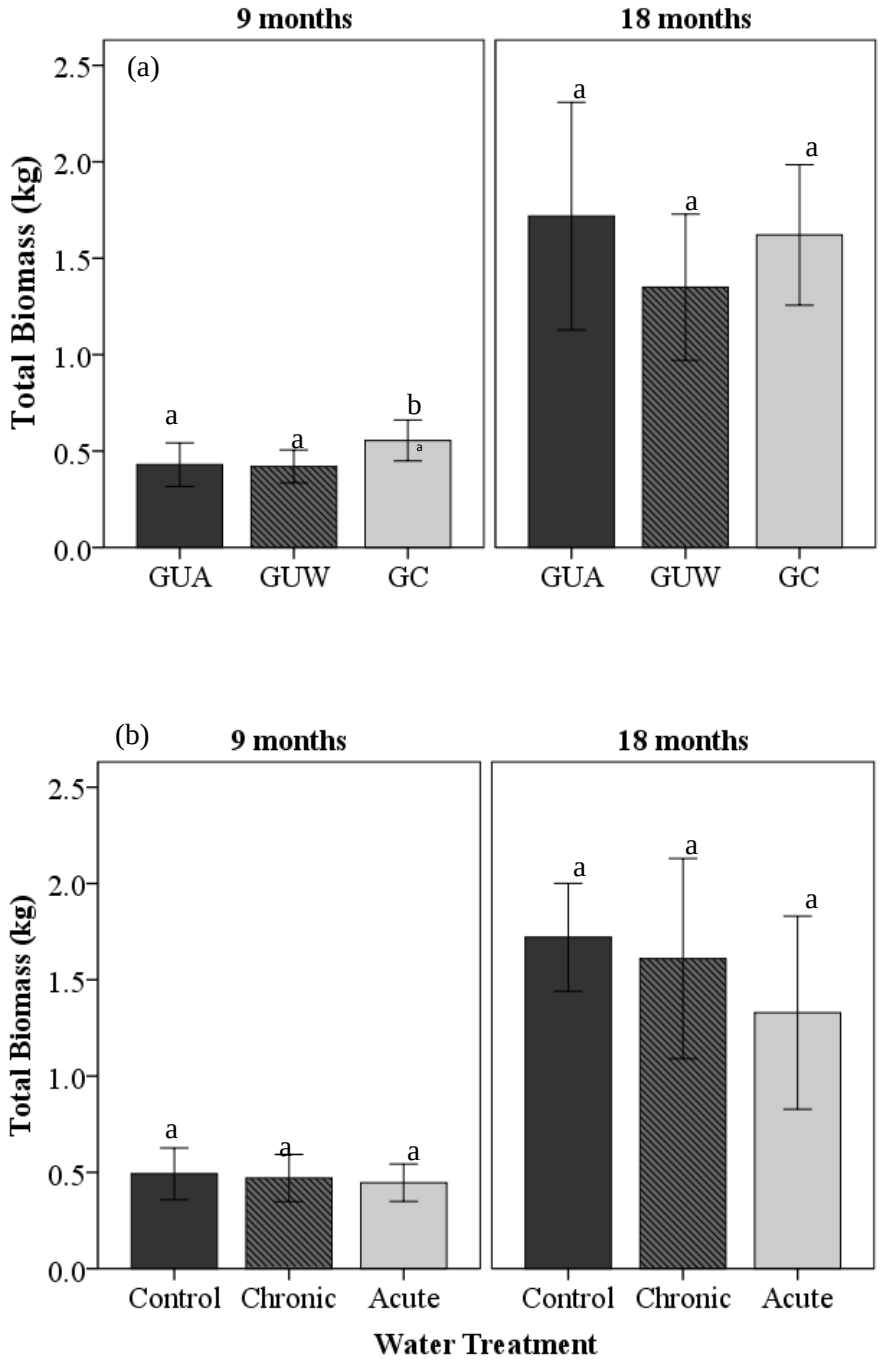


Figure 3.26: Total biomass (kg) of three *Eucalyptus* clones (a) clonal effect after 9 months and 18 months growth; (b) water treatment effect after 9 months growth and 18 months growth (Different letters denote significant difference between clones or treatments).

The GUA clone had the greatest leaf and stem biomass after nine and 18 months growth (Table 3.3). At both harvests, the GC clone had significantly less leaf biomass than the GUA and GUW clones. Leaf biomass was not different between water treatments after nine months but was significantly reduced (by 30%) in the acute stress treatment after 18 months growth (Table. 3.3). The GUA clone had significantly more stem biomass after 18 months compared with GUW and GC ($p = 0.041$). The control treatment had the greatest stem biomass after nine and 18 months but was not significantly higher than chronic or acute treatments for either harvest. GC had up to 50% more root biomass than GUA and GUW after nine months growth ($p < 0.0001$). Although the difference was still significant ($p < 0.0001$) after 18 months, GC was only 15 - 25% higher than the GU clones (Table 3.3). The acute stress treatment showed significantly less root biomass (reduction of 25%) than the control and chronic treatment after 18 months tree growth.

Table 3.3: Biomass parameters (leaf, stem and root dry mass) of *Eucalyptus* clonal hybrids in response to drought stress (p values derived from a two-way analysis of variance)

Parameter	**Treatment/**	**Mean ± SE**	**Mean ± SE**	**p-value**	
	Clone	**9 months**	**18 months**	**9 months**	**18 months**
Leaf	GUA	0.155 ± 0.07[a]	0.364 ± 0.16[a]		
Biomass	GUW	0.130 ± 0.03[b]	0.281 ± 0.08[ab]		
(kg)	GC	0.124 ± 0.01[b]	0.219 ± 0.06[b]	**0.029**	**0.003**
	Control	0.135 ± 0.05[a]	0.340 ± 0.08[a]		
	Chronic	0.143 ± 0.03[a]	0.307 ± 0.13[a]		
	Acute	0.131 ± 0.02[a]	0.210 ± 0.11[b]	**0.567**	**0.002**
Stem	GUA	0.137 ± 0.04[a]	0.720 ± 0.28[a]		
Biomass	GUW	0.116 ± 0.02[a]	0.507 ± 0.20[b]		
(kg)	GC	0.133± 0.02[a]	0.522 ± 0.15[b]	**0.096**	**0.041**
	Control	0.142± 0.03[a]	0.620 ± 0.15[a]		
	Chronic	0.126± 0.02[a]	0.578 ± 0.29[a]		
	Acute	0.119± 0.02[a]	0.534 ± 0.24[a]	**0.092**	**0.632**
Root	GUA	0.143 ± 0.05[a]	0.634 ± 0.20[a]		
Biomass	GUW	0.174 ± 0.06[a]	0.560 ± 0.17[a]		
(kg)	GC	0.298± 0.09[b]	0.880 ± 0.22[b]	**0.000**	**0.000**
	Control	0.217± 0.08[a]	0.760 ± 0.22[a]		
	Chronic	0.201± 0.12[a]	0.730 ± 0.23[a]		
	Acute	0.196± 0.09[a]	0.580 ± 0.24[b]	**0.726**	**0.030**

Figure 3.27 (a) showed that the GC clone allocated biomass differently from the GU clones. GC allocated more than 50% of its biomass to roots after both nine and 18 months, and allocated the least amount of biomass to the stems, which are the plant component responsible for tree productivity. The GUA clone allocated the majority of its biomass to stem (36 and 42%, at 9 and 18 months respectively). It can also be noted that allocation of biomass to leaves decreased with age, whereas allocation to stem increased (Fig. 3.27 (a)).

The proportions of biomass allocated to roots, stems and leaves did not differ by more than 4% between water treatments at both nine and 18 months (Fig. 3.27 (b)). Biomass allocation, in response to tree age, showed that allocation to leaf biomass reduced by 30% and stem biomass increased after 18 months tree growth, irrespective of watering treatment.

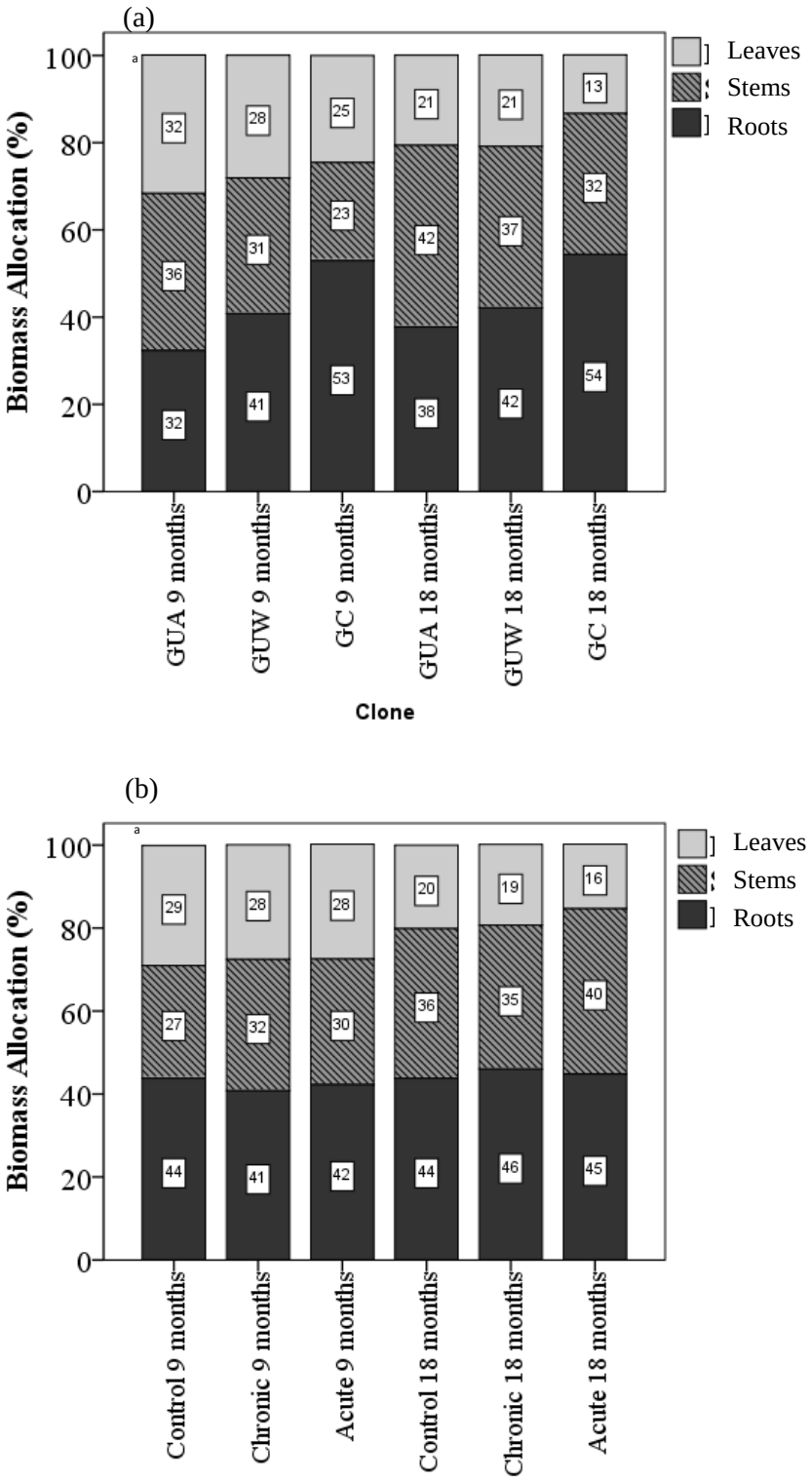
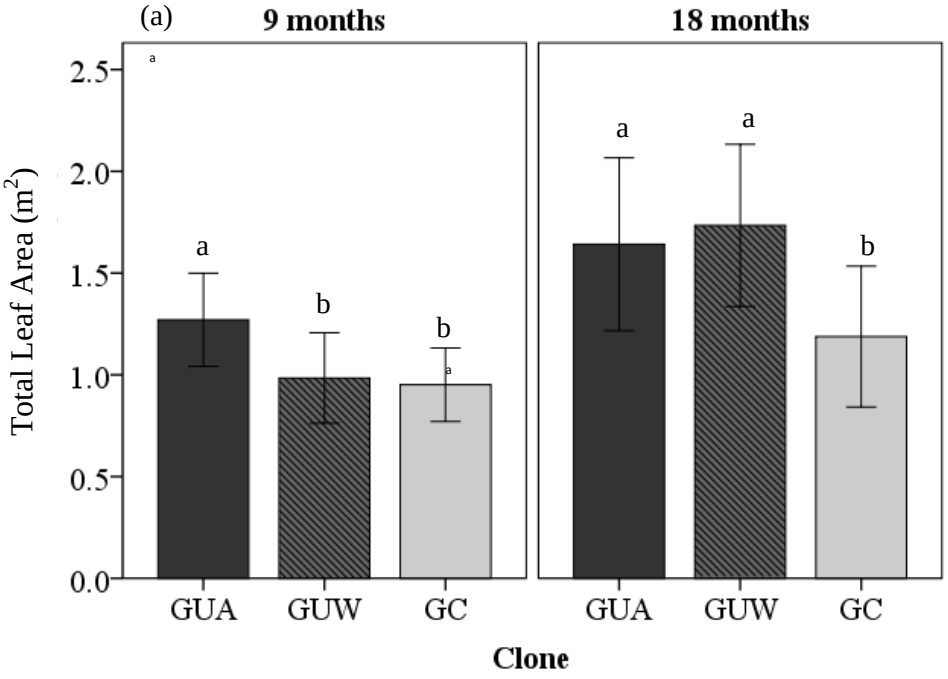


Figure 3.27: Proportional (%) allocation of biomass to roots, stems and leaves (%) by the three *Eucalyptus* clones (a) clonal effect after 9 and 18 months growth; (b) water treatment effect after 9 and 18 months growth.

Total leaf area was greatest in the GUA clone after nine months (Fig. 3.28 (a); $p < 0.0001$; mean = 1.3 m^2). After 18 months, the GUW clone had more leaf area than GUA, although the difference was not significant. The GC clone had significantly less (25 – 35%) leaf area after 9 and 18 months. Figure 3.28 (b) showed that there was no difference between leaf area in all three treatments ($p = 0.862$) after 9 months. Leaf area differed in water treatments (control > chronic > acute) after 18 months ($p = 0.008$). Acute (periodic and cyclic drought stress) most negatively reduced leaf area, by up to 25% compared with the control.



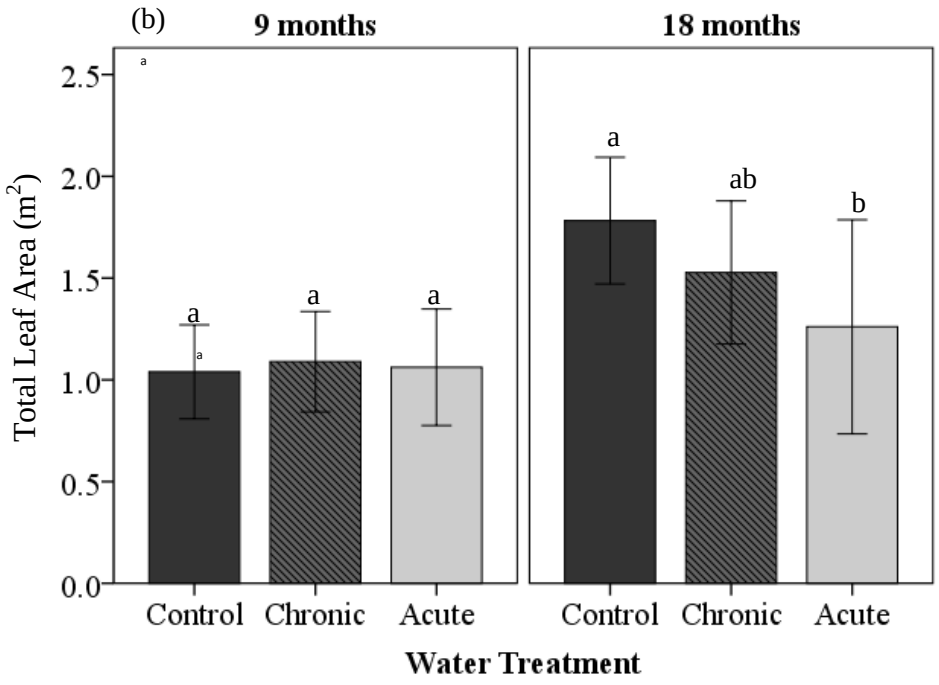


Figure 3.28: Total leaf area (m^2) of three *Eucalyptus* clones (a) clonal effect after 9 months and 18 months growth; (b) water treatment effect after 9 months growth and 18 months growth (Different letters denote significant difference between clones or treatments).

Specific leaf area (SLA) is expressed as the area of leaf per unit leaf biomass. There was no difference in SLA between clones or water treatments after nine months (Table 3.4). SLA was 50 – 60% greater after 18 months relative to 6 months in all clones and water treatments. The GUW clone had significantly greater SLA than the GUA and GC clones ($p = 0.031$) thereby inferring that GUW leaves have more area per unit leaf mass after 18 months growth. Plants subjected to the acute stress had significantly higher SLA than the chronic and control treatment (Table 3.4). GC clones had a significantly greater (40 – 60% greater) root:shoot than GU clones after both 9 and 18 months (Table 3.4). Previously, Fig. 3.27 showed that GC clones allocated 50% of their biomass exclusively to roots. There was however no difference in root:shoot between the water treatments at both harvest intervals (Table 3.4).

Table 3.4: Biomass parameters (Specific leaf area and Root : Shoot) of *Eucalyptus* clonal hybrids in response to drought stress (p values derived from a two-way analysis of variance)

Parameter	**Treatment**	**Mean ± SE**	**Mean ± SE**	**p-value**	
		9 months	**18 months**	**9 months**	**18 months**
SLA	GUA	2.66 ± 0.09[a]	5.13 ± 0.35[a]		
(m^2/kg)	GUW	2.51 ± 0.09[b]	6.43 ± 0.33[b]		
	GC	2.45± 0.09[a]	5.55 ± 0.34[a]	**0.281**	**0.031**
	Control	2.56± 0.09[a]	5.39 ± 0.34[ab]		
	Chronic	2.54± 0.09[a]	5.23 ± 0.34[a]		
	Acute	2.51± 0.09[a]	6.49 ± 0.34[b]	**0.921**	**0.027**
root:shoot	GUA	0.49 ± 0.07[a]	0.62± 0.06[a]		
	GUW	0.71± 0.07[a]	0.75± 0.06[a]		
	GC	1.16± 0.07[b]	1.22± 0.06[b]	**0.0001**	**0.0001**
	Control	0.80± 0.07[a]	0.83± 0.06[a]		
	Chronic	0.77±0.07[a]	0.92± 0.06[a]		
	Acute	0.80± 0.07[a]	0.84± 0.06[a]	**0.933**	**0.553**

3.5 Destructive measurements: Whole Plant Hydraulic Characteristics

Hydraulic conductance (K_h) was measured and expressed as a function of flow rate normalized per unit leaf area (m^2). K_h was significantly lower in the GUA clones after nine months growth (Fig 3.29 (a); $p < 0.0001$). GC clones had a 50% greater K_h than GUA. Figure 3.29 (a) shows that K_h increased by approximately three times in all three clones after 18 months. An increase in total biomass accompanies a concurrent increase in K_h because more biomass provides more hydraulic pathways to conduct water thereby increasing K_h. The variability within clones was very high, largely in part due to the difficulty of accurately measuring hydraulic flow of trees of greater than 2.0 meters height with the available equipment. There was no difference in K_h between *Eucalyptus* clones after 18 months growth, although GUW did have the highest mean K_h (Fig. 3.29 (a); $p = 0.116$). K_h was not significantly different between water treatments after 9 and 18 months (Fig. 3.29 (b); $p = 0.730$ and 0.290 respectively). The variability within measurements of the same water treatment were relatively even after nine months, but variability after 18 months was extremely high. The chronic treatment had the highest K_h by approximately 20% compared with the control (Fig. 3.29 (b)).

Hydraulic resistance to water flow (R_h) is the inverse of K_h and was also expressed normalized per unit leaf area (m^2). The GUA clone had the highest R_h after 9 and 18 months (Fig. 3.30 (a); $p < 0.0001$; $p = 0.174$ respectively). R_h also decreased with age because the number of hydraulic pathways increased with age, therefore decreasing overall resistance to water flow. Fig 3.30(b) shows that R_h was not significantly different between water treatments after 9 and 18 months ($p = 0.91$; $p = 0.081$). R_h was highest in the control treatment, after 18 months, which was the opposite trend compared with trees measured at nine months. It would be expected that the control treatment would have the lowest R_h because no water stress was imposed. On the other hand, there could be a decrease in R_h with stress as a compensating method to increase the flow of the limited water through the plant to the leaves.

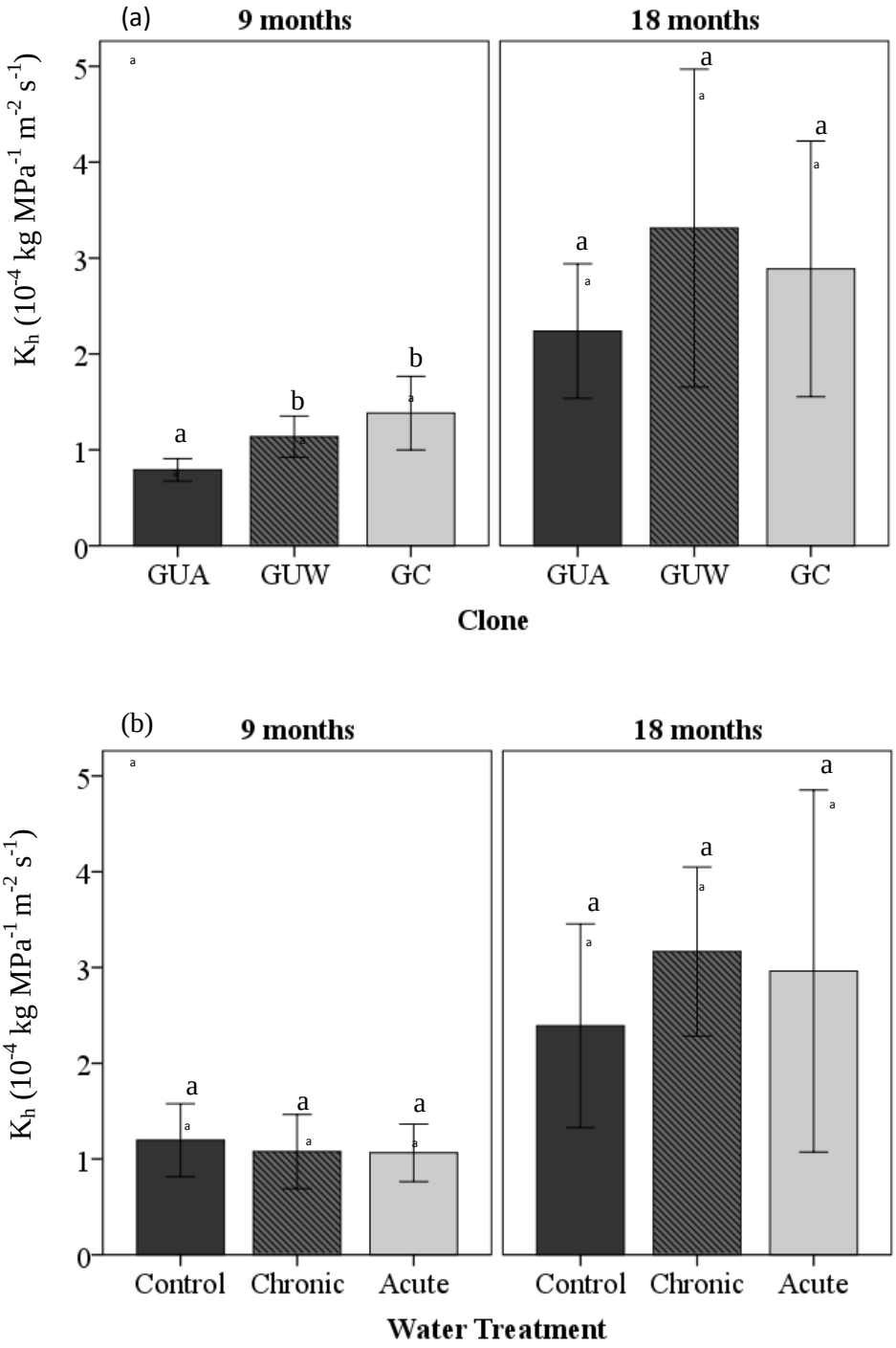


Figure 3.29: Total hydraulic conductance (K_h) of three *Eucalyptus* clones (a) clonal effect after 9 months and 18 months growth; (b) water treatment effect after 9 months growth and 18 months growth (Different letters denote statistical significance between clones or treatments).

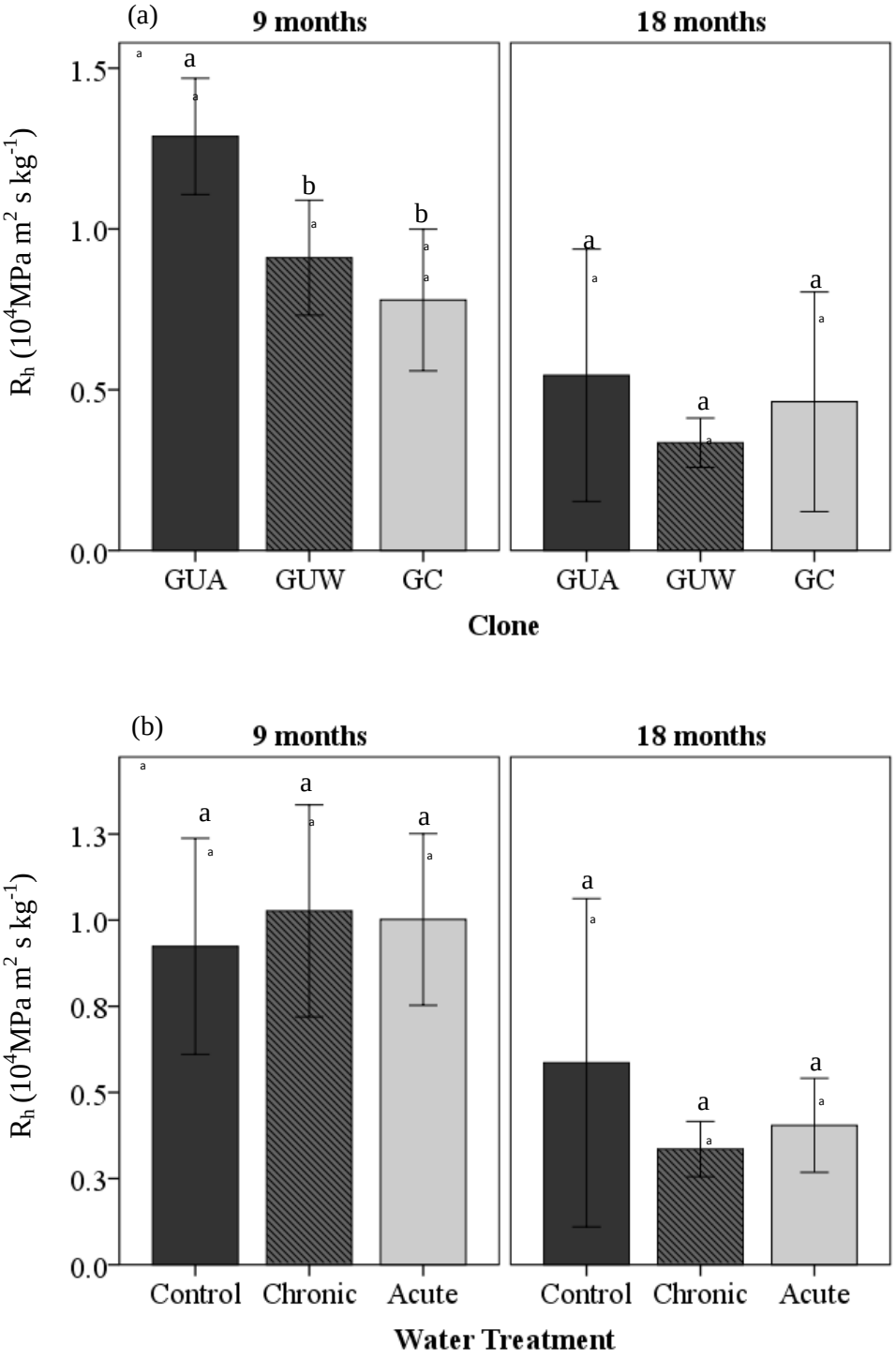


Figure 3.30: Total hydraulic resistance (R_h) of three *Eucalyptus* clones (a) clonal effect after 9 months and 18 months growth; (b) water treatment effect after 9 months growth and 18 months growth (Different letters denote statistical significance between clones or treatments).

Table 3.5 shows the hydraulic resistance to water flow in individual plant components (roots, stems and leaves) which are expressed as components of R_n. The GC clone had significantly lower R_{root} at nine months, primarily due to the high root biomass possessed by GC clones. GUA had significantly higher R_{stem} and R_{leaves} than GUW and GC after 9 and 18 months. There was however no difference in hydraulic characteristics among water treatments at both harvest periods (Table 3.5).

The allocation of hydraulic resistance to plant components (root, stems and leaves) is shown in Figure 3.31. Panel (a) displays allocation of resistance in clones after 9 and 18 months. At nine months, more than 55% of the total R_h resided in the roots, in all three clones. This proportion was similar to that seen in Figure 3.26 (a), where more than 50% of the total biomass was found in the roots. GUW clones had 10% more resistance in the roots than GUA and GC. A completely different pattern of allocation of resistance was seen after 18 months growth (Fig. 3.31 (a)). The proportion of hydraulic resistance was lowest in the roots, in all three clones. The allocation of resistances to roots, stems and leaves did not differ significantly between clones after 9 and 18 months growth.

When assessing the allocation of resistance in response to water treatments, Figure 3.31 (b) shows that up to 60% of the resistance can be attributed to the roots at nine months. After 18 months however, the proportion of the resistance in the leaves was significantly greater. The proportion of resistance in the above-ground plant components (stems and leaves) increased by 50% by 18 months (Fig. 3.31 (b)). There was no difference between water treatments in the allocation of resistances to plant components at both harvests.

Table 3.5: Hydraulic characteristics (leaf, stem and root hydraulic resistance, R_h (10^4MPa m^2 s kg^{-1})) of *Eucalyptus* clonal hybrids in response to drought stress (p values derived from a two-way analysis of variance; clone*water treatment interactions reported only if significant)

Parameter	Treatment/ Clone	Mean ± SE 9 months	Mean ± SE 18 months	p-value 9 months	p-value 18 months
$\mathbf{R_{root}}$	GUA	0.736 ± 0.05[a]	0.051 ± 0.02[a]		
	GUW	0.601 ± 0.04[a]	0.048 ± 0.02[a]		
	GC	0.421± 0.04[b]	0.061 ± 0.02[a]	**0.0001**	**0.849**
	Control	0.570± 0.05[a]	0.340 ± 0.08[a]		
	Chronic	0.630± 0.04[a]	0.307 ± 0.13[a]		
	Acute	0.559± 0.04[a]	0.210 ± 0.11[a]	**0.483**	**0.669**
$\mathbf{R_{stem}}$	GUA	0.209 ± 0.01[a]	0.159± 0.01[a]		
	GUW	0.129± 0.01[b]	0.117± 0.01[b]		
	GC	0.132± 0.01[b]	0.124± 0.01[ab]	**0.0001**	**0.036**
	Control	0.150± 0.008[a]	0.150± 0.01[a]		
	Chronic	0.152± 0.008[a]	0.117± 0.01[a]		
	Acute	0.168± 0.008[a]	0.133± 0.01[a]	**0.227**	**0.146**
$\mathbf{R_{leaves}}$	GUA	0.346 ± 0.03[a]	0.355± 0.07[a]		
	GUW	0.180± 0.02[b]	0.168± 0.07[a]		
	GC	0.226 ± 0.02[b]	0.277± 0.07[a]	**0.0001**	**0.229**
	Control	0.232± 0.03[a]	0.391± 0.07[a]		
	Chronic	0.246± 0.02[a]	0.177± 0.07[a]		
	Acute	0.275 ± 0.02[a]	0.212 ± 0.07[a]	**0.450**	**0.076**

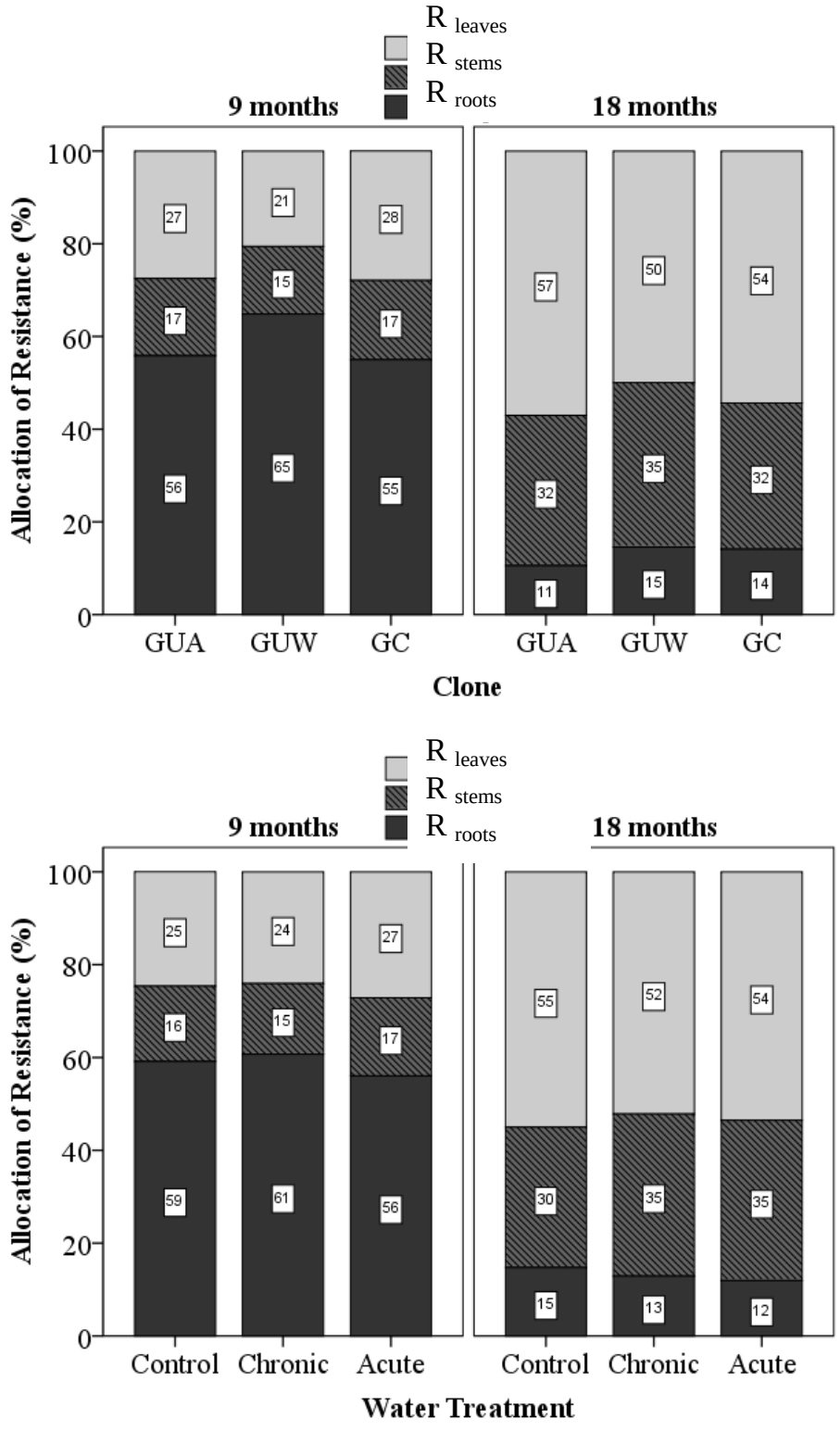


Figure 3.31: Allocation of hydraulic resistance to plant components (roots, stems and leaves) of three *Eucalyptus* clones (a) clonal effect after 9 and 18 months growth; (b) water treatment effect after 9 and 18 months growth.

3.6 Growth and Physiology Characteristics: Correlated Biomass and R_h Parameters

Biomass and whole-plant hydraulic characteristics were assessed to determine whether there were any relationships between these parameters. Establishing a clear relationship between, for example, biomass and hydraulic conductance (K_h) will determine if an increase in the number of hydraulic pathways in a *Eucalyptus* tree will ensure an increase in total biomass long-term. Figure 3.32 shows that as K_h increased, total biomass increased. An increase in biomass increased the total number of hydraulic pathways available for water flow thereby increasing K_h. Additionally, an increase in hydraulic conductivity could lead to higher leaf water potentials, higher stomatal conductance and higher CO_2 assimilation (the 'hydraulic limitation hypothesis'). The correlation between total biomass and K_h was significantly positive ($p = 0.03$). Considering that R_h is the inverse of K_h, Figure 3.33 shows that R_h was significantly and negatively correlated with total biomass.

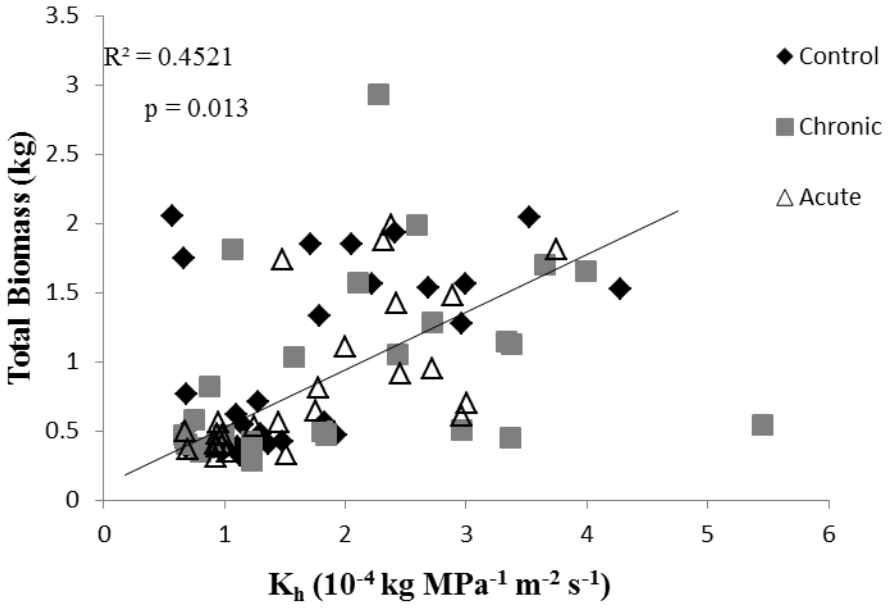


Figure 3.32: Correlation between hydraulic conductance (K_h) and total biomass (kg) of plants subjected to the watering treatments (control, chronic and acute stress).

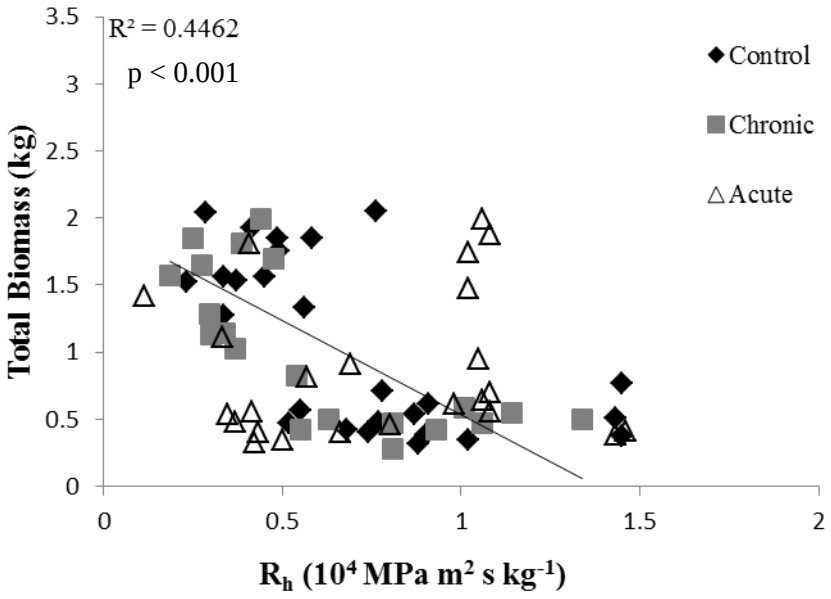


Figure 3.33: Correlation between hydraulic resistance (R_h) and total biomass (kg) of plants subjected to watering treatments (control, chronic and acute stress).

The allocation of biomass to roots (expressed as a percentage of total biomass) was significantly and negatively correlated with allocation of biomass to stems (Fig. 3.34; $p < 0.0001$). The proportion of biomass allocated to leaves was 25% less than the stems. Figure 3.35 shows that percentage biomass allocated to leaves was significantly and negatively correlated with that of percentage root biomass ($p = 0.001$).

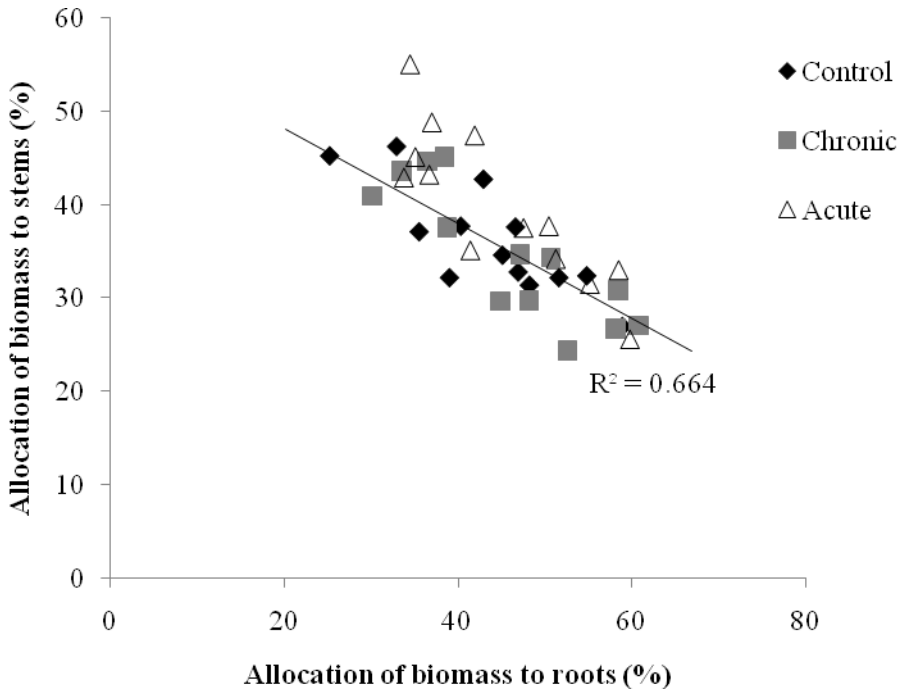


Figure 3.34: Correlation between allocation of biomass to roots and stems in plants subjected to water treatments (control, chronic and acute stress) at 18 months ($p = 0.014$).

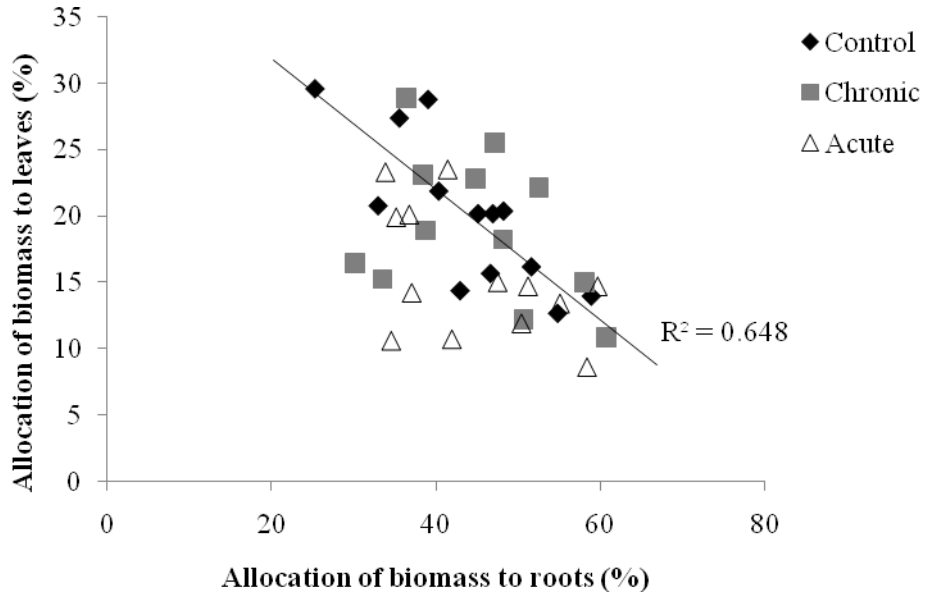


Figure 3.35: Correlation between allocation of biomass to roots and leaves in plants subjected to water treatments (control, chronic and acute stress) at 18 months ($p < 0.0001$).

3.7 Growth and Physiology Characteristics: Correlated Photosynthetic and Hydraulic Parameters

Photosynthetic (actual/spot measurements), growth efficiency and whole-plant hydraulic characteristics (particularly K_h) were investigated for any relationship between individual parameters. Figure 3.36 shows that as K_h increases, growth efficiency increases (R^2 = 0.320). Growth efficiency is considered wood volume per total leaf area, and eucalypt trees subjected to the acute water treatment had significantly less leaf area and therefore higher growth efficiency in relation to K_h.

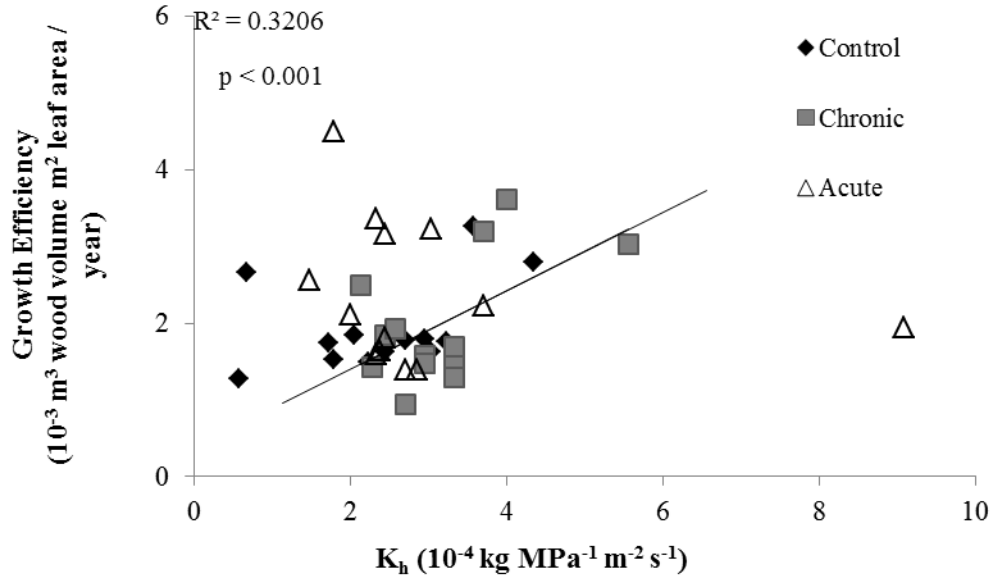


Figure 3.36: Correlation between K_h (hydraulic conductance, normalized by leaf area) and growth efficiency in plants subjected to watering treatments (control, chronic and acute stress) at 18 months.

Figure 3.37 and 3.38 show the positive, significant correlation between K_h and A_n at 9 and 18 months growth, respectively (R^2 = 0.15 and 0.45). An increase in K_h accompanied an increase in A_n. Maximum A_n was lower after 18 months tree growth, whereas K_h was higher. A positive relationship was also evident between K_h and g_s (Fig. 3.39 and 3.40 at 9 and 18 months tree growth, respectively).

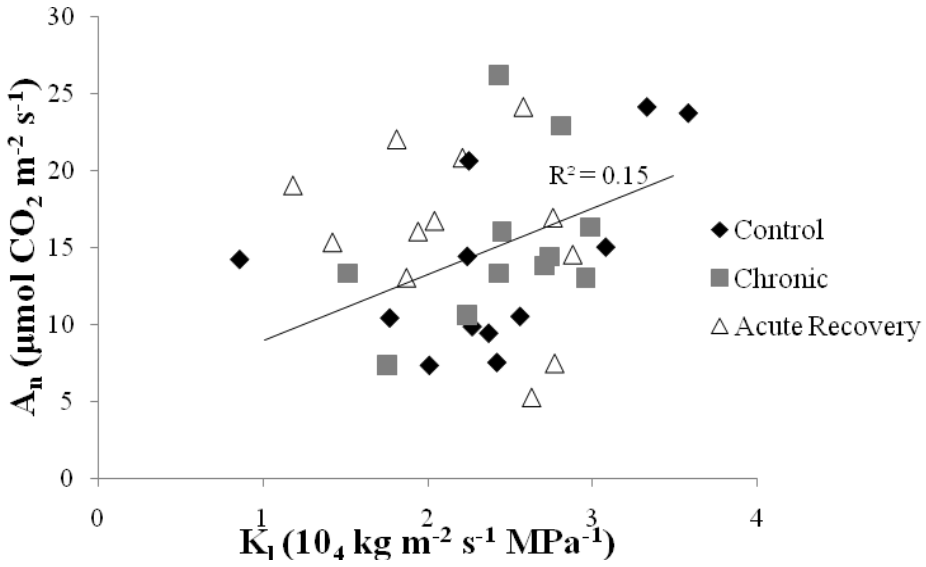


Figure 3.37: Correlation between K_h (hydraulic conductance, normalized by leaf area) and A_n in plants subjected to watering treatments (control, chronic and acute stress) at 9 months (p = 0.041).

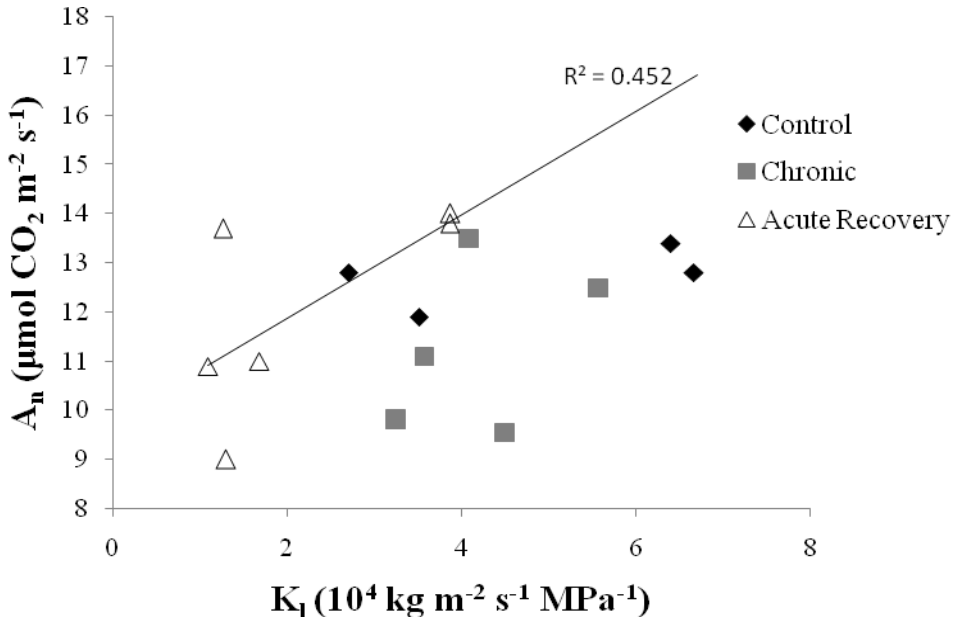


Figure 3.38: Correlation between K_h (hydraulic conductance, normalized by leaf area) and A_n in planted subjected to watering treatments (control, chronic and acute stress) at 18 months (p = 0.032).

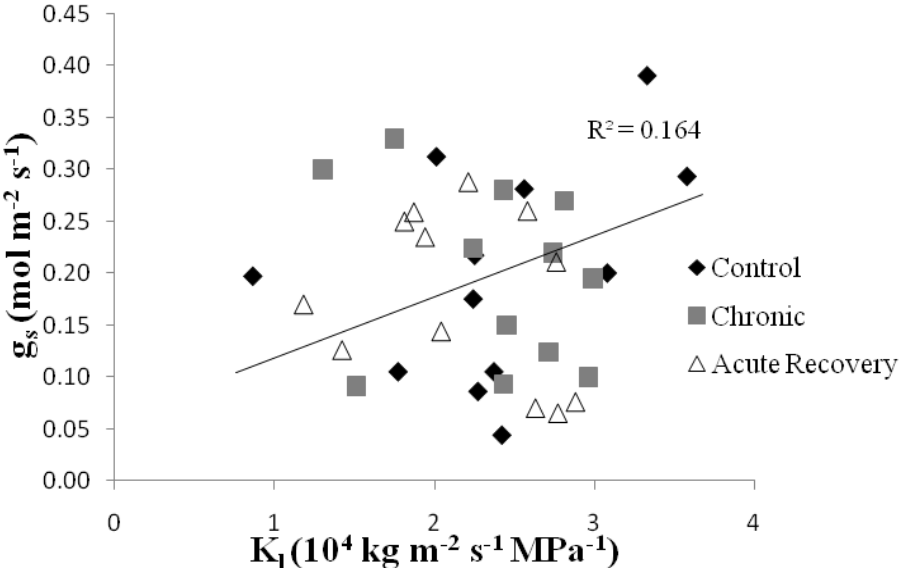


Figure 3.39: Correlation between K_h (hydraulic conductance, normalized by leaf area) and g_s in plants subjected to watering treatments (control, chronic and acute stress) at 9 months ($p = 0.031$).

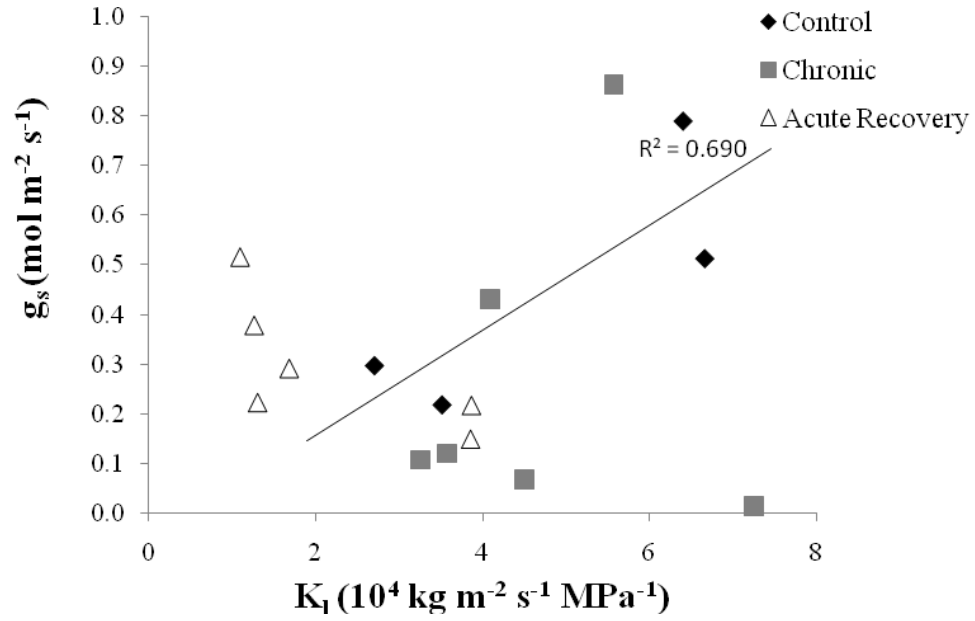


Figure 3.40: Correlation between K_h (hydraulic conductance, normalized by leaf area) and g_s in plants subjected to watering treatments (control, chronic and acute stress) at 18 months ($p = 0.04$).

3.8 Discussion

Sappi Forests Research embarked on a tree breeding program with eucalypts in 1984 (Morris, 2008). The program has made Sappi self-sufficient in genetically improved orchard seed and hybrid combinations are continuously produced for vegetative propagation (Morris, 2008). The clones chosen for planting are determined based on market requirements, yield improvement and the need to decrease risk, particularly in terms of drought risk (Morris, 2008). The selection of clones has to be matched with the site i.e. site-species matching, to ensure productivity in that specific climate (Pallett, 2005; Boreham and Pallett, 2009). Demands for improved productivity have created a need for research into the understanding of morphological and physiological characteristics of *Eucalyptus* trees of different clones. Water stress is one of the most important environmental factors limiting plant productivity and research knowledge of the response to water stress in *Eucalyptus* clones is essential for maintaining productivity and site-species matching.

Considering that few studies have been carried out on the morphological and physiological response to water stress of *Eucalyptus* clones in South Africa, the aim of this study was to investigate these responses in three South African produced *Eucalyptus* clones under three different watering regimes. Morphological assessments were measured by means of height, diameter, biomass and leaf area over an 18 month trial period. Physiological responses of the clones by water stress included the assessment of photosynthetic characteristics and hydraulic conductance to water flow. The first objective of the current study was to measure the impact of watering regime on the morphology (height and diameter) of three *Eucalyptus* clonal hybrids.

Figure 3.41 displays a results summary diagram showing which morphological or physiological parameters were significantly affected by clone, water treatment or age.

RESULTS SUMMARY

Physiological and Morphological Parameters Are Affected By:

CLONE

- Height (GC > GU)
- Diameter Growth Rate (GUW > GUA & GC)
- Growth Efficiency (GC > GU)
- Volume (GC & GUW > GUA)
- A_n (GUW signif lower at 6 months)
- Root:shoot (GC > GU)
- SLA (GUW > GUA & GC)
- K_h (signif lower in GUA at 9 months)
- Leaf Area (GU > GC)

GC: Growth efficiency
GUW: Diameter growth
GUA: Leaf area supporting growth

WATER TREATMENT

- Diameter (control > stress)
- Volume (control > stress)
- J_{max} (↓ at acute wilting point)
- V_{cmax} (↓ at acute wilting point)
- E_n + WUE (↓ in chronic treatment)
- g_s (↑ in control treatment)
- SLA (acute > control & chronic)
- Leaf Area (control > stress)

Control: Diameter; leaf area
Chronic: Improved WUE
Acute: SLA signif higher

AGE

- A_n & E_n (↓ with age and winter)
- g_s (↑ with age and winter)
- Biomass allocation (↓ to leaves; ↑ to stems)
- SLA (significant only at 18 months)
- Allocation of resistance to water flow (↑ to leaves; ↓ to roots)
- Leaf Area (significant only at 18 months)

At 18 months: change in allocation to biomass and resistance; leaf area maintained is significantly different between clone and water treatment

CONCLUSIONS

Clone: GUW clones maintain greater diameters, improved WUE, and greater above-ground biomass

Water treatment: Chronic water stress (small water deficits long-term) improve WUE, maintain greater diameters, more leaf area than acute water stress (severe, short-term water stress)

Age: Leaf area and allocation of biomass and resistance are controlled ontogenetically

Leaf Area is the morphological parameter driving physiological changes

Figure 3.41: Summary of results of physiological and morphological parameters that are affected by eucalypt clone, water treatment or tree age in plants grown for 9 or 18 months.

Eucalyptus clones show different patterns of response depending on the morphological parameter being assessed. The GC clone was significantly taller than GU clones (Fig. 3.2-3) whereas GU clones (in particular GUW) reached greater diameters, although not significantly greater (Fig. 3.5-6). Absolute height and diameter were reflected by the fact that growth rate (in height) was greater in GC clones and growth rate (in diameter) was greater in GU clones (Fig. 3.4 and 3.7).

In contrast to clonal response, eucalypt water treatment response was significant in terms of diameter but not height growth (Fig. 3.3 and 3.6). Height growth was not affected by water treatment but diameter growth was significantly greater in the control treatment. The acute water stress most negatively affected diameter growth (Fig. 3.7). GU clones exposed to the control water treatment had the greatest diameter but when exposed to the acute water treatment, GU growth rate (in diameter) was less than GC clones (Fig. 3.5). Although GU clones had the greatest diameter growth under favourable water balance, GU had greater water stress susceptibility than that of GC. This has been shown in field trials, where drought stress more negatively affected GU clones compared with GC clones (Drew *et al*, 2009; Drew and Pammenter, 2006; van der Willigen and Pammenter, 1998). GC has been shown to display slow and steady diameter growth ("tortoise" growth) but GU displays fast growth under favourable conditions ("hare" growth habit) (Drew *et al.*, 2009). Differing growth habits have been observed in other *Eucalyptus* species (Alijaro *et al.*, 1972; Downes *et al*., 1999; Morgan and Barton, 2008). Height growth was therefore determined by genetics (GC > GU) whereas diameter growth was firstly determined by water treatment imposition (control > chronic > acute) and secondarily by genetics (GU > GC).

Tree volume was greater in GC clones (primarily because of greater heights attained) but the tree volume was not significantly different from the GUW clones (which display greater diameters) (Fig. 3.9). Tree volume was significantly greater in the control and lowest in the acute water treatment. Similar findings have been reported for *Eucalyptus* clones grown in field trials at mesic and xeric sites, where trees grown at mesic sites attained up to 100% more wood volume than trees grown at xeric sites (vander Willigen and Pammenter, 1998). When expressing growth as tree volume per leaf area per year (i.e. growth efficiency, GE), GC clones had a greater GE

than GU (Fig. 3.10). GC clones maintain more volume of wood per unit leaf area (m^2) compared with GU clones, primarily because GC clones have significantly lower total leaf area than GU clones (Fig. 3.10). GE was greatest, although not significantly, in the acute water treatment, i.e. more volume of wood per unit leaf area than the control treatment. Expressing GE per unit leaf area does not take into account the significantly lower total leaf area maintained by the acute treatment trees due to leaf loss from water stress (senescence).

As previously mentioned, water stress is the main factor limiting plant yield in semi-arid regions (Egea *et al*., 2011). The degree to which growth is affected by water stress is of utmost importance as to whether *Eucalyptus* species can tolerate environmental conditions at a specific plantation site (Gindaba *et al*., 2005). Understanding the relationship between water stress and plant physiological processes in *Eucalyptus* depends on the severity, duration and rapidity of the drought event (Rouhi *et al*., 2007). Studies focussing on photosynthesis have been used as tools to explore the physiological basis of tree growth under water-limited conditions (Lambers *et al*., 1998; Gindaba *et al*., 2005). Stomata also play an important role in controlling carbon balance and negatively affected carbon balance when closed in response to water limitation (Rouhi *et al*., 2007). An objective of the current study was to assess whether physiological characteristics e.g. photosynthetic capacity and plant water relations of the three *Eucalyptus* clones differed with water availability and with tree age.

Acute water stress negatively and significantly affected maximum photosynthetic potential (J_{max}, at saturating CO_2 concentration) of all three *Eucalyptus* clones, when measured at leaf wilting point (Fig. 3.11). After re-watering the eucalypt trees subjected to the acute water treatment, there was no significant difference in J_{max} compared with chronic and control water treatments. GU clones showed the greatest J_{max} in control water treatments, whereas J_{max} in GC clones was greatest in the chronic treatment (Fig. 3.11). Whitehead *et al*. (2004) showed that *Eucalyptus* species have relatively high photosynthetic capacity and the average J_{max} values exceed those found across woody species. The results presented in the current study are comparable with the results found by Whitehead *et al*. (2004). Although J_{max} was negatively affected by acute water stress at wilting, the recovery of J_{max} is in agreement with other *Eucalyptus* studies regarding photosynthetic capacity (Searson *et al*., 2004).

E. occidentalis showed no difference in J_{max} between well-watered and water-limited plants (Searson *et al*., 2004) and J_{max} of *E. camaldulensis* seedlings was unaffected by water limitation in a glasshouse study (Gibson *et al*., 1994). J_{max} declined with age in all three *Eucalyptus* clones (Fig. 3.11).

Γ (CO_2 compensation point) and photorespiration did not differ between clones, watering treatment or age (Fig. 3.15 and 3.18). V_{cmax} (carboxylation efficiency) did however decline with leaf age. J_{max} and V_{cmax} have been shown to decrease with tree age in almond and olive trees (Diaz-Espejo *et al*., 2007; Egea *et al*., 2011). Ontogenetic changes (i.e. leaf age or seasonal differences) were shown to decrease J_{max} and V_{cmax} more than water limitation.

The GC clone showed higher instantaneous photosynthetic rates (A_n) than GU clones over three seasons within the 18 month growth trial period (Table 3.1). A_n decreased significantly during the winter season in all three *Eucalyptus* clones, and by 18 months tree age (summer season), A_n was between 2-4 µmol CO_2 m^{-2} s^{-1} less than the previous summer. Seasonal and age-related declines in A_n have been reported in numerous studies (Diaz-Espejo *et al*., 2007; Egea *et al*., 2011) and ontogenetic changes were shown to affect almond trees more than water restriction (Nortes *et al*., 2009).

Water stress did not affect A_n when the eucalypt trees were juvenile saplings (six months tree growth. There was a seasonal decline in photosynthetic rate (A_n) during winter of 2010, and A_n was significantly lower in water stressed treatments. Decrease in A_n during different seasons has been observed in deciduous (Flexas *et al*., 2009) and evergreen species (Niinemets *et al*., 2005). The decrease is usually associated with a decrease in J_{max} and V_{cmax}, which was observed in *Eucalyptus* clones in the current study (Fig. 3.10 and 3.19). Decreases in A_n in response to water stress have also been observed in almond trees (Egea *et al*., 2011) and olive trees (Niinemets *et al*., 2009) and the reduction was attributed to non-stomatal limitations e.g. decreased carboxylation efficiency i.e. a reduction in V_{cmax}. For *Eucalyptus* clones (GU and GC), instantaneous photosynthetic rate was affected more negatively by seasonal and tree age factors than it was by a reduction in water supply.

Stomatal conductance (g_s) and transpiration (E_n) were highest in the GC clone over all three seasons (Table 3.2). g_s was greatest after 12 months of tree growth (during winter) and was lowest in the chronic stress treatment at 12 and 18 months. E_n was greatest during the juvenile growing phase (at 9 months tree growth), and declined with tree age, similar to A_n.

E_n in the chronic treatment was also consistently lower than the control or acute water treatment. g_s was found to have the same degree of seasonal plasticity as that found in almond leaves (Egea *et al*., 2011). The results of the current *Eucalyptus* study were in contrast to those found by Egea *et al*. (2011) as g_s increased with eucalypt tree age, rather than the decrease found in almond trees. Therefore after 18 months growth, the reduction in A_n was not attributed to stomatal reduction. A decline in g_s has been reported for other eucalypt species (Whitehead and Beadle, 2004), but the current study shows that photosynthetic reduction could be attributed to non-stomatal limitation e.g. less efficient biochemical processes in the photosynthetic cycle. Water stress had a significant negative effect on g_s up to 18 months tree growth. Reductions in g_s due to water limitation in woody angiosperms have been reported in a number of other studies (Flexas *et al*., 2002; Niienemets *et al*., 2005; Warren, 2008).

E_n did not differ between the clones during any growth season, for the duration of the growth trial period (Table 3.2). What was of particular interest with regards to E_n, was that E_n was significantly lower in the chronic water treatments, when compared with the control and acute water treatments. Even though g_s was lower in chronic treatment, although not always significantly over 18 months, E_n was limited in the chronic treatment. A reduction in E_n has important consequences in terms of theoretical water use efficiency (WUE, A_n/E_n) because *Eucalyptus* have been reported to optimise WUE in water limited environments (Mooney *et al*., 1978; Anderson *et al*., 1996).

Instantaneous water use efficiency (WUE) was higher, although not significantly so, in the chronic water treatment and in GUW clones (Fig. 3.22). WUE was higher in the chronic treatment primarily because of a significant reduction in E_n (Table 3.2). WUE estimates are described to vary widely between *Eucalyptus* species and environmental conditions (Whitehead and Beadle, 2004). Dunin and Mackay (1982) showed seasonal variations in WUE for *E. maculata*, although significant seasonal variation in WUE for the current study was not evident.

Soil water deficit has been reported to reduce WUE in severely water-stressed *E. camaldulensis* and *E. globulus*, but the reduction in WUE was driven by a reduction in A_n (Gindaba *et al*., 2004). Due to the constant, low soil water availability that facilitated long-term, mild soil water deficit, eucalypt clones growing in the chronic treatment showed a measure of physiological plasticity in terms of E_n, that ensured improved WUE. *Eucalyptus* species grown in xeric environments do not usually have high WUE (Searson *et al*., 2004) but two studies have been shown to find improved WUE in xeric environments (Anderson *et al*., 1996; Sefton *et al*., 2002). One might have expected the acute water treatment to show a measure of WUE improvement, but perhaps the recovery periods following a severe drought or water stress cycle facilitated no need for optimised WUE over the growth trial period.

A_n was correlated with g_s for eucalypt clone and water treatment at both six and 18 months tree growth (Fig. 3.23-25). Numerous studies have reported that A_n is correlated with g_s for *Eucalyptus* species (Ngugi *et al*., 2004; Searson *et al*., 2004; Gindaba *et al*., 2005; Niienemets *et al*., 2009; Egea *et al*., 2011). As g_s increased in clone or water treatment, A_n increased as displayed by the significant positive correlation in Fig. 3.23 – 25. Stomatal response of GC and GU leaves may have increased the supply of CO_2 to maintain high A_n during favourable conditions. Searson *et al*. (2005) reported that an increase in CO_2 supply to the mesophyll of *E. occidentalis* and *E. sideroxylon* leaves assisted in the improvement of nitrogen use efficiency (NUE). Close co-ordination between A_n and g_s has been shown in *Ficus sylvatica* (Montpied *et al*., 2009) and the co-ordination is considered to be associated with a decrease in the efficiency of the photosynthetic apparatus. The reduction in A_n is later accompanied by a reduction in g_s, which could be understood as a down-regulation process due to leaf age or residual water stress effect (Egea *et al*., 2011). Co-ordination of mesophyll photosynthetic capacity and stomatal aperture remains a question in plant science (Lawson *et al*., 2003).

The evaluation of biomass and biomass allocation, leaf area and root:shoot interactions under a controlled watering regime could provide important information on the potential performance of eucalypts under field conditions (Nilsen and Orcutt, 1996; Gindaba *et al*., 2005).

Trees can change biomass allocation patterns when the soil environment changes e.g. changes in soil water deficit (Sands and Mulligan, 1990). Soil water deficits have been shown to cause reductions in total biomass, leaf production and patterns of allocation (Osorio *et al*., 1998; Pereira and Chaves, 1993). Consequences of moderate soil water deficit involve acclimation to a water-limited environment e.g. increase in root:shoot, leaf loss and stomatal closure (Osorio *et al*., 1998). Although more than 500 000 hectares of eucalypts are currently planted in South Africa, the demand for sustainable harvest is not being met (DWAF, 2005; Morris, 2008).

Forest research output has considerably increased eucalypt wood production per unit area but a thorough understanding of biomass allocations (especially below-ground) in response to soil water deficit will improve understanding of the specific clonal response to water stress. The objective of the current study in terms of biomass allocation was to evaluate the effects of water stress and eucalypt clone on biomass partitioning at the juvenile (9 months) and early adult (18 months) growth stages.

Total dry biomass was negatively affected by water stress after 18 months growth, although the reduction was not significant (Fig. 3.26). Total biomass was lowest in the GUW clones (after 18 months) and highest in the GUA clone. Above-ground biomass was significantly greater in GUA, as GUA had significantly more leaf and stem dry matter than GUW and GC (Table 3.3). In terms of wood production, the GUA clone had up to 30% more stem dry matter than GUW and GC. The control water treatment had the greatest biomass (in terms of root, stem and total) compared with the chronic and acute treatment. The acute treatment was most negatively affected with respect to biomass in response to water stress (Table 3.3). Stem productivity was never more than 15% greater in the control treatment, implying that moderate-severe soil water deficit does not detrimentally affect wood productivity, especially if there is a period of recovery from prolonged or acute water stress. These results are in contrast to those obtained by Mokotedi (2010) for *E. nitens* x *nitens* where drought stress (similar to the acute water treatment in the current study) reduced total biomass by up to 50% in comparison with control plants.

Moderate and severe soil water deficit were also shown to reduce total biomass of *E. globulus* by up to 50% (Osorio *et al.*, 1998). The most noticeable difference in the results found (i.e. total biomass decreases in response to water stress) is that the eucalypt plants of the two studies were grown in relatively small pots (10 or 25 litre pots) and the plants were not more than six months old (Mokotedi, 2010; Osorio *et al*., 1998). Perhaps, with tree age, reduction in total biomass becomes less significant, depending on eucalypt clone and the degree of soil water deficit.

Biomass allocation patterns differ between GU and GC clones (Fig. 3.28). The GC clone allocates more than 50% of total biomass to the roots, whereas in the GU clone it is approximately 40%. The proportion of biomass allocated to roots remains relatively the same over the 18 month growth trial period. The proportion of biomass allocated to leaves decreases with tree age by up to 12% in all three eucalypt clones (Fig. 3.28). The GC clone had 40% less leaf biomass than GU at the early adult growth stage. In terms of productivity, trees of the GC clone would have the necessary root biomass to withstand drought more effectively than GU. However, 10% more stem biomass in the GU clones would yield significantly more wood per unit area in a plantation. The genotype has been shown to affect total biomass and allocation of carbon to plant components in *E. globulus*, therefore providing evidence that genotypes do differ in their biomass allocation patterns.

Root biomass does not differ by more than 5% between water treatments at both nine and 18 months growth (Fig. 3.27). Larcher (1995) stated that higher biomass allocation to roots could increase the amount of water available to the plant when soil water deficits are experienced. Eucalypts in the current study did not show increased biomass allocation to the roots and it may have been possible that some degree of root restriction had occurred by the 18 month growth stage. As evident in clonal response, allocation to stem increased by up to 10% with tree age, with a concurrent reduction in leaf biomass. In the current study, it appears that genetic traits i.e. clone and ontogenetic traits determine biomass allocation more than soil water deficit. Root:shoot did not differ with tree age or change in response to water stress, which suggests that root:shoot ratios are also genetically controlled.

GU and GC clones are known to maintain different leaf areas and possibly different rooting patterns (Drew *et al*., 2009). The current study produced similar findings and GC was shown to consistently maintain significantly less total leaf area than GU (Fig. 3.28). Specific leaf area (SLA) increased by double to the early adult growth stage (18 months) and GUW retained significantly more leaf area per dry mass than GUA and GC clones and this was reflected in biomass partitioning (Fig. 3.26). The GC clone allocates at least 50% of total biomass to roots, and during field trials it was found that GC trees were not prone to uprooting in high winds, whereas GU clones were (Drew *et al*., 2009). Total leaf area was significantly reduced by 18 months growth only in the acute treatment (Fig. 3.28). Acute water stress reduced total leaf area by 30% across all three clones. Total water loss from leaves (experiencing water stress) can be decreased by reducing the total evaporative surface area of leaves. Leaf senescence usually follows severe drought stress cycles and future leaf growth is then also decreased (Sands and Mulligan, 1990). A reduction in total leaf area can conserve soil water but the total growth achieved is then also reduced. If total leaf area is reduced in the long term, a reduction in biomass accumulation will ultimately be observed. Although the difference was not significant, there was a reduction in total biomass of the trees exposed to the acute water stress treatments, compared with the control and chronic treatments. The same reduction of total leaf area was found between water treatments (control > chronic > acute). Long-term biomass reduction in water-stressed plants has been attributed to a reduction in total leaf area in *E. globulus* (Osorio *et al.*, 1998), *E. microtheca* (Li and Wang, 2003), *E. maculata* (Whitehead and Beadle, 2004) and *E. nitens x nitens* (Mokotedi, 2010).

Acute water stressed leaves had significantly greater SLA (more leaf area per unit dry mass), perhaps to maximise photosynthetic capacity while making use of a limited amount of dry matter (Table 3.4). Values for SLA are known to vary from 2 – 8 m^2 kg^{-1} in the field but can reach up to 15 m^2 kg^{-1} in controlled, potted studies (Whitehead and Beadle, 2004). These results are in contrast to that shown for native or plantation studies because SLA was less than half that of other *E. grandis* SLA values reported (Whitehead and Beadle, 2004). SLA is also usually lower at sites with lower water supply, and this was not the case in the current study (Table 3.4).

SLA is reported to be greatest in emerging eucalypt leaves and lower in mature leaves and following growth seasons (White *et al*., 2000). The current results for SLA in *Eucalyptus* GU and GC clones show an increase with tree age, in contrast to the response reported by White *et al*. (2000) and Whitehead and Beadle (2004).

The current study had as one of its objectives, the evaluation of the effects of water stress and clonal hybrid on hydraulic characteristics at juvenile and young adult stages. Hydraulic characteristics were measured using a high pressure flow meter (HPFM, Tyree, 1993) which uses positive pressure to refill any embolised xylem in the hydraulic system. The HPFM measures only maximum K_h and so cannot be used to assess ψ-related changes in hydraulic conductivity (Sperry *et al*., 2002). Previous research on hydraulic conductance of *Eucalyptus* clones has shown that hydraulic conductance (normalised by leaf area) did not differ (Mokotedi, 2006). Clones grown at sites differing in water availability showed that higher absolute K_s values were found at mesic sites, compared with xeric sites (vander Willigen and Pammenter, 1998). The results of the present study expressed hydraulic conductance or resistance normalised by leaf area. The GC clone had the highest hydraulic efficiency (K_h, normalised by leaf area) at the juvenile growth stage, whereas GUW had the greatest K_h after 18 months growth (Fig. 3.30). GUA leaves sustain significantly more leaf area than GUW and GC, and had the lowest hydraulic efficiency. These results imply then that GUA trees conduct less water per unit leaf area than other eucalypt clones.

There were no significant differences in K_h in response to water stress treatment, but the chronic treatment was found to conduct more water per unit leaf area than the control and acute treatment. The control treatment had the lowest (but not significantly lower) hydraulic efficiency, presumably due to the higher leaf area maintained by the control treatment trees. The trees exposed to the chronic water treatment showed a level of phenotypic plasticity by responding to mild long-term water stress by reducing leaf area sufficiently while ensuring a greater hydraulic supply of water to the remaining leaves. Manoharan (2002) found similar results, reporting that K_h for three eucalypt clones was higher in plants in the low water treatment.

Lower leaf area was also developed as a result of water stress in this study, and this may have increased the hydraulic conductance of the shoots relative to the well watered plants which had a greater leaf area (Manoharan, 2002). Increased hydraulic efficiency of shoots and roots (when normalised by leaf area) under water stress can be considered a drought avoidance strategy (Nardini and Tyree, 1999). Although Sperry and Sullivan (1992) showed that reductions in K_h in response to drought were attributed to xylem hydraulic conductance, the current study does not show any clear hydraulic pattern in roots, stems and leaves of eucalypts in response to drought stress (Table 3.4).

The proportion of total hydraulic resistance residing in the roots was greatest in the GUW clone after nine months growth (Fig. 3.31). At the nine month growth stage, more than 50% of the hydraulic resistance (R_h) was located in the roots, regardless of clone or water treatment. In three month old *Eucalyptus* clones, between 59-81% of the total R_h was located in the roots (Manoharan, 2002), and these results are in agreement with the present study. High root resistances have been reported for two different *Eucalyptus grandis* x *urophylla* hybrids, suggesting that high root resistances may be a genetic characteristic of *Eucalyptus* plants (Manoharan, 2002). The pattern of proportional allocation of resistances was similar to that of the proportional allocation of biomass to roots, stems and leaves (Fig. 3.27). The implication of high root resistance is that less water will be conducted but whether high root resistance reduces embolism potential is yet to be established.

There were no significant differences in allocation of resistances to plant parts between clones or water treatment. There was however a significant difference in R_h with tree age (Fig. 3.30). There appeared to be a complete reversal of the majority of total R_h between roots and leaves. Hydraulic resistance of leaves at 18 months represented more than 50% of the total hydraulic resistance. Above-ground biomass after 18 months tree growth, contributed up to 85% of R_h. K_h increases by up to three times with a 12 month increase in tree age (Fig. 3.29). Increase in K_h with tree age could be explained by increase in biomass that causes an increase in the number of xylem pathways within the tree.

Van der Willigen and Pammenter (1998) concluded that hydraulic characteristics measured in closely related eucalypt clones were influenced primarily by water availability and secondarily by genetics. The current study shows equal influence of both water availability and genetic make-up, and the hydraulic efficiency was ultimately determined by leaf area. Similar findings were found by Manoharan (2002) with four eucalypt clones, showing that the hydraulic efficiency of *Eucalyptus* clones is driven by changes in leaf area, which differed significantly with both water treatment and clone in this study.

Total biomass shows concurrent increase with K_h, implying again that as eucalypt trees increase in size, the number of hydraulic pathways increase thus causing an increase in K_h (Fig. 3.33). The relationship was inversely confirmed because R_h decreased proportionately with total biomass (Fig. 3.35). Contrary to the results found by vander Willigen and Pammenter (1998) for *Eucalyptus* species, growth efficiency (GE) was negatively related to K_h (Fig. 3.36). Due to the highly variable nature of leaf area in response to water treatment, expressing GE as a function of leaf area meant that GE was greater in most of the acute stress treatments.

In response to water stress K_h of the clones grown in this study, was positively correlated with A_n and g_s at both nine and 18 months tree growth (Fig. 3.37-40). Co-ordination of hydraulic characteristics and gaseous exchange properties has been reported for a number of species e.g. Scots pine (Mencuccini and Grace, 1996) and *Callitris* species (Brodribb *et al.*, 2010). Tyree and Sperry (1998) emphasize the strict regulation between g_s and hydraulic characteristics of the xylem flow pathway. Transpiration and mean g_s are determined by the properties of the hydraulic pathway, and A_n is determined by mean maximum g_s (Whitehead, 1998). Close correlation between K_h, A_n, and g_s of eucalypt trees over an 18 month growth period show the intricate balance of physiological processes to attain tree growth over a wide range of water availabilities.

Conclusions

Eucalypt tree physiology in the current study could be considered to be governed by genetics, environmental variables (e.g. water stress) and / or ontogenetics (tree or leaf age). *Eucalyptus grandis* x *camaldulensis* achieved significantly greater tree heights than *E. grandis* x *urophylla* clones. Tree height appeared to be controlled primarily by clone genetics. In terms of plant water relations, GC clones maintained consistently higher E_n and g_s rates over all three seasons across the 18 month growing period. The GUW clone however, attained moderately improved instantaneous WUE, implying that GUW is more efficient at assimilating carbon per unit of water lost. Root:shoot and SLA were controlled by genetics, and were independent of water stress. The GC clone allocated the majority of their biomass to roots, whereas GUA this was to stems. In terms of wood productivity, GUA clones out-compete that of the GC and GUW clones. GC however would have greater ability to withstand water stress in the long term (due to high root biomass) whereas GUW uses water more efficiently while accumulating carbon biomass.

While tree height was considered to be genetically controlled, tree diameter was significantly affected by water stress i.e. watering regime. The GUW clone reached the greatest stem diameters but this was not reflected in terms of wood volume. Wood volume takes tree height (genetically 'governed') and tree diameter (environmentally 'governed') into account. GC clones maintained moderately greater tree volume because of significantly greater tree height.

J_{max} was significantly affected by watering regime, but only at the wilting point of eucalypt leaves. In terms of plant water relations and actual photosynthetic values, the trees subjected to the chronic treatment had improved instantaneous WUE in comparison with the control and acute treatments. *Eucalyptus* clones show some degree of phenotypic plasticity because the amount of carbon that is assimilated per unit water transpired changes in response to water stress. Total biomass was not significantly affected by water stress. The acute water stress treatment achieved the lowest total biomass.

Although water stress significantly and negatively affected J_{max} only at leaf wilting point, the duration of wilting over multiple water stress cycles was sufficient to reduce total biomass. Had J_{max} been significantly less during the recovery period (which it was not) total biomass would have been more significantly reduced.

Physiologically, *Eucalyptus* clones were affected by ontogenetic changes i.e. leaf or tree age. J_{max} and V_{cmax} decreased with leaf age and the 'down-regulation' of photosynthetic parameters was not due to constraints imposed by stomatal conductance. Photosynthetic down-regulation was explained by non-stomatal limitations enforced by less efficient biochemical pathways of the photosynthetic cycle i.e carboxylation efficiency of Rubisco (V_{cmax}).

As tree age increased with concurrent increase in biomass, K_h showed a corresponding increase in all three clones across all water treatments. The correlation of biomass and K_h was understood to be caused by an increase in the number of hydraulic (increasing K_h) associated with increase in biomass.

Perhaps the most important morphological and physiological parameter identified in the current study was that of leaf area. Leaf area was different between clones (GU > GC), therefore a genetic constraint was inherent in total leaf area. Leaf area was negatively and significantly affected by reduction in watering regime, thereby imposing an environmental constraint in terms of leaf area. With tree age there was also a change in leaf area. SLA in particular was significantly greater with an increase in tree age. Leaf area affects the expression of growth efficiency, hydraulic efficiency, total carbon assimilation and total biomass achieved. For GU and GC *Eucalyptus* clones in the current study, the primary parameter driving physiological interactions and ultimately determining wood productivity could be considered to be leaf area.

4. Leaf Characteristics

During the investigation of physiological and morphological characteristics of three *Eucalyptus* clones in response to water stress, a number of leaf characteristics were concurrently measured, all of which were destructive to individual leaves. Following the first harvest, after nine months growth, it was noted that a considerable portion of the above-ground resistance to water flow was located in the leaves. While measuring R_{leaves}, of a whole tree using the high pressure flow meter, it was observed that most of the leaves would "fill up" and liquid water would be expressed through the stomata. There were however, particular leaves that would still remain "unfilled" with water even under conditions measuring maximum K_h. Another aspect that was noted was that during acute drought stress and subsequent drought stress recovery, leaves of GUA clones were observed to dry out completely and die, whereas GUW and GC leaves showed some degree of drought stress tolerance and recovery. This prompted further investigation into the measurement of the resistance to water flow of individual leaves. Measurements were performed when leaves were completely water stressed and when recovering from water stress over a period of seven days, and then compared with the control (Fig. 4.1). Gaseous exchange measurements (actual maximum photosynthetic rate and stomatal conductance) were also measured on the same water-stressed trees, in order to correlate R_{leaf} with A_{max} and g_s.

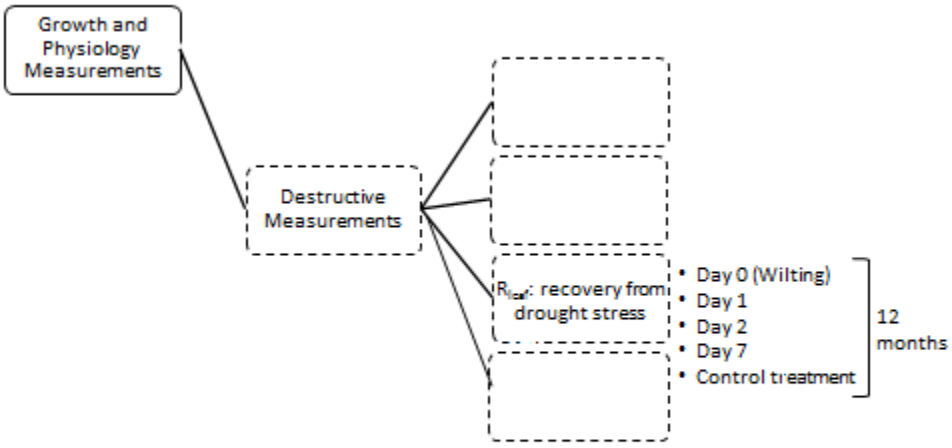


Figure 4.1: Growth and physiology measurements indicating the leaf hydraulic characteristics measurements pertaining to recovery from drought stress after 12 months growth.

4.1. Leaf Hydraulic Characteristics

Hydraulic resistance was measured in individual leaves and expressed normalized by leaf area (R_{leaf}). R_{leaf} was not significantly different between *Eucalyptus* clones (Fig. 4.2 (a); p = 0.313). Figure 4.2 (b) shows that R_{leaf} was significantly greater in the acute stress treatment, by 40%, compared with the control and chronic treatment (p < 0.0001). There was also a significant interaction between water treatment and clone (Fig. 4.3; p< 0.0001). In all three *Eucalyptus* clones, the control treatment had the lowest R_{leaf}. The acute stress treatment generated significantly greater R_{leaf} in GUA and GC clones (Fig. 4.3). The GUW clone had the least variable R_{leaf} among water treatments, and R_{leaf} did not differ by more than 15% between the treatments.

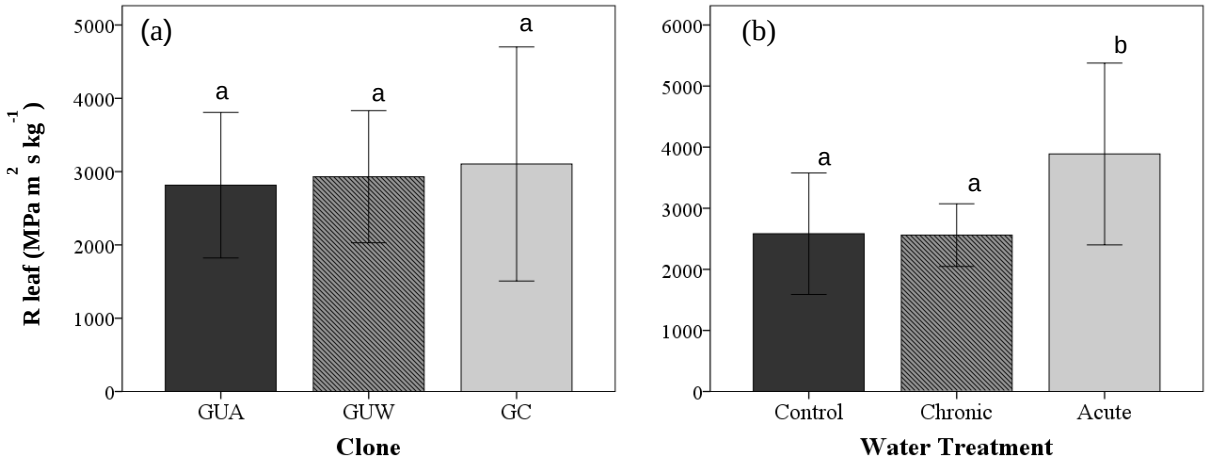


Figure 4.2: Leaf hydraulic resistance (R_{leaf}) of *Eucalyptus* (a) clonal hybrids (p = 0.313) (b) in response to water treatment (p < 0.0001); (Different letters denote statistical significance between clones or treatments).

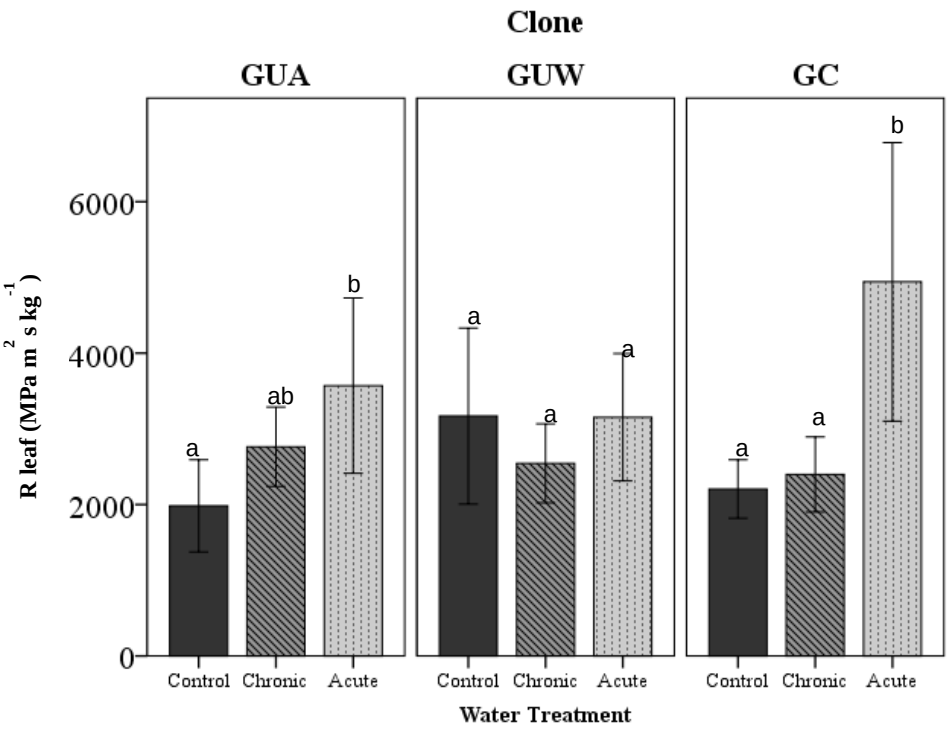


Figure 4.3: Leaf hydraulic resistance of *Eucalyptus* clones in response to water stress (Clone*Water treatment interaction: $p < 0.0001$); (Different letters denote statistical significance between clones or treatments).

Leaf hydraulic resistance was also measured in response to drought stress and subsequent water stress recovery. GUA had significantly higher R_{leaf} (by 100%) after day 2 of water stress recovery compared with the control and chronic treatment (Fig. 4.4 (a)). R_{leaf} was significantly greater (by 75%) after one day recovery from water stress in the GUW clone (Fig. 4.4 (b)). The GC clone had significantly higher R_{leaf} only on the day which leaves were wilting (at the end of the water stress cycle, before re-watering) (Fig. 4.4 (c)). GUA leaves recover in terms of hydraulic resistance, by day 7 of re-watering, in comparison with the control. GUW leaves recover hydraulically by day 2 and GC leaves recover from acute drought stress by day 1 of re-watering, when compared with the control treatment.

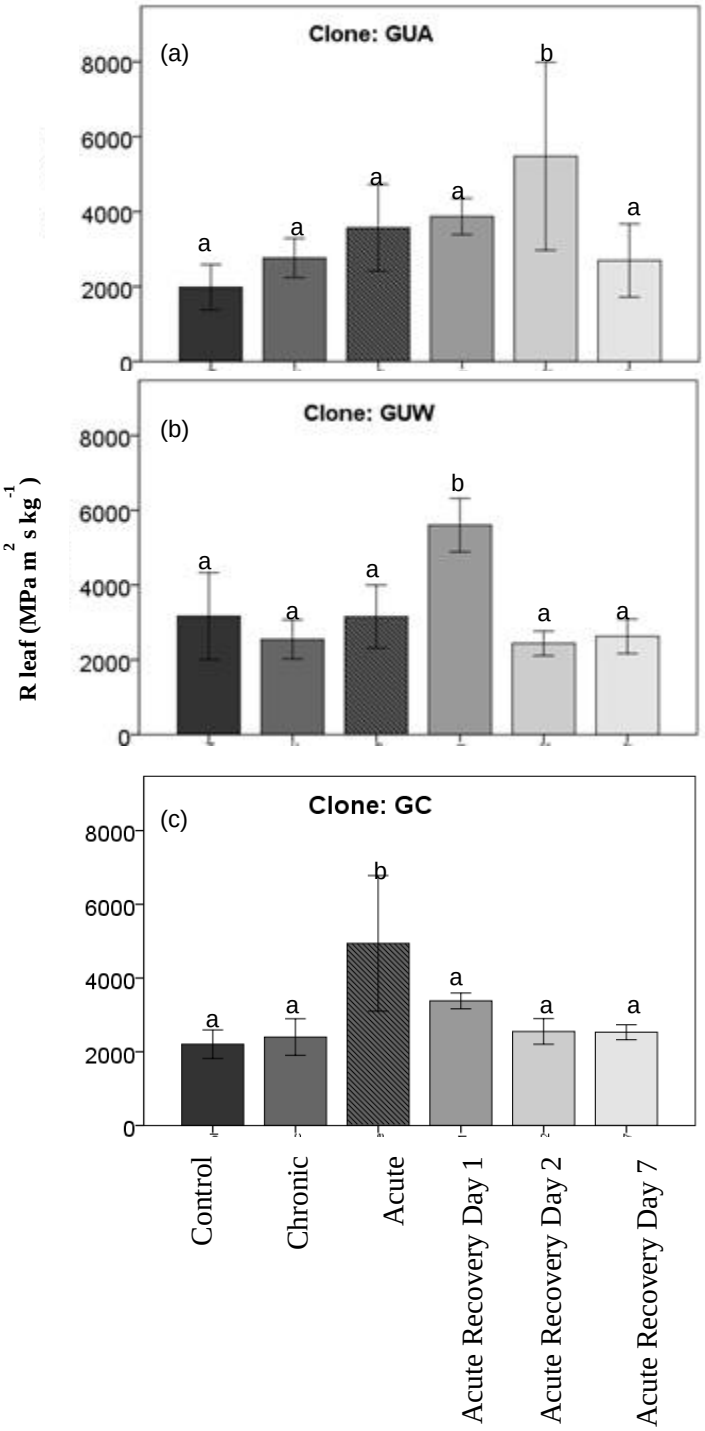


Figure 4.4: Mean leaf hydraulic resistance of *Eucalyptus* clonal hybrids ((a) GUA, (b) GUW and (c) GC) in response to water stress and subsequent water stress recovery.

R_{leaf} was expressed as a proportional contribution between the three leaf components that constitute an individual leaf: the petiole, venation and extravascular tissue. $R_{petiole}$, $R_{venation}$ and $R_{extravascular}$ were measured in all three water treatments and in the acute water stress recovery treatment at leaf wilting point (day 0) and day 1, 2 and 7 recovery after re-watering (Fig. 4.5). $R_{petiole}$ was approximately 500 MPa m^2 s kg^{-1} in GU clones and was 200 MPa m^2 s kg^{-1} in the GC clone (Fig. 4.5 (a) – (c)). $R_{petiole}$ was not significantly different between water treatments of all three clones ($p = 0.89$). $R_{petiole}$ was a minimum of 50% less than $R_{venation}$ and $R_{extravascular}$ across all treatments.

$R_{extravascular}$ was higher than $R_{venation}$ in all three clones for the control treatment. $R_{venation}$ and $R_{extravascular}$ in the chronic treatment did not differ significantly from the control for GU and GC clones. During acute drought stress recovery, the GC clone had a significantly higher $R_{venation}$ than $R_{extravascular}$ (Fig. 4.5 (c)). Although $R_{venation}$ decreased with drought stress recovery (from re-watering) the resistance observed was significantly different from the control.

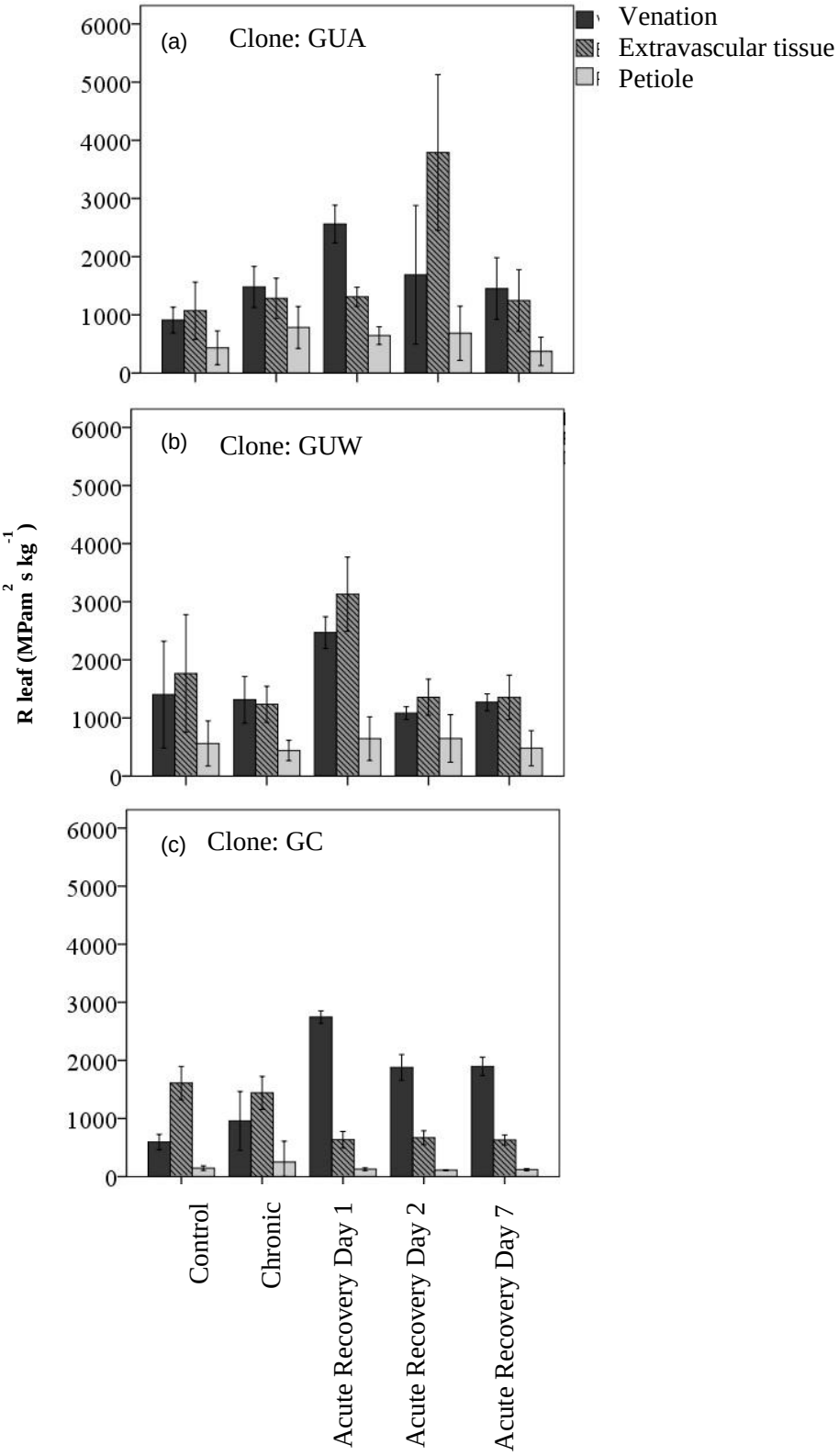


Figure 4.5: Leaf hydraulic resistance components (petiole, venation and extravascular tissue) of *Eucalyptus* clonal hybrids ((a) GUA, (b) GUW and (c) GC) in response to water stress and subsequent water stress recovery.

4.1.2 Leaf hydraulic characteristics and correlation with photosynthetic parameters

Actual photosynthetic rates were measured in leaves of the clones in response to water treatment. Measurements were performed on leaves of a similar age on days concurrent with R_{leaf} measurement. Two-way analysis of variance showed that mean assimilation rate was not significantly different among clones after 12 months growth (Fig. 4.6 (a); p = 0.366). Water treatment had a significant effect (p = 0.02) with Figure 4.6 (b) showing that the control had the highest assimilation rate compared with the chronic and acute treatment (p = 0.002). The chronic treatment was 30% lower than the control, and the acute treatment (at wilting) was 0 µmol CO_2 m^{-2} s^{-1}. Photosynthetic measurement of leaves at their wilting point had to be considered as a zero value because stomatal conductance was zero and this prompted inaccurate negative photosynthetic recording.

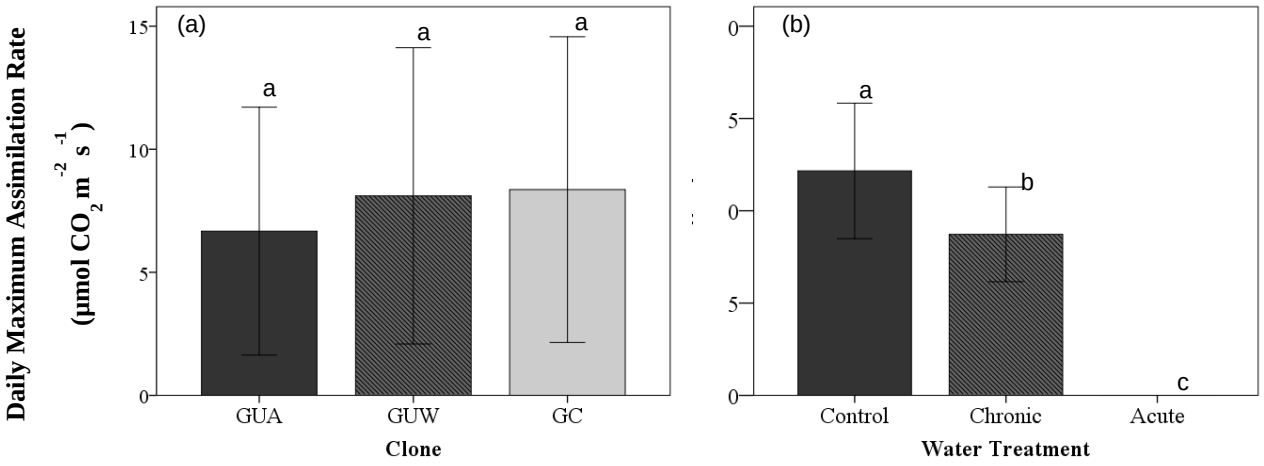


Figure 4.6: Mean maximum assimilation rate of *Eucalyptus* (a) clonal hybrids (p = 0.366) (b) in response to drought stress (p = 0.002) when measured concurrently with R_{leaf}; (Different letters denote statistical significance between clones or treatments).

Stomatal conductance (g_s) was also not significantly different among clones (Fig. 4.7 (a); p = 0.871). g_s in the chronic treatment was significantly reduced (60% less) compared with the control, and the acute treatment was considered to be zero (Fig. 4.7 (b); p = 0.002).

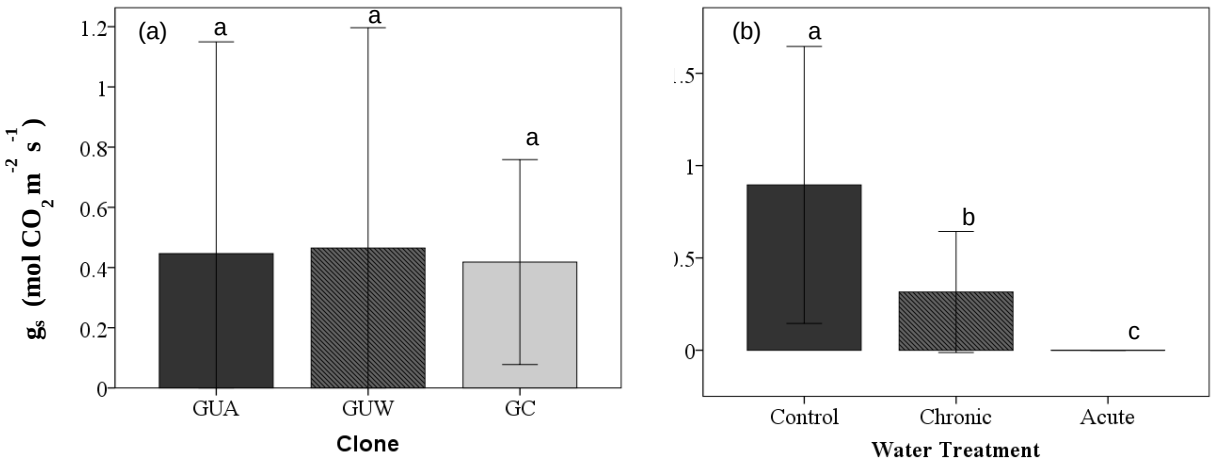


Figure 4.7: Mean maximum stomatal conductance of *Eucalyptus* (a) clonal hybrids (p = 0.871) in response to (b) drought stress (p = 0.002) when measured simultaneously with R_{leaf}; (Different letters denote statistical significance between clones or treatments).

Mean assimilation rate (A_n) was measured in the control, chronic and acute stress recovery treatments on day 1, 2 and 7 in all three clones. Figure 4.8 (a) shows that A_n was significantly higher in the control treatment compared with the water stress treatments (mean = 11.7 ± 1.4; $p < 0.0001$). A_n was significantly reduced after acute stress and was 85% and 76% lower after day 1 and 2 of re-watering, respectively (Fig. 4.8 (a)). On day 7, A_n was not significantly different from the control but was still 21% lower. GUW showed a similar assimilation rate pattern to that of GUA (Fig. 4.8 (b)). A_n in the control of GUW was significantly higher than the other water stress treatments (mean = 13.7 ± 1.4; $p < 0.0001$). On day 7 of re-watering however, A_n was significantly lower than the control (mean = 7.6 ± 1.4; 45 % less). Figure 4.8 (c) shows that GC leaves had a significantly lower A_n in the acute stress treatment compared with the control after one day of re-watering (mean = 5.20 ± 1.4; 59% less than the control). GC showed the greatest capacity to recover photosynthetically from water stress, but under control conditions, GUW had the greatest A_n.

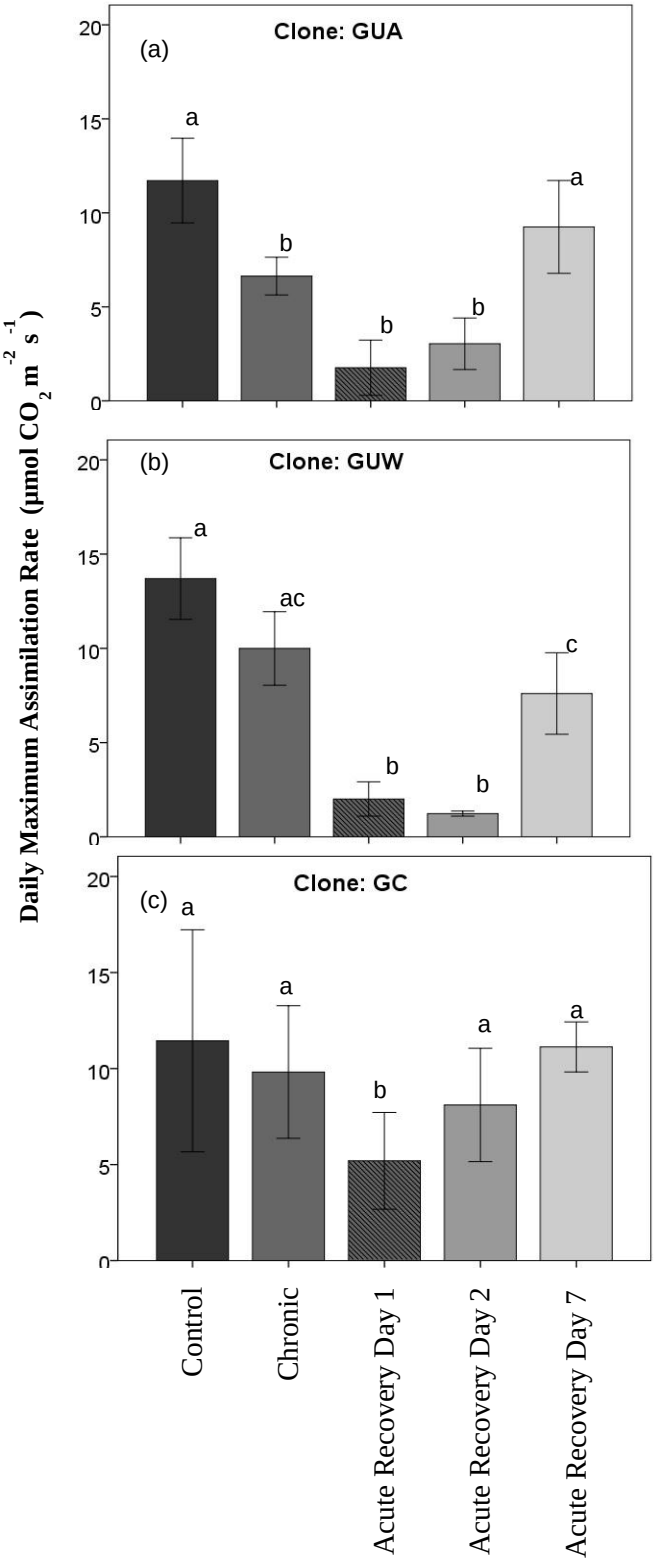


Figure 4.8: Mean maximum assimilation rates of *Eucalyptus* clonal hybrids (a) GUA, (b) GUW and (c) GC in response to water stress and subsequent water stress recovery.

Stomatal conductance (g_s) was significantly higher in the control treatment of all three *Eucalyptus* clones (mean GUA = 0.60 ± 0.27; GUW = 0.51 ± 0.23; GC = 0.49 ± 0.03 mol CO_2 m^{-2} s^{-1}; $p < 0.01$ in all three clones; Fig. 4.9 (a) – (c)). Fig 4.9 (a) shows that g_s of GUA leaves was significantly reduced in the chronic and acute day 1 and 2 recovery treatments compared with the control. The chronic treatment was 78% lower (mean = 0.13 ± 0.06) whereas after day 1 and 2 of re-watering in the acute treatment g_s was 98% and 87% lower, respectively (mean = 0.01 ± 0.01; 0.08 ± 0.03). g_s was reduced after seven days of re-watering in the acute treatment but was not significantly different from the control (37% less; $p = 0.338$).

GUW leaves showed a reduction in g_s in the acute treatment compared with the control, which was comparable with the GUA clone (Fig. 4.9 (b); $p = 0.016$). Stomatal conductance was 98% and 94% lower after day 1 and 2 of re-watering in the acute treatment, compared with the control. As observed in GUA, g_s in GUW was not significantly different after seven days of re-watering in the acute treatment, compared with the control, but was 47% lower (mean = 0.27 ± 0.21; $p = 0.436$).

Leaves of the GC clone showed the same g_s in the control, chronic and day 7 of re-watering in the acute recovery treatments (mean = 0.49 ± 0.03; 0.48 ± 0.03; 0.49 ± 0.15, respectively; $p = 1.00$; Fig.4.9 (c)). Stomatal conductance was significantly reduced in the acute treatment after day 1 and 2 of re-watering (mean = 0.06 ± 0.04 and 0.14 ±0.09; 88% and 71% lower than the control; $p = 0.001$). GC leaves showed that the least variability of g_s in response to water stress treatment when compared with the GUA and GUW clones and also had the highest values under stress conditions.

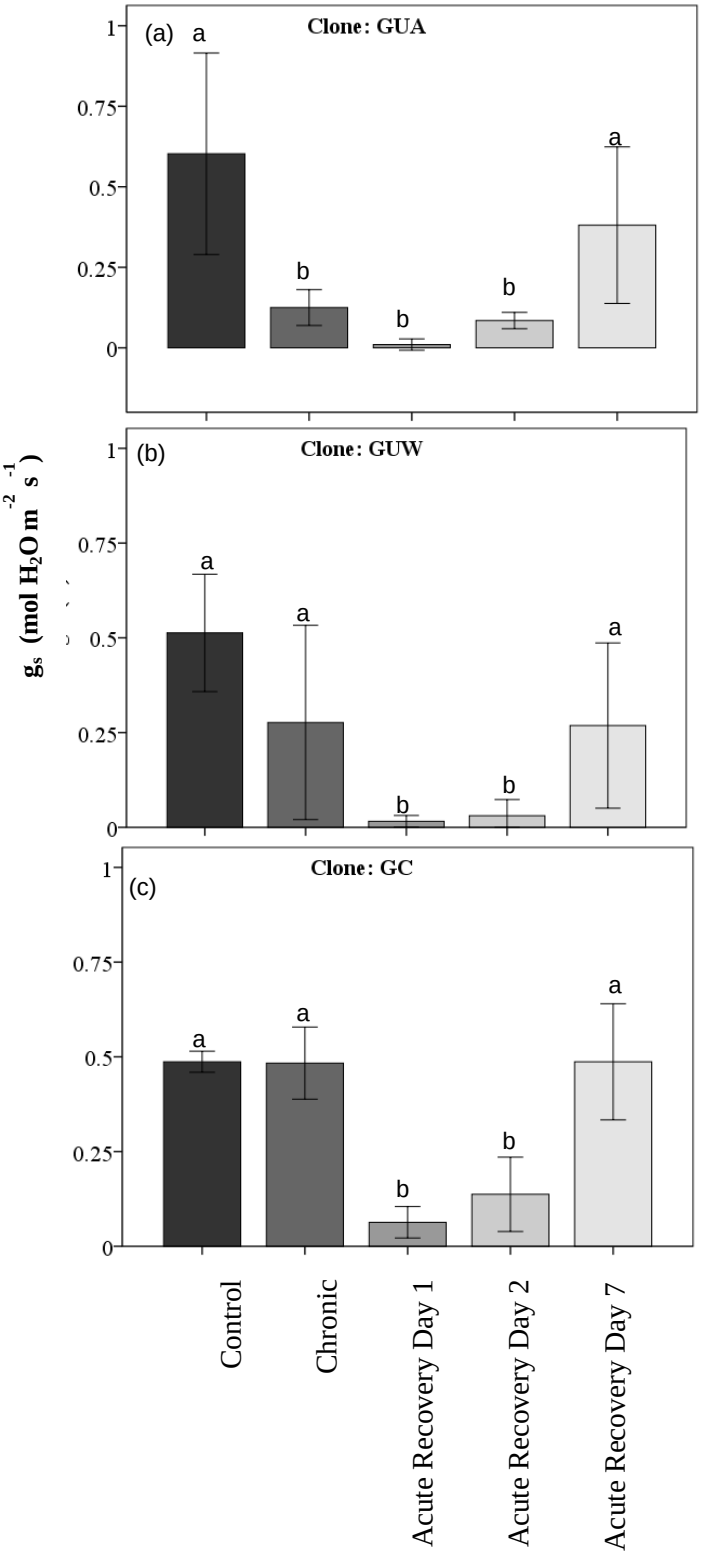


Figure 4.9: Mean maximum stomatal conductance of *Eucalyptus* clonal hybrids (a) GUA, (b) GUW and (c) GC in response to water stress and subsequent water stress recovery.

4.1.3 Leaf hydraulic resistance and photosynthetic correlation

R_{leaf} was negatively and significantly correlated with both A_n and g_s when expressed in terms of clone (p = 0.001 and 0.026; Figure 4.10 and 4.11, respectively). R_{leaf} was not expressed in terms of water treatment because it did not show a significant relationship. If the inverse of R_{leaf} was expressed i.e. K_{leaf}, the relationship with A_n and g_s was also found to be positively and significantly correlated.

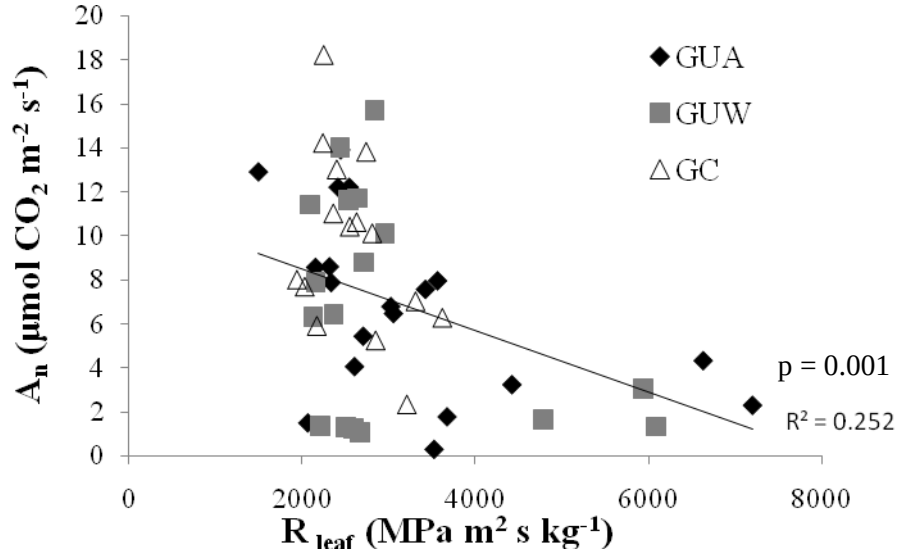


Figure 4.10: Correlation between R_{leaf} and A_n, expressed in terms of eucalypt clone.

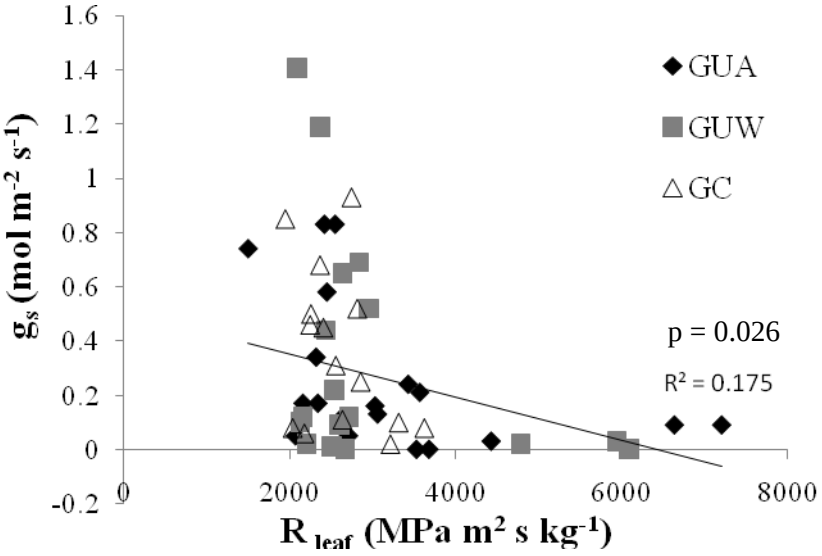


Figure 4.11: Correlation between R_{leaf} and g_s, expressed in terms of eucalypt clone.

4.2 Stomatal Characteristics

Before the second harvest was completed, after 18 months growth, it was suggested that further attention be paid to leaf anatomical characteristics including stomatal characteristics. Stomatal size and density were examined in order to assess why the water stress recovery response differed among the clones. On completion of the stomatal characteristics study, an article by Pinheiro *et al.* (2005) stimulated investigation of $\delta^{13}C$ and $\delta^{15}N$ of *Eucalyptus* leaves (Fig. 4.12). The ratio of A_n:E_n is a measure of instantaneous WUE, which may not be translated into long-term WUE. A more positive $\delta^{13}C$ would imply that leaves that were exposed to drought stress over a long period of time would have improved long-term water use efficiency (WUE). Long-term WUE measured by $\delta^{13}C$ would be important information for breeders, particularly if one clone shows improvement in long-term WUE.

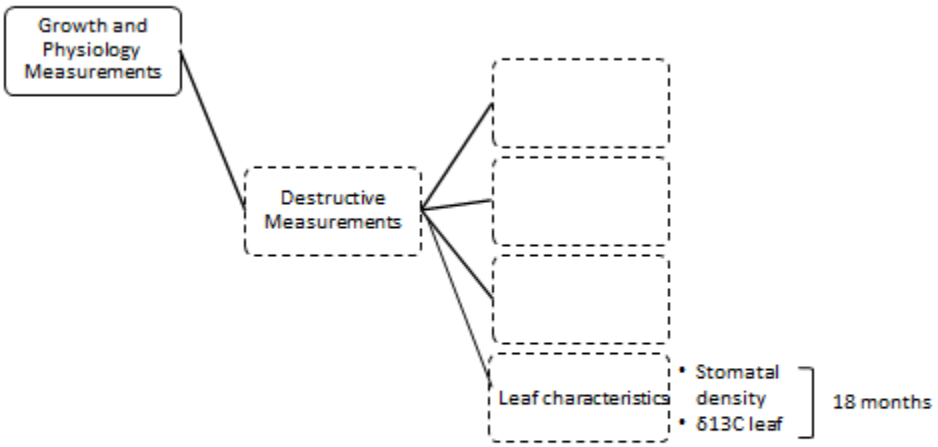


Figure 4.12: Growth and physiology measurements showing leaf characteristics measurements pertaining to recovery from drought stress after 18 months growth.

Stomatal density (per mm^2) was measured on the upper (adaxial) and lower (abaxial) leaf surfaces to assess for clonal differences and differences in response to water treatment. Stomatal density on the lower leaf surface was similar across all three *Eucalyptus* clones (mean GUA = 60 ± 13; GUW = 67 ± 7; GC = 66 ± 8 mm^{-2}; $p = 0.116$; Fig. 4.13 (a)). GUA leaves had no stomata on the upper surface (mean = 0; Fig. 4.13 (a)). Stomatal density on the upper surface of GUA leaves was therefore significantly less than GUW and GC leaves (mean = 18 ± 3 and 25 ± 4, respectively; $p < 0.0001$). The upper surface of GUW leaves also had significantly fewer stomata than GC leaves ($p < 0.0001$). GU clones therefore exhibit significantly lower stomatal densities on the upper leaf surface than GC *Eucalyptu*s clones.

Figure 4.13 (b) shows that stomatal density on the lower leaf surface was significantly higher in the acute treatment (mean = 68 ± 20; $p = 0.037$). The control and chronic treatments had 19% and 8% less stomata on the lower leaf surfaces, respectively (mean = 61 ± 17; and 63 ± 9; Fig 4.13 (b)). There were no differences between stomatal densities on the upper leaf surfaces in response to water treatment ($p = 0.195$; Fig. 4.13 (b)).

There was a significant interaction between clone and water treatment with regards to stomatal density ($p < 0.0001$; Fig. 4.14). The lower leaf surface had a significantly higher number of stomata across all treatments. In all three water treatments, there were no stomata on the upper surface of GUA leaves. There was no clear pattern in terms of stomatal density on the lower leaf surface when looking across water treatments in each *Eucalyptus* clone. These measurements were performed during the second harvest, after 18 months growth on leaves of a similar age. Unfortunately, the idea to assess this parameter arose only just before the second harvest. It was therefore not possible to track stomatal development on growing leaves or check for differences between leaves on juvenile and adult *Eucalyptus* trees.

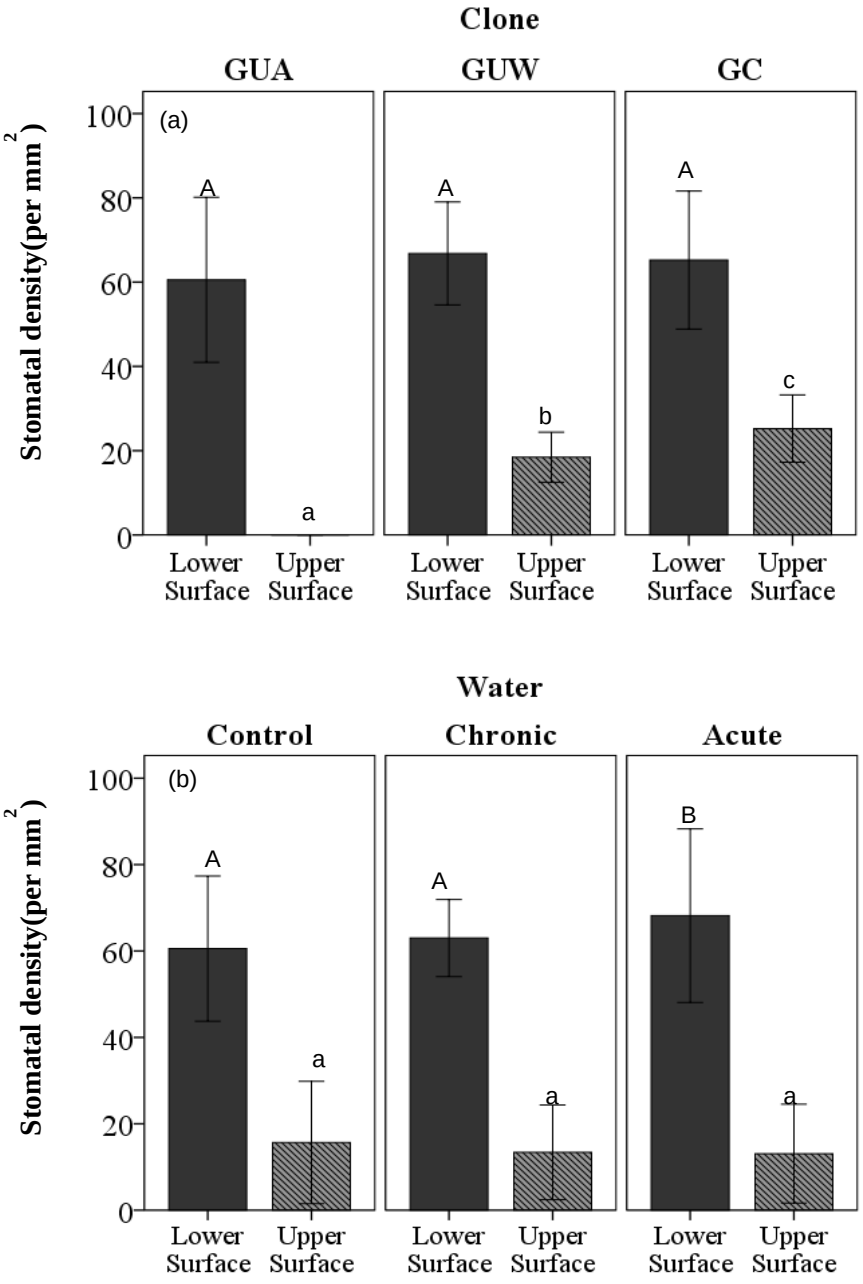


Figure 4.13: Stomatal density on the lower and upper surfaces of *Eucalyptus*
(a) clonal hybrids (p = 0.116 (lower surface) and p = 0.0001 (upper surface)) (b) in response to water stress (p = 0.037 (lower surface) and p = 0.195 (upper surface));
(Different letters denote statistical significance between clones or treatments).

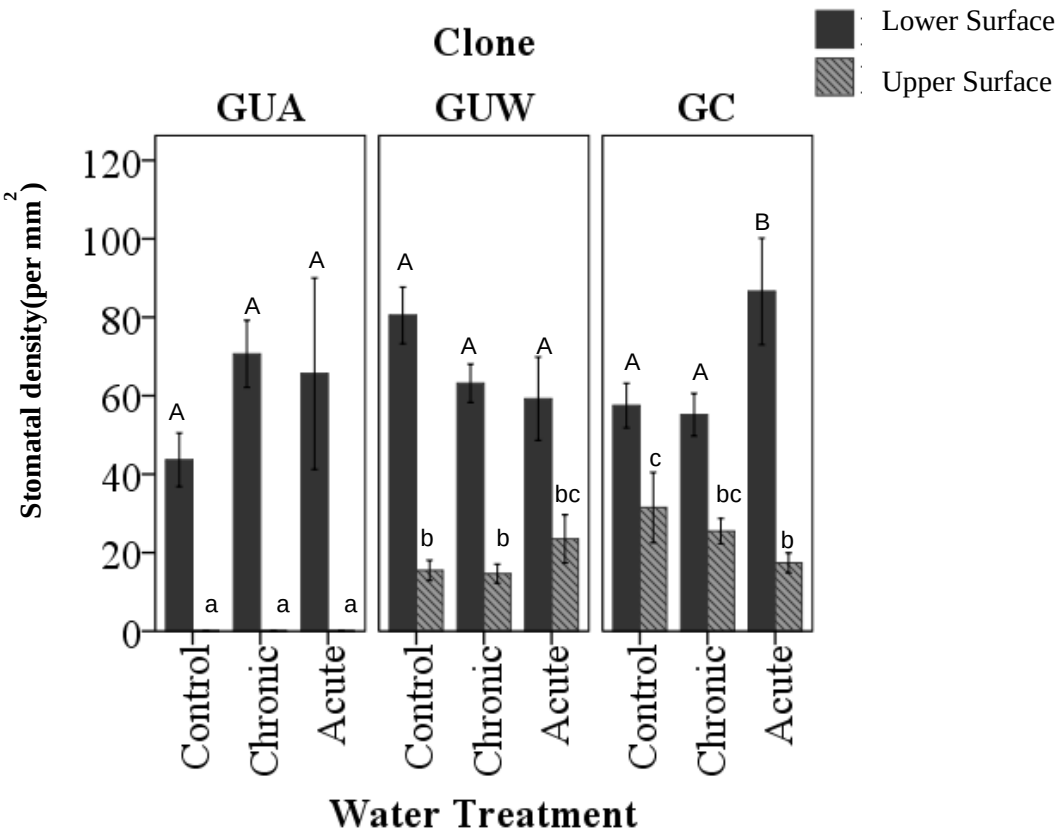


Figure 4.14: Stomatal density of *Eucalyptus* clones in response to water stress (Clone*Water treatment interaction: $p < 0.0001$).

Stomatal size (mean guard cell length) (mm) on the lower leaf surface did not differ among clones ($p = 0.104$; Fig. 4.15 (a)). There was no difference in stomatal size on the upper surface of GUW and GC leaves, but because GUA leaves did not have stomata, there was a significant difference in stomatal size ($p < 0.001$; Fig. 4.15 (a)). Figure 4.15 (b) shows that stomata are smaller on the upper surface of *Eucalyptus* leaves in all three water treatments (mean = 0.03 mm; $p = 0.845$). On the lower leaf surface, stomata are larger, but there was no difference in stomatal size between water treatments (mean = 0.05mm; Fig. 4.15 (b)).

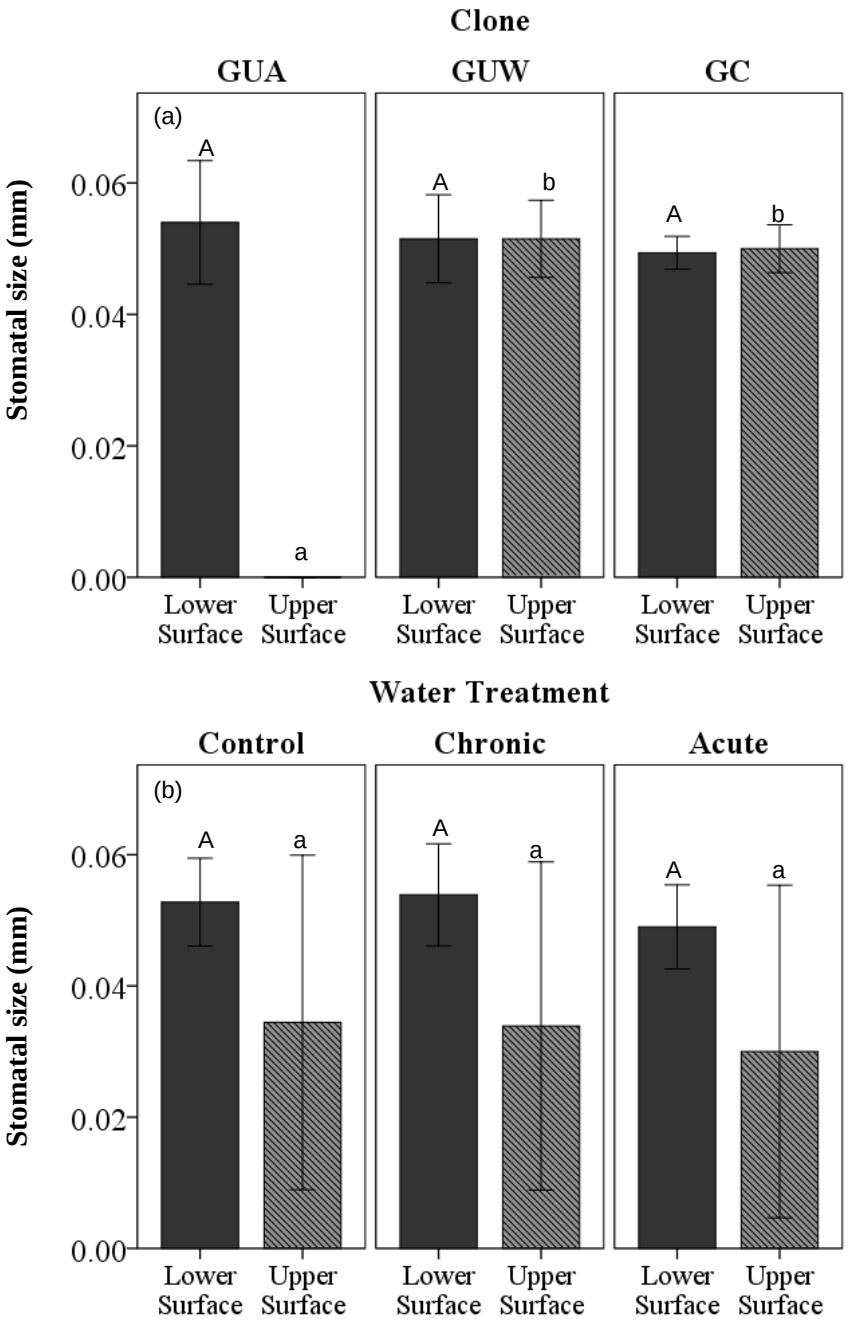
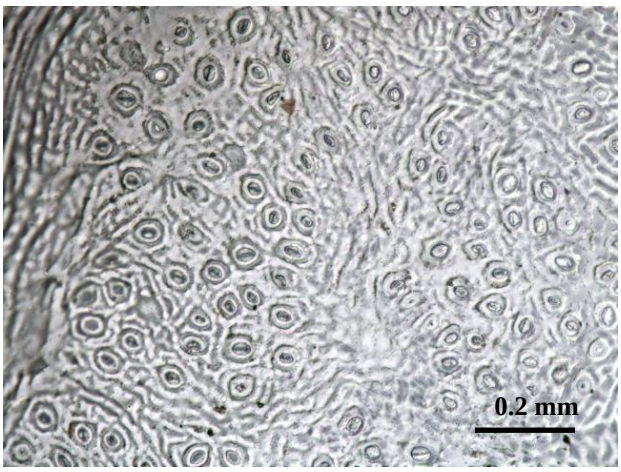
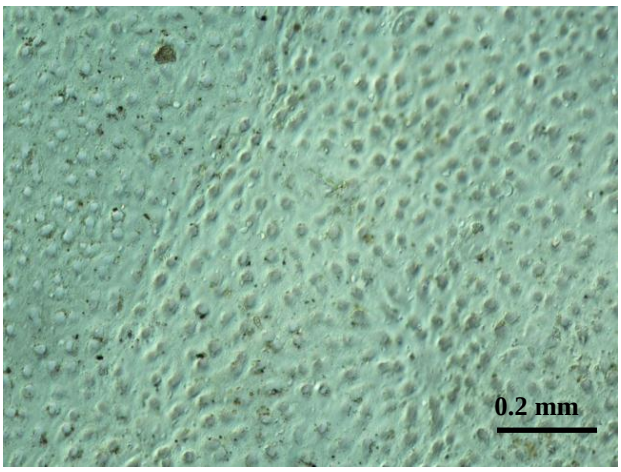


Figure 4.15: Stomatal size on the lower and upper surfaces of *Eucalyptus*
(a) clonal hybrids (p = 0.104 (lower surface) and p = 0.0001 (upper surface)) (b) in response to water stress (p = 0.102 (lower surface) and p = 0.845 (upper surface));
(Different letters denote statistical significance between clones or treatments).

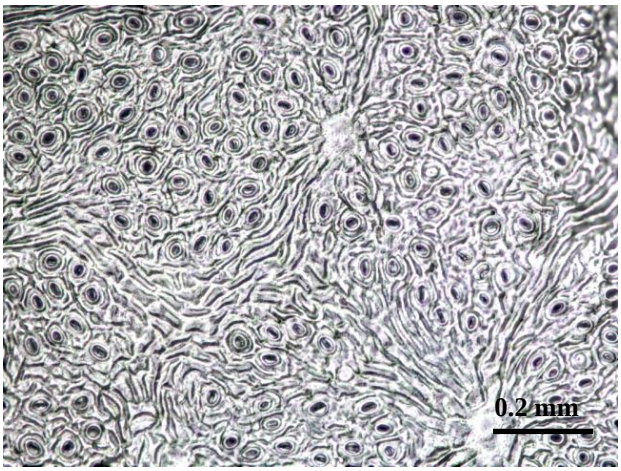
Figure 4.16 (a-f) show light microscope images of lower and upper leaf surfaces of the clones. Figure 4.16 (a) and (b) show the lower and upper of GUA leaves. There were no stomata present on the upper leaf surface (Fig. 4.16 (b)). On the upper surface of GUW and GC leaves, stomata were present, although they were smaller and less numerous than on the lower leaf surfaces (Fig. 4.16 (d) and (f)).



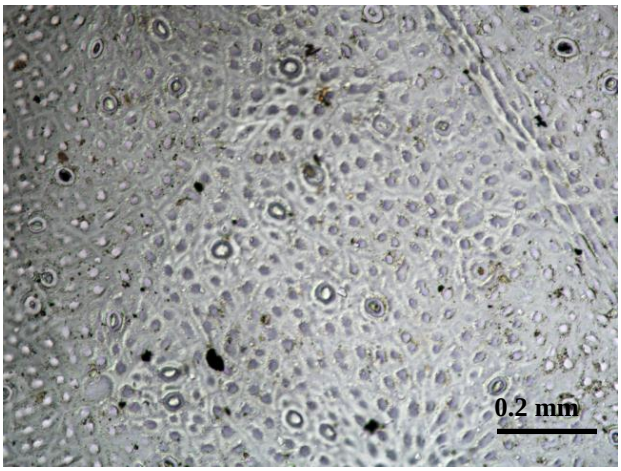
(a) GUA: Lower Surface



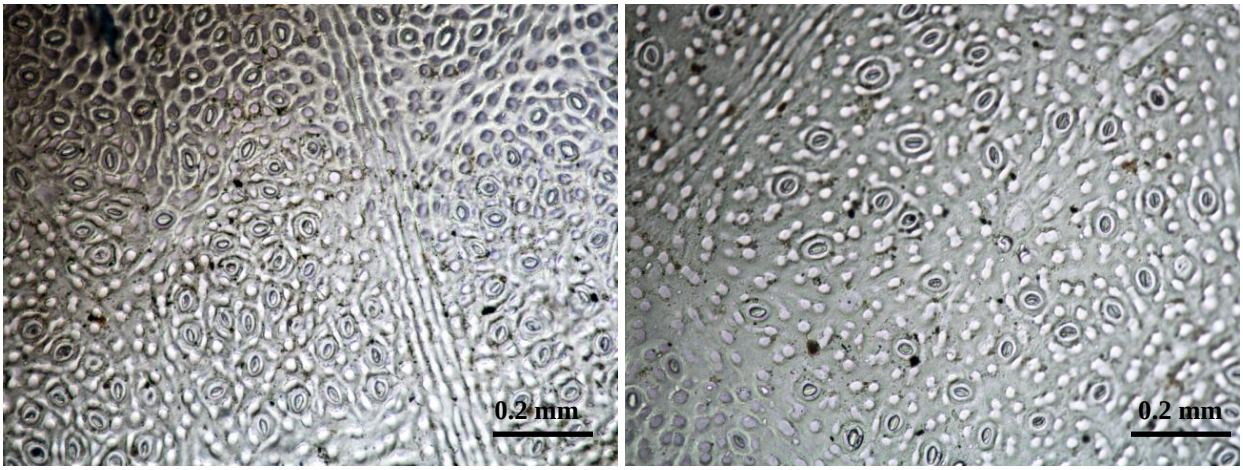
(b) GUA: Upper Surface



(c) GUW: Lower Surface



(d) GUW: Upper Surface



(e) GC: Lower Surface (f) GC: Upper Surface

Figure 4.16: Light microscope images of cellulose acetate replicas of lower and upper *Eucalyptus* leaf surfaces. (Note the absence of stomata on the upper surface of GUA leaves (b)).

4.3 Leaf δ ^{13}C measurements

$\delta^{13}C$ was not different between the clones ($p = 0.283$; Fig. 4.17 (a)). $\delta^{13}C$ was significantly less negative in the chronic treatment (mean = -27.34 ± 0.88; $p < 0.0001$; Fig. 4.17 (b)). There was no difference between $\delta^{13}C$ in the control and the acute treatments (-28.73 ± 0.85 and -28.78 ±0.75, respectively). A difference in $\delta^{13}C$ infers improved water use efficiency in the chronic treatment compared with the control and acute treatments.

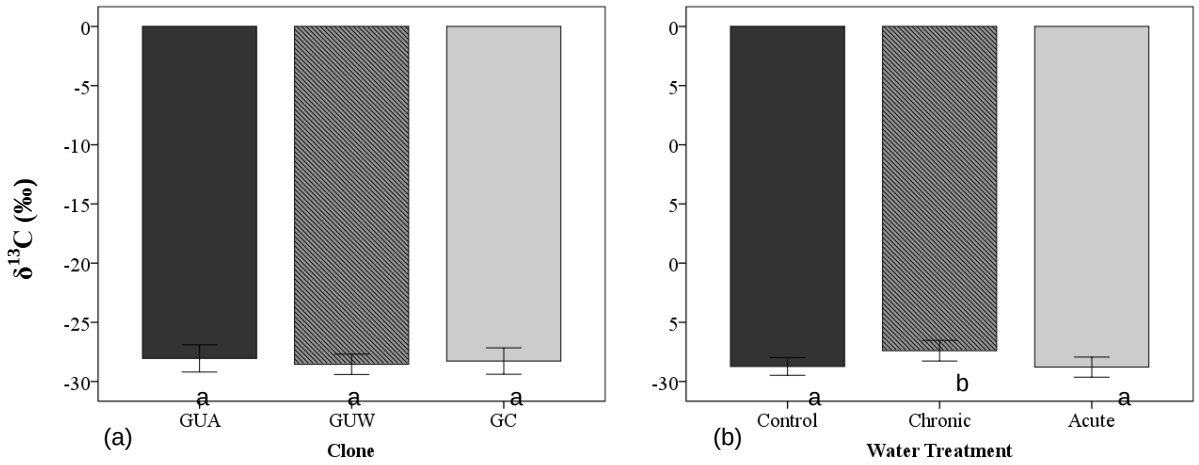


Figure 4.17: Mean $\delta^{13}C$ of *Eucalyptus* (a) clonal hybrids (p = 0.283) and (b) in response to water stress (p = 0.0001) (Different letters denote statistical significance between clones or treatments).

$\delta^{15}N$ was not different between *Eucalyptus* clones, although $\delta^{15}N$ in GC leaves was less negative than GUA and GUW (mean (GC) = -1.35 ±1.2; p = 0.54; Fig. 4.18 (a)). There was also no difference in $\delta^{15}N$ between water treatments (p = 0.55; Fig. 4.18 (b)).

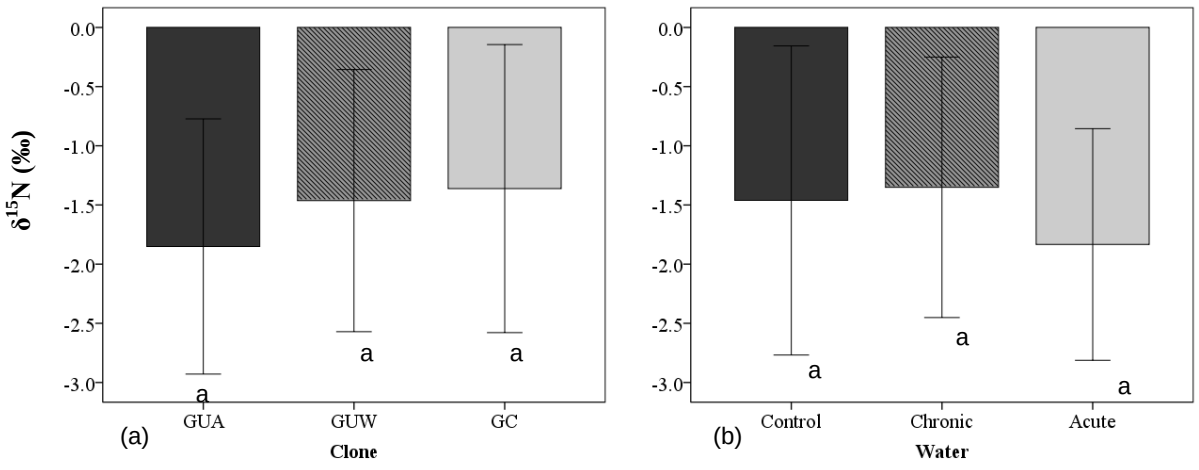


Figure 4.18: Mean $\delta^{15}N$ of *Eucalyptus* (a) clonal hybrids (p = 0.54) and (b) in response to drought stress (p = 0.55) (Different letters denote statistical significance between clones or treatments).

Figure 4.19 shows a significant, negative correlation between g_s and $\delta^{13}C$ (R^2 = 0.699; p = 0.025). As stomatal conductance increases, $\delta^{13}C$ becomes more negative. $\delta^{13}C$ was also correlated with K_l (whole-plant hydraulic conductance, normalized by leaf area), and the relationship was found to be significant and negative (R^2 = 0.212; p = 0.03; Fig. 4.20).

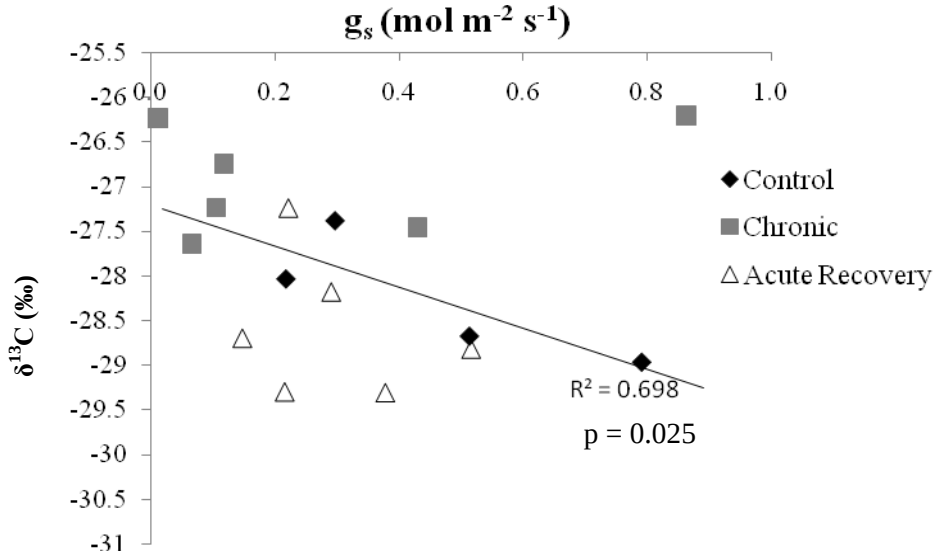


Figure 4.19: Correlation between g_s and $\delta^{13}C$ when expressed in terms of eucalypt water treatment.

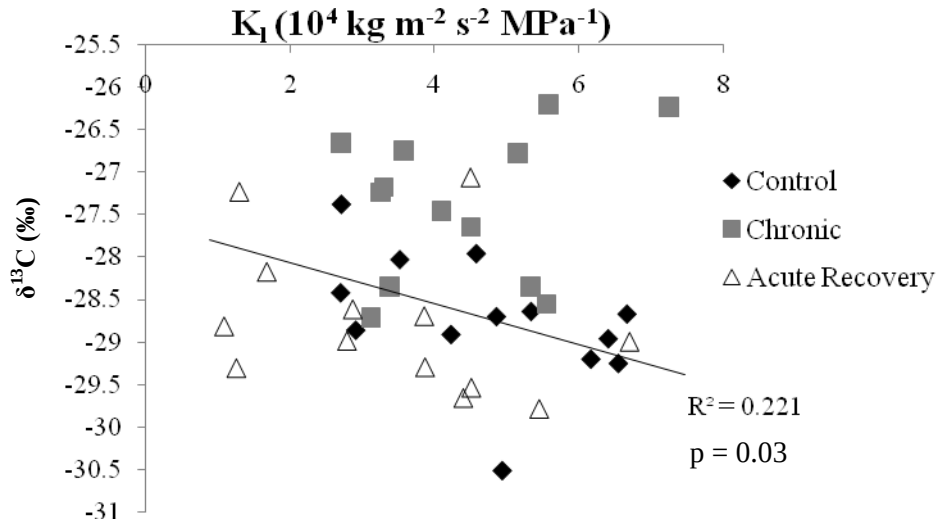


Figure 4.20: Correlation between K_l and $\delta^{13}C$ when expressed in terms of eucalypt water treatment.

4.4 Discussion

The original aim of the current leaf characteristics study was to assess leaf morphological and physiological response of hybrid clones when exposed to different levels of drought stress. Considering that up to 40% of the total hydraulic resistance of the GU and GC clones resided in the leaves, it was suggested that investigation of leaf characteristics (especially physiology) would be an important addition to the current study. Figure 4.21 shows a results summary diagram of the physiological and morphological parameters of leaves that may or may not be affected by eucalypt clone or water treatment.

Much recent research on leaves has focused on the biochemical and molecular processes in response to drought (Flexas *et al*., 2008). Leaf physiological responses to drought have now received more attention (Blackman *et al*., 2009; Brodribb and Cochard, 2009; Resco *et al*., 2009), particularly that of plant hydraulic characteristics. Plant hydraulic characteristics have been identified as a principle or primary governor of gas exchange characteristics during periods of water stress and water stress recovery. Water potential in response to water stress becomes more negative, increasing hydraulic tension, cavitation events and thus hydraulic resistance (Tyree and Sperry, 1989). Plants are capable of recovering from a loss in hydraulic conductivity but the recovery mechanisms employed by water stressed leaves are largely unknown (Bucci *et al*., 2003; Brodribb and Holbrook, 2004). R_{leaf} can contribute more than 30% of R_{total} (Sack and Holbrook, 2006) and R_{leaf} can constrain or reduce maximum g_s and photosynthetic capacity (Brodribb *et al*., 2005). As R_{leaf} increases there is known to be a corresponding decrease in g_s therefore hydraulic vulnerability has the potential to indicate plant response to water stress.

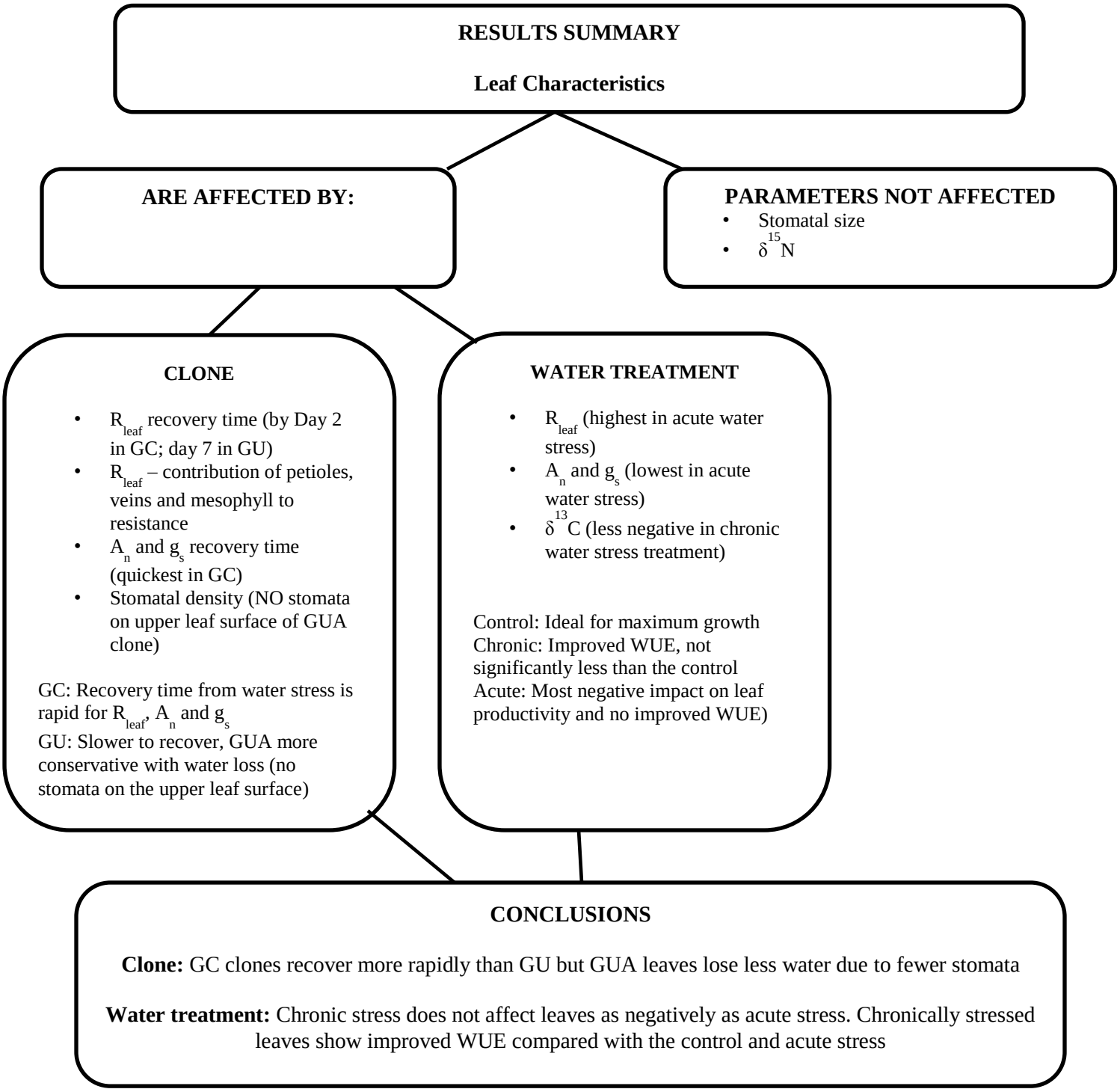


Figure 4.21: Summary of leaf characteristics that are affected or not affected by eucalypt clone or water treatment.

There was no difference between in R_{leaf} between eucalypt clones (Fig. 4.2). Leaf hydraulic resistance appears to be similar across clones of unstressed leaves. Water stress recovery was however different in each clone (Fig. 4.4). GC clones showed the quickest recovery (after re-watering in the acute treatment) as R_{leaf} on day 1 of recovery was not different from the R_{leaf} in the control treatment. This shows that GC leaves have a rapid recovery response, provided that the soil water deficit returns to unstressed conditions and trees are relieved of long-term water stress. GUW clones showed higher R_{leaf} values during initial stages of recovery and with recovery from water stress (in terms of R_{leaf}) within 2 days after re-watering (Fig. 4.4). GUA clones show a gradual build-up of R_{leaf} in response to water stress and have the longest recovery period of 7 days after re-watering.

When investigating the components that contribute to total R_{leaf} ($R_{petiole}$, $R_{venation}$ and $R_{extravascular}$ tissue), the resistance found in the petioles of all 3 clones was minimal i.e. less than 10% of R_{leaf} (Fig. 4.5). These results are agreement with those of Sack and Tyree (2005) who found 14% of R_{total} resided in the petioles of *Acer saccharum* and 5% of R_{total} resided in the petioles of *Quercus rubra*. $R_{petiole}$ did not change significantly in response to water stress and subsequent water stress recovery, suggesting that $R_{petiole}$ is influenced genetically and not environmentally, for these 3 eucalypt clones.

The majority of the "hydraulic bottlenecks" to water flow in leaves are considered to be in the xylem ($R_{venation}$) or in the mesophyll ($R_{extravascular}$). What is of particular importance is the fact that there is no agreement upon whether most of the leaf resistance to water flow is located in the xylem veins or in the mesophyll tissue. In sugar maple and red oak, more than 60% of total R_{leaf} was located in the xylem ($R_{venation}$) (Sack *et al*., 2004; Sack and Tyree, 2005; Sack and Holbrook, 2006). Experiments on *Coffea arabi*ca have yielded results concluding that 75% of the total R_{leaf} resided in the mesophyll tissue ($R_{extravascular}$) (Gasco *et al*., 2004). There are also different implications attached to whether leaf resistance is located primarily in the xylem or in the mesophyll. In the current study, leaf hydraulic resistance is located differently between the xylem and mesophyll, and is dependent on the eucalypt clone. In GU clones, resistance in the mesophyll tissue ($R_{extravascular}$) increases significantly in response to water stress (Fig. 4.5). The point at which $R_{extravascular}$ was the greatest corresponded with the highest R_{leaf} value.

However, when GU leaves were unstressed (control treatment), $R_{extravascular}$ and $R_{venation}$ were relatively equal (~ 40-45% each). $R_{extravascular}$ was relatively higher in GC clones (25% higher than $R_{venation}$) when the leaves were not water stressed. GC leaves showed a substantial increase (by more than twice) in $R_{venation}$ during water stress and during water stress recovery. The increase in R_{leaf} (in response to water stress) is usually explained by xylem cavitation, but leaf turgor loss could have also contributed to increased mesophyll (extravascular) resistance (Brodribb and Holbrook, 2005; Knipfer and Steudle, 2008). GU clones exhibited higher extravascular resistance in response to water stress, whereas GC clones displayed increased R_{leaf} explained by xylem caivitation.

The results of the current leaf characteristics study are rather intriguing as the allocation of the components of leaf hydraulic resistance (petiole, venation and extravascular tissue) would be expected to be the same in closely related eucalypt clones such as GU and GC. The fact that one clone showed an increase in $R_{extravascular}$ (GU) and the other increased in $R_{venation}$ (GC) in response to water stress showed that the hydraulic junctions restricting water flow may be different depending on the leaf morphology and anatomy of specific eucalypt clones. Regardless of whether R_{leaf} increased in response to water stress by xylem cavitation or extravascular resistance increase, the prompt reversibility of increased R_{leaf} in mildly or acutely stressed plants implies that other physiological processes are operating. In all three clones, increased R_{leaf} from water stress was reversed (back to R_{leaf} values comparable with the control) within seven days.

A_n and g_s were measured concurrently with R_{leaf} in order to investigate the relationship between plant water relations, gaseous exchange and hydraulic characteristics. A_n and g_s were measured during winter and values were of the lower range for eucalypts (± 8 -12 µmol CO_2 m^{-2} s^{-1}; Fig. 4.6 – 7). A_n and g_s were not significantly different between *Eucalyptus* clones, although GUA clones did have 20% lower A_n values. A_n and g_s appeared to be less variable between clones during winter due to lower E_n and daily lower temperatures.

Control treatments had significantly greater A_n and g_s than leaves of the acute treatment at wilting point and A_n and g_s values were considered to be zero (at leaf wilting point) as negative values and "noise" were measured due to stomatal closure (Fig. 4.6). The same pattern of response was evident across all three eucalypt clones, where A_n and g_s were significantly higher in the control treatment (Fig. 4.8 and 4.9). A_n of GUA clones "recovered" i.e. was not significantly different from the control by day 7 of re-watering (Fig. 4.8). The same response of recovery was seen for GUA clones with respect to R_{leaf}. A_n of GC leaves had recovered photosynthetically from water stress by day 2 of re-watering. GUW leaves exposed to the acute stress treatment however showed a significant reduction in A_n (compared with the control) even after 7 days of re-watering. Photosynthetic recovery of GUW leaves after water stress appeared to be the slowest of all three clones.

For all three clones, g_s had recovered from acute water stress by day 7 and g_s was no longer limiting assimilation rates. Perhaps the lack of photosynthetic recovery in GUW leaves was caused by non-stomatal limitations e.g. V_{cmax} (carboxylation efficiency) of J_{max} where photosynthetic biochemistry of Rubisco regeneration was temporally and negatively affected by water stress. Recovery from water stress in the three clones was found to be similar with a "hydraulic-stomatal limitation model" (Blackman *et al*., 2009; Blackman *et al*., 2010) where the recovery of gaseous exchange characteristics was strongly influenced by the recovery of leaf hydraulic conductance i.e. leaf hydraulic resistance decreased. Other woody temperate species have been reported to recover from increased hydraulic resistance and reduced gaseous exchange (due to water stress) within a 24 hour period (Blackman *et al*., 2009).

Brodribb and Jordan (2008) stated that the study of the relationship between hydraulic characteristics and stomatal response to changes in soil water deficit demonstrate the constraints and the dynamics of gaseous exchange characteristics. Recent research has focused on the stringent co-ordination of K_{leaf} and g_s and positive relationships have been found when correlating K_{leaf} and g_s (Sack *et al*., 2003; Brodribb and Holbrook, 2005; Meinzer *et al*., 2004; Blackman *et al*., 2009). The close co-ordination of K_{leaf} and g_s has been correlated with leaf characteristics such as stomatal pore parameters (Sack *et al*., 2003) and vessel diameters (Aasamaa *et al*., 2001).

Stomatal characteristics vary between species and different trees have different "safety margins" at stomatal closure before K_{leaf} is reduced (Sack and Holbrook, 2006; Johnson *et al*., 2009). Although the majority of the literature discusses leaf hydraulic characteristics in terms of K_{leaf}, for the current study the inverse of K_{leaf} i.e. R_{leaf} was used to correlate photosynthetic parameters e.g. A_n and plant water relations e.g. g_s. A negative, significant correlation was determined between R_{leaf} and both A_n and g_s for all three *Eucalyptus* clones (Fig. 4.10-11). The co-ordination between leaf hydraulic characteristics and g_s indicates that leaf hydraulic characteristics are of primary importance in determining water loss from the tree crown (Brodribb *et al*., 2005). Whether the majority of the hydraulic resistance resides in the leaf venation or extravascular mesophyll tissue will impose different constraints upon gaseous exchange recovery from water stress.

GC clones showed increased hydraulic resistance in leaf venation in response to water stress but gaseous exchange recovery from water stress was rapid i.e. within 48 hours. GU clones however show increased hydraulic resistance in the mesophyll tissue in response to water stress and the increase in R_{leaf} ensured slower gaseous exchange recovery from water stress. Increased hydraulic resistance located in the mesophyll (as evident in GU clones) could possibly restrict water flow to the photosynthetic apparatus and hence cause slower photosynthetic recovery.

Stomatal density was measured only during the second harvest at 18 months tree growth. Unfortunately, stomatal density was not measured at the same time as R_{leaf}, A_n and g_s in order to correlate stomatal pore characteristics with leaf hydraulic characteristics. The most remarkable discovery from the investigation into stomatal pore characteristics was the finding that stomata were absent on the upper leaf surface of GUA clones (Fig. 4.13-16). GUA and GUW eucalypt clones are both *E. grandis x urophylla* hybrids and the complete absence of stomata on the upper leaf surface of GUA clones, but not GUW, was perplexing. GC leaves had significantly more stomata on the upper leaf surface than GUA and GUW and perhaps GC stomatal density may be correlated with the evidence that GC leaves had significantly higher g_s values (Table 3.2). Stomatal density and size did not differ between water treatments, but there could possibly have been different stomatal pore characteristics if these parameters were monitored over the course of the growth trial.

Water use efficiency (WUE) was measured instantaneously as a function of carbon assimilation per unit water transpired (Fig. 3. 21 and 3.22). The current study found the GUW clone and trees grown in the chronic water treatment had relatively higher WUE compared with other treatments. The instantaneous results expressed for WUE prompted further investigation into the $\delta^{13}C$ values for *Eucalyptus* clonal hybrids in response to water stress. $\delta^{13}C$ has been shown to be an alternative measure of demonstrating improved long-term WUE. The degree of discrimination between $^{13}CO_2$ and $^{12}CO_2$ i.e. $\delta^{13}C$, indicates the ratio of c_i:c_a which is related to long-term WUE (Farquhar and Richards, 1984; Prentice *et al*., 2010; Whitehead and Beadle, 2004).

The only indication of improved long-term WUE for the current study (with regards to $\delta^{13}C$) was found by significantly greater $\delta^{13}C$ values for the chronic water treatment (Fig. 4.17). The corresponding measurements of instantaneous WUE and WUE (in terms of $\delta^{13}C$) were interesting because they showed that trees growing in the chronic water stress treatment maintained improved WUE compared with the control and acute stress treatments. Increases in $\delta^{13}C$ values have been found in water-stressed *Coffea canephora* clones, implying improved long-term WUE (Pinheiro *et al*., 2005). Similar $\delta^{13}C$ values were obtained by Schultze *et al*. (2006) for a variety of *Eucalyptus* species along a rainfall gradient, however there were no direct correlations between total rainfall and $\delta^{13}C$ of eucalypt leaves. $\delta^{13}C$ was also found to be significantly and negatively correlated with g_s and K_{leaf} (Fig. 4.19 and 4.20). Less negative $\delta^{13}C$ values were associated with lower g_s and K_{leaf} values implying that long-term WUE was controlled in terms of lower g_s and more strictly regulated K_{leaf}. Less negative $\delta^{13}C$ values were shown by Farquhar *et al*. (1989) to arise from lower g_s values in leaves.

Conclusions

Even in closely related *Eucalyptus grandis* clonal hybrids, leaf response to water stress and subsequent water stress recovery was different. *E. gandis* x *camaldulensis* leaves experienced high increases in hydraulic resistance of veins ($R_{venation}$) under acute water stress. During water stress recovery, GC leaves achieved typical unstressed R_{leaf} values within one day of re-watering. Photosynthetic recovery of GC leaves was also rapid, with A_n values within control treatment range after only two days of re-watering. *E. grandis* x *urophylla* leaves were slower to recover from the imposition of acute water stress.

Both GUA 380 and GUW 1700 leaves showed drastic increases in hydraulic resistance of the mesophyll or extravascular tissue ($R_{extravascular}$) in response to water stress. R_{leaf} and photosynthetic recovery in GU leaves were slower than GC. GUW leaves did not fully recover from water stress (in terms of A_n) after seven days of re-watering. If leaf venation affects the hydraulic resistance to water flow, cavitation events occur during periods of water stress. Subsequent water stress recovery can easily reverse mild cavitation events. Water stress within the mesophyll tissue cannot be reversed easily, even by positive pressure. It therefore appears that an increase in $R_{extravascular}$ during water stress more negatively affects photosynthetic processes and recovery from water stress. For GU clones, the increase in $R_{extravascular}$ during water stress could be considered as a major disadvantage affecting photosynthesis and ultimately stem productivity.

Interestingly, GUA and GUW leaves exhibit different stomatal densities. The absence of stomata on the upper surface of GUA leaves was intriguing. GUA 380 is one of the most widely grown eucalypt hybrid clones (by SAPPI) and research has not been performed yet on the stomatal properties of GU or GC leaves. Perhaps the lack of stomata on the upper leaf surface has an advantage with respect to plant water relations and the prevention of water loss during g_s and A_n. During periods of water stress, fewer stomata would be highly favourable in maintaining strict water control. The absence of stomata could explain the lower stomatal conductances achieved by GUA leaves. If speculating wildly, perhaps the lack of stomata on GUA leaves could be seen as an adaptation to prevent excessive water loss in response to water stress. Water stress in GUA leaves affects photosynthetic parameters negatively because the majority of the resistance to water flow resides in the mesophyll tissue.

Another exciting discovery found from further investigation into eucalypt leaf characteristics was the correlation of WUE, both instantaneously and in terms of $\delta^{13}C$. In both WUE measurements, WUE was greatest in the chronic water treatments. Chronic water stress treatments appear to have improved long-term WUE and leaves showed some degree of plasticity when responding to mild, long-term water stress. GUW leaves showed more promising instantaneous WUE than GUA and GC, but WUE in terms of $\delta^{13}C$ did not reflect the same relationship.

FINAL CONCLUSIONS

5.1 Growth and Physiology of *Euclayptus* clones in response to drought stress

The current study aimed to explain and build on the differing growth responses displayed in the GC and GU clones, in response to rainfall events, reported by Drew *et al*. (2009). The objectives of the study were to measure the impact of watering regime on the morphology (height, diameter and biomass) and physiology (hydraulic conductance, leaf water relations and photosynthetic characteristics) of three *Eucalyptus* clonal hybrids. The physiological and morphological characteristics of the three clones were then assessed for differences with tree age, water availability and among the clones. The influence of drought and subsequent drought stress recovery after re-watering the plants was also evaluated in leaves (resistance to water flow, stomatal conductance and photosynthetic rate). Figures 5.1 and 5.2 were used to summarise the significant differences in physiological and morphological characteristics (and leaf characteristics) of tree age, water availability and among the clones.

The GC clone showed significantly better growth efficiency (GE, cm^3 wood m^{-2} leaf area $year^{-1}$) than the GU clones. The higher GE was due to the significantly lower leaf area maintained by the GC clone, and not because the GC clone accumulated more wood than the GU clones. The GC clone appeared to be the most drought tolerant clone as it was less affected in terms of diameter and total biomass in response to water stress. The primary reason for the drought tolerance was that 50% of the total biomass of the GC clone was below-ground (root biomass). While high root biomass may confer traits of drought tolerance (more root surface area for water absorption and a greater volume of soil exploited), in the GC clone in this study, it was at the expense of lower above-ground biomass (especially leaf area). Higher root biomass would imply greater hydraulic efficiency to the tree, and this was reflected by the higher photosynthetic rates and stomatal conductance attained by the GC clone. The GC 438 clone can be used in commercial plantings at sites that are known to experience lower than sufficient rainfall. Stem productivity of the GC clone on these sites would possibly be greater than that of a higher biomass, more drought-susceptible clone. However, the continued commercial planting of the GC clone at mesic sites would not accomplish the increased long-term productivity objectives of SAPPI, due to the lower leaf area and reduced stem biomass achieved by the GC clone, compared with the GU clones available for planting.

RESULTS SUMMARY

Physiological and Morphological Parameters Are Affected By:

CLONE

- Height (GC > GU)
- Diameter Growth Rate (GUW > GUA & GC)
- Growth Efficiency (GC > GU)
- Volume (GC & GUW > GUA)
- A_n (GUW was signif lower at 6 months)
- Root:shoot (GC > GU)
- SLA (GUW > GUA & GC)
- K_h (signif lower in GUA at 9 months)
- Leaf Area (GU > GC)

GC: Growth efficiency
GUW: Diameter growth
GUA: Leaf area supporting growth

WATER TREATMENT

- Diameter (control > stress)
- Volume (control > stress)
- J_{max} (↓ at acute wilting point)
- V_{cmax} (↓ at acute wilting point)
- E_n + WUE (↓ in chronic treatment)
- g_s (↑ in control treatment)
- SLA (acute > control & chronic)
- Leaf Area (control > stress)

Control: Diameter; leaf area
Chronic: Improved WUE
Acute: SLA was greatest

AGE

- A_n & E_n (↓ with age and winter)
- g_s (↑ with age and winter)
- Biomass allocation (↓ to leaves; ↑ to stems)
- SLA (significant only at 18 months)
- Allocation of resistance to water flow (↑ to leaves; ↓ to roots)
- Leaf Area (significant only at 18 months)

At 18 months: change in allocation to biomass and resistance; leaf area maintained is significantly different between clone and water treatment

CONCLUSIONS

Clone: GUW clones maintain greater diameters, improved WUE, and greater above-ground biomass

Water treatment: Chronic water stress (small water deficits long-term) improve WUE, maintain greater diameters, more leaf area than acute water stress (severe, short-term water stress)

Age: Leaf area and allocation of biomass and resistance are controlled ontogenetically

Leaf Area is the morphological parameter driving physiological changes

Figure 5.1: Summary of results of physiological and morphological parameters that are affected by eucalypt clone, water treatment or tree age in plants grown for 9 or 18 months.

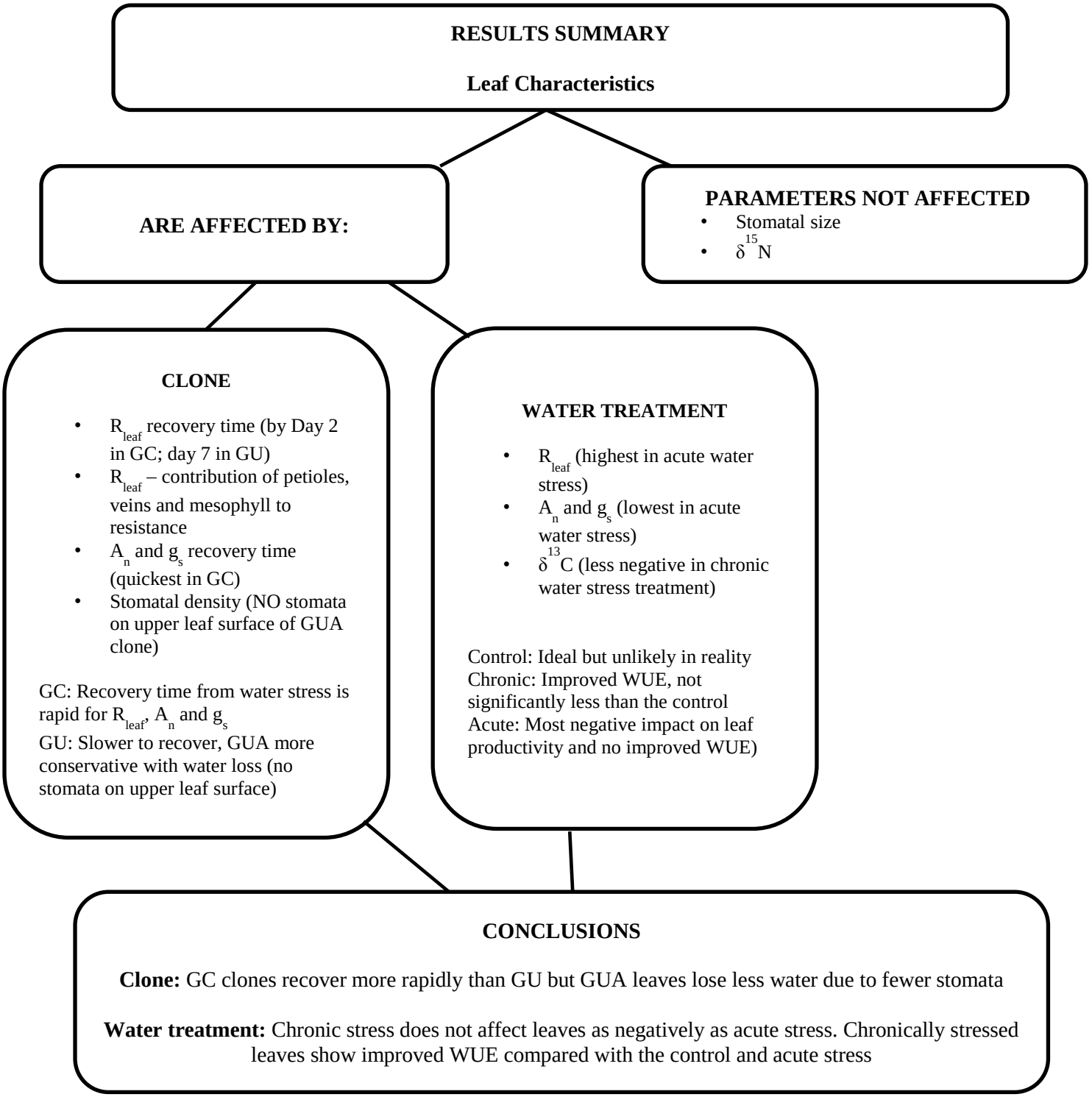


Figure 5.2: Summary of leaf characteristics that are affected or not affected by eucalypt clone or water treatment.

Both the GU clones (GUA380 and GUW1700) produced more stem biomass than did the GC clone. While plants of the GUW clone achieved greater diameters than those of the GUA clone, the GUA clone produced 30% more stem biomass than the GUW clone after 18 months growth. From personal observations, the GUA clone lost a significant amount of leaf area after an acute water stress event. Leaf dieback in response to water stress could be considered a drought avoidance strategy that prevents further water loss. Beyond a certain point, hydraulic dysfunction from drought can be non-recoverable, and the sacrificial death of disposable plant organs (e.g. leaves) will improve hydraulic conductance and water status in the remaining foliage (Holloway-Phillips and Brodribb, 2011). Recovery of leaf area after water stress is time-consuming, and can take up to 100 days in some woody species (Brodribb and Cochard, 2009), which therefore slows down the maximum diameter growth attained.

The GUW clone produced less stem biomass, but it lost 20% less leaf area than the GUA clone following an acute drought stress event. The GUW clone showed enhanced traits of drought tolerance compared with the GUA clone in response to acute drought stress. Moderately improved instantaneous water use efficiency (WUE) was also shown in the GUW clone in plants subjected to the chronic water stress treatment. Commercial planting of GU clones should be assessed in conjunction with long-term weather and soil data of the sites to be planted. The GUA380 clone would produce significantly greater stem biomass, provided only that extreme weather conditions (e.g. severe drought) were not experienced or predicted in the long-term. The GUW1700 clone, while less productive, was considerably more drought tolerant and would be a less risky clone choice, at a time where climate change is predicted to cause further extreme weather events.

Clone or water treatments affected some, but not all leaf characteristics (Fig. 5.2). Acute water stress increased the leaf hydraulic resistance to water flow while simultaneously decreasing assimilation rate and stomatal conductance. The leaf recovery time (after re-watering) was however, clone dependent. Leaves of the GC clone recovered from stress (i.e. R_{leaf}, A_n, and g_s were not significantly different from the control treatment) as soon as 2 days of re-watering. The GU clone recovered from stress after approximately 7 days after re-watering.

Rapid recovery of K_{leaf} (inverse of R_{leaf}), seen in the GC leaves, is likely if the primary cause of leaf hydraulic dysfunction is cell collapse of the minor veins (Blackman *et al*., 2010). Beyond a certain point non-recoverable hydraulic dysfunction can occur, and this phenomenon was noted with the GUA clone in response to the acute water stress treatment. Brodribb and Feild (2010) reported that K_{leaf} can be related to vein density and hydraulic architecture and there is an influence on gaseous exchange and water flux. Leaf hydraulic recovery could be considered a key functional trait linked to leaf structure, leaf anatomy and ecological tolerance.

One of the fascinating observations from the study was that stomata were not present on the upper surface of the GUA clone unlike the closely related GUW clone. No information could be found regarding stomatal density studies on southern African *Eucalyptus grandis* clonal hybrids. The absence of stomata on the upper leaf surface could be deemed to be a drought avoidance strategy of the GUA clone, reducing transpiration and preventing leaf dieback during drought stress.

5.2 Growth and Physiology of *Euclayptus* clones in response to tree age

A number of physiological parameters of the plants were influenced by tree age. Assimilation rate and transpiration decreased with age and were lowest during the winter season in all three clones. The reduction in carbon fixed (and hence growth rate) and water transpired with age are experienced in most C_3 plants. Water, nutrients and soil space for roots (in the current study) can become limiting with tree age and size, and this constrains the efficiency with which carbon can be assimilated (Way, 2011).

The proportional allocation of resistance to water flow (R_h) and biomass changed with tree age. Allocation of biomass to leaves decreased with age, whereas allocation of resistance (to water flow) in leaves increased with age. The reverse was evident in root biomass and root hydraulic resistance. Although root biomass did not increase in response to drought stress, as evident in *Eucalyptus globulus* clones (Costa E Silva *et al*., 2004), the allocation of biomass to roots and the change in the hydraulic properties of the root system could be considered a successful drought acclimation strategy. For the *E. grandis* clones in the current study, changes in the hydraulic properties of the plant organs were driven by primarily by age, and not by strategic drought stress acclimation.

5.3 The Implications of Drought Stress Severity and Duration

Drought is one of the most important environmental factors that limit crop yield. A reduction in plant yield can occur by reducing the effect of intercepted light that can be converted into plant material though photosynthesis (Bunce, 2009). Photosynthetic constraint during drought can be highly correlated with the reduction in stomatal conductance (Flexas *et al.*, 2004). The improvement of photosynthesis during drought needs to be based on efforts to understand the physiological processes that reduce photosynthesis (Flexas *et al.*, 2004).

Mild or moderate drought causes reductions in photosynthesis by early stomatal closure whereas severe drought stress can result in non-stomatal limitations of photosynthesis i.e. biochemistry of the photosynthetic cycle is interrupted (Bunce, 2009). Therefore the severity and duration of drought affects the underlying physiological responses of the tree.

Trees can respond to drought by different physiological mechanisms:

- A reduction in leaf water potential
- Stomatal closure
- Leaf dieback, that reduces transpiration and photosynthesis
- Carbon partitioning to the roots and storage changes
- Drought can "weaken" trees by making them more susceptible to insect attacks and pathogens

The tree response to drought is expected to be complex and variable, and it is likely that the entire tree would be involved in the physiological and morphological response to drought. Assessing the physiological and morphological characteristics in response to water stress in the current study yielded some interesting, although unforeseen results (Fig. 5.1). Unsurprisingly, the control treatment (little or no water stress was applied) yielded significantly greater tree diameters, volumes and leaf area than the trees subjected to water stress. Total biomass, although lower in plants subjected to the water stress treatments, was not significantly reduced by water stress. Acute water stress (severe, short-term cyclical drought, with periods of recovery from re-watering) had a more negative influence on the reduction of leaf and root biomass compared with the chronic stress treatment (mild, long-term water stress).

Plants subjected to chronic water stress showed moderately (but not significantly) improved instantaneous WUE. Commercial planting sites experiencing little or no water stress in the South African Zululand area are not common, and moderate to severe water stress would be considered the norm in certain regions.

When assessing the influence of water stress on eucalypt productivity, chronic water stress confers plant traits that include increased WUE and maintenance of greater tree diameters. Acute water stress was shown to decrease stem biomass by 15% compared with the control after 18 months growth. Long-term drought stress similar to the acute water stress treatment applied here could possibly progressively reduce stem biomass over the approximately 7 year growth rotation.

Studies of the investigation of $\delta^{13}C$ values have been performed in natural *Eucalyptus* forests in Australia, but there was found to be no correlation between the $\delta^{13}C$ in leaves and along a rainfall gradient (Schultze *et al*., 2006). In pot-grown studies of *Coffea arabica* however, $\delta^{13}C$ in leaves was significantly greater (i.e. less negative) in water-stressed plants, suggesting some degree of higher long-term water use efficiency. The $\delta^{13}C$ values for the current study indicate that the plants in the chronic treatment showed some degree of increased long-term water use efficiency because $\delta^{13}C$ was significantly greater under this treatment. The finding that plants in the chronic treatment had better WUE (in terms of $\delta^{13}C$) than the control and acute stress treatment was in accordance with data that showed instantaneous WUE was moderately higher in plants subjected to chronic water stress.

5.4 Assessing the objectives of the current study

a) Measure the impact of watering regime on the morphology (height and diameter) of three *Eucalyptus* clonal hybrids:

Watering regime was shown to have significantly different impacts on the height and diameter of the three *Eucalyptus* clones. Tree height was significantly different among clones, where the GC clone was taller the GU clones, regardless of water treatment. Watering treatment had no significant effect on the height of the eucalypt clones in the current study.

Tree diameter was primarily and significantly affected by watering regime. Water stress (acute and chronic) significantly reduced the diameter of all three clones.

b) Evaluate the effects of water stress and clonal hybrid on hydraulic characteristics and biomass partitioning at the juvenile (9 months) and early adult (18 months) growth stages:

Biomass partitioning to roots was driven by genetics (the GC clone had significantly more root biomass than the GU clones), whereas biomass partitioning to leaves was governed by genetics and the imposition of water stress. The proportion of biomass accumulated in the stems increased substantially with tree age. Hydraulic characteristics changed with tree age, but not among clones or watering regime. It was evident that there was a reversal in the proportion of the allocation to resistance in the leaves and roots. As tree age increased, and the number of drought stress events increased, leaves of eucalypt trees represented the "hydraulic bottlenecks to water flow", a phenomenon seen in many other tree species. Even though biomass partitioning was driven primarily by genetics, and the proportional allocation of hydraulic resistance was determined by tree age, there still existed a positive, significant relationship between hydraulic conductance and total biomass.

c) Determine the influence the drought stress and consequent drought stress recovery has on resistance to water flow in the leaf, stomatal conductance and instantaneous photosynthetic rate:

The results from the investigation of leaf characteristics, in response to water stress, showed positive, significant correlations between K_{leaf}, A_n and g_s. While K_{leaf}, R_{leaf}, A_n and g_s were governed by the imposition of drought stress, the leaf recovery time (i.e. once the parameter was not different from the control) was determined by eucalypt clone. The GC clone showed rapid leaf recovery (in terms of K_{leaf}, A_n and g_s) essentially because water stress negatively affected the leaf venation, and not the mesophyll tissue (where photosynthesis occurs).

d) Assess whether the physiological characteristics (plant water relations, photosynthetic capacity and hydraulic conductance) of the three *Eucalyptus* clonal hybrids differ with tree age, water availability and among clones:

Tree age was shown to cause changes in allocation of resistance to water flow, a reduction in A_n and E_n and changes in leaf area. Reduction of water availability (in the chronic and acute stress treatments) decreased J_{max}, V_{cmax}, E_n, g_s and leaf area.

Genetics (clonal hybrid) significantly affected differences in SLA, K_h and leaf area. The interaction of age, water availability and clone affects tree physiology on a whole-plant level. The only morphological parameter that can drive physiological processes, that was different with tree age, watering regime and among clones, was that of leaf area.

5.5 Future suggestions for research

The onset of climate change has significant consequences with regards to the future productivity of plant ecosystems worldwide. Climate change predictions suggest that drought events will be more common, earlier snowmelts will occur, higher temperatures and greater variability of rainfall are highly probable (IPCC, 2007). It is likely that there will be significantly longer periods without rainfall, in a number of regions, as well as less rainfall captured by the soil because of more intense storm events (IPCC, 2007). There is no detailed assessment and research dedicated to the potential impacts of climate change on the forestry industry in southern Africa (Warburton and Schulze, 2008). Significant shifts in areas that experience high rainfall are expected, and this is likely to change commercial productivity of the forestry sector. Warburton and Schulze (2008) have suggested that the current areas optimal for planting trees may have to shift in response to changing rainfall patterns.

Conflicting evidence pertaining to physiological responses of trees to elevated CO_2, as a consequence of climate change, have been reported. Way (2011) suggested that tree size class and species differences are more important factors determining tree response to drought under elevated CO_2 than nutrient availability. An undervalued area of research when considering tree response to changes in water availability is that of the physiology of the water transport system of trees (Brodribb *et al*., 2010). On a smaller scale, recent research has focussed on venation traits possessed by leaves that correlate with climatic variables or can be directly decoded through models as predictors of plant function (Blonder *et al*., 2011). “Venation networks” within leaves can be important predictors of leaf function. Understanding the importance of leaf venation, xylem transport and resistance to cavitation events, in response to drought, are likely to progressively improve predictions of plant and environmental water use.

The improvement of predictions that govern maximum plant productivity can then be used on local and global scales and in response to future environmental change. There is underestimated potential for the use of water transport physiology to predict the growth and mortality of trees where climate change has altered predictable and reliable rainfall patterns. The use of FACE experiments to simulate elevated CO_2 response in real crop ecosystems has provided interesting results for a number of studies worldwide, but these systems are extremely expensive and substantial maintenance and labour costs are required for meaningful results.

Research that requires great investment cost is not a priority during a period when global financial crisis and budget cuts are prevalent, and the forestry sector in southern Africa is not excluded from the current situation where financial priorities are to cut spending. It would be foolish to make grand, hugely expensive suggestions for future work at SAPPI when it is unlikely, at the current time, that these suggestions would be viable.

Technology transfer of forestry research requires cost-benefit analysis and there is a pressing need to produce conclusions from research undertaken that align with management systems. Presently, refining choice of species and clones than maintain optimal productivity in response to changing rainfall events and patterns, is more important that continually releasing new clonal varieties.

A number of small-scale research studies could be initiated as a continuation of the current study. Considering the interesting results found in terms of stomatal density (i.e. lack of stomata on the upper leaf surface of GUA leaves), eucalypt clones could be screened for stomatal anatomical differences. Stomatal density determination is rapid, inexpensive and does not require highly skilled technical equipment or staff. It may be useful to assess whether lack of stomata on the upper leaf surface is a common occurrence in all GUA clones, or if it is age-related.

The GUA clone also showed the most pronounced leaf dieback in response to water stress. Correlations between stomatal absence and leaf dieback in response to soil water deficit of closely related clones could be examined.

Another relatively inexpensive investigation could be determination of $\delta^{13}C$ of leaves of clones in areas experiencing significantly different rainfall. Leaf samples could be collected in the field or at tree breeding stations, and the dried leaf samples can be processed locally. Determination of a site or rainfall gradient can be related to improved water use efficiency may be valuable for future site predictions and commercial clone planting. Another suggestion by Schultze *et al*. (2006) would be to relate wood $\delta^{13}C$ to leaf $\delta^{13}C$, and evaluate whether a relationship occurs between long-term WUE in the leaves and carbon assimilated in the wood.

Existing and future short-term research should focus on facilitating continuous improvement of site-species matching and production management. Should or when the global financial crisis be alleviated and more funds become available for more extensive research programs, there exists great potential in further investigation of water transport physiology (leaf and whole plant hydraulic conductance characteristics) in response to prolonged or severe water stress. The link between plant hydraulic systems, stomatal control of transpiration rate and the concurrent correlation of photosynthetic rates is imperative for comprehending whole-plant responses to changes in water availability. There also exists a huge gap in knowledge of how climate change, especially elevated CO_2 and rainfall variability, will change *Eucalyptus* productivity in southern Africa. Climate change research requires great investment that would require funding from additional research institutions or the corporate sector in order to produce the quality of research being performed worldwide. Research objectives should continuously aim to align with management strategies that benefit the forestry sector in southern Africa.

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