

**SOME PHYSIOLOGICAL AND GROWTH RESPONSES
OF THREE EUCALYPTUS CLONES TO SOIL WATER
SUPPLY**

By

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As the candidate's supervisor I have/have not approved this thesis for submission.

Signed: _____ Name: _____ Date: _____

*If a tree is treated as a living organism, with an understanding of its
vital
functions, it will be a constant source of profit and pleasure to men.*

- N.T.Mirov

ABSTRACT

The response of three *Eucalyptus* spp. clones (GC550, GU210 and TAG14) to water availability was assessed in terms of growth, plant water status, leaf gas exchange, whole plant hydraulic characteristics (both root and shoot), stem xylem vulnerability. Furthermore, to experimentally assess the influence of hydraulic conductance on leaf physiology and plant growth, specimens of two of the clones were subjected to long-term root chilling. Prior to harvesting data were collected on the diurnal variation in leaf water potential (ψ_L), transpiration rate (E), stomatal conductance (g_s) and net CO_2 assimilation rate (A). Main stem xylem vulnerability was assessed using ultrasonic acoustic emissions (UAE). Vulnerability of the main stem was assessed as the leaf water potential corresponding to the maximum rate of acoustic emissions (ψ_L , EPH_{max}), and as the critical water potential triggering cavitation events, calculated as the mean of the water potentials of data points lying between 5 and 10% of the total accumulated emissions ($\psi_{CAV,cUAE,\%}$). Hydraulic conductance was measured on roots and shoots using the high-pressure flow meter (HPFM). Root data were expressed per unit root dry mass ($K_{r/trdw}$) and per unit leaf areas ($K_{r/LA}$), shoot data expressed per unit shoot dry mass ($K_{S/tsdw}$) and per unit leaf area ($K_{S/LA}$), and whole plant conductance was expressed per unit leaf area ($K_{P/LA}$). Soil-to-leaf hydraulic conductance was also assessed as the inverse of the slope of the relationship between leaf water potential and transpiration rate (the evaporative flux, EF, method).

A field study was undertaken on three month old TAG14 and GU210 plants. Diurnal values of leaf water potential ψ_L , E and g_s were consistently higher in TAG14 than GU210, but A did not differ among the clones. Main stem xylem vulnerability ($\psi_{CAV,cUAE,\%}$) was higher in TAG14 than GU210. In both clones midday ψ_L fell below $\psi_{CAV,cUAE,\%}$, suggesting lack of stomatal control of xylem cavitation. $K_{r/LA}$ was higher in TAG14 than GU210, whereas, $K_{S/LA}$ and $K_{S/tsdw}$ was higher in GU210 than TAG14. A greater proportion of hydraulic resistances resided in the roots, particularly in GU210. $K_{P/LA}$ was higher in TAG14 than GU210 clone, although the significance was marginal ($P=0.089$). However, all the physiological measurements were consistent with the concept of higher hydraulic conductances in TAG14 leading to

lower leaf level water stress. Above ground biomass was higher in TAG14 than GU210, in agreement with this concept, although this clone was more vulnerable than GU210.

Material grown for 14 months in 25 l pots clones showed no differences in ψ_{Soil} between the high and low watering supply, indicating that even the 'high' supply was inadequate to prevent water stress. In accordance with this, diurnal values of ψ_L , g_s , E and A did not differ significantly between treatments and clones. Early stomatal closure was apparent, maintaining ψ_L constant during the middle of the day. Stem xylem vulnerability, assessed as both $\psi_{L,EPH_{\text{max}}}$ and $\psi_{CAV,cUAE,\%}$ showed that the main stem of GC550 was more vulnerable than other two clones, and that low watered plants were more resistant to xylem cavitation than those receiving high water. Midday ψ_L fell below the vulnerability values assessed by both measures across treatments and clones, suggesting lack of stomatal control preventing stem xylem cavitation. There was no relationship between stem xylem cavitation and the shoot hydraulic conductances. Root pressures did not differ between either treatment or clones. $K_{r/LA}$ was marginally higher in high watered plants, and $K_{S/LA}$ and $K_{S/tsdw}$ were higher in low watered plants, possibly by adjustment of leaf hydraulic architecture, and there were no clonal differences. $K_{r/LA}$ was much lower than $K_{S/LA}$. $K_{P/LA}$ did not differ between the watering treatment, but there was a clonal effect. Growth in dry mass was higher in high watered than low watered plants, but there were no differences among clones. As $K_{P/LA}$ was not affected by watering treatment there was no relationship between $K_{P/LA}$ and growth in total biomass.

In plants grown for 21 months in 85 l pots low water treatment decreased midday ψ_L , g_s , E and A relative to high watered plants. Interclonal differences occurred at midday. Stem xylem vulnerability assessed as $\psi_{CAV,cUAE,\%}$ and as $\psi_{L,EPH_{\text{max}}}$ show similar trends as in the 14 months saplings, clonal differences being significant in ψ_L , EPH_{max} . There was a 1:1 relationship between minimum leaf water potential and ψ_L , EPH_{max} , suggesting that the water potential developed was limited by stem vulnerability. This implies stomatal control to reduce transpiration rates to prevent

extensive cavitation occurring. These plants did not develop positive root pressures, indicating that recovery from xylem cavitations occurred through some other process. $K_{r/LA}$ was higher in high watered plants than those receiving low water, and clonal differences were observed in $K_{r/trdw}$. There was no treatment effect in $K_{S/LA}$ and $K_{S/tsdw}$, but a clonal effect was apparent. $K_{P/LA}$ was significantly different between treatment, and was reduced by low water in two clones, and increased by this in TAG14. Reduced water availability reduced biomass production, with a greater effect on roots than shoots, such that low watering reduced root:shoot ratios. There was a weak but significant relationship between whole plant hydraulic conductance and maximum stomatal conductance, and between plant conductance and total biomass produced; these data are consistent with the concept of some hydraulic limitation to growth.

Root chilling (achieved through chilling the soil) of two of the clones was used to experimentally manipulate hydraulic conductance to test the hydraulic limitation hypothesis. Short-term root chilling decreased both $K_{r/LA}$ and $K_{P/LA}$ in both clones, but had marginal effects on leaf gas exchange. With long-term chilling the decrease in $K_{r/LA}$ was observed only in GU210, with TAG14 showing some adjustment to the treatment. As the roots constitute the major hydraulic resistance, $K_{P/LA}$ largely reflected those of the roots. Long-term root chilling significantly affected leaf physiological characteristics, despite the lack of effect on hydraulic conductance in TAG14. Long term chilling decreased the whole plant dry mass, but the effect was smaller in TAG14, and this clone also showed morphological adjustment, in that root growth was less adversely affected than shoot growth. The data from GU210 support the hydraulic limitation hypothesis; because of the morphological and physiological adjustment to long-term root chilling in TAG14, the data are unsuitable to directly assess the hypothesis.

PREFACE

The experimental work described in this thesis was carried out in the School of Life and Environmental Sciences, University of Natal, Durban-4041, South Africa, from February 1999 to December 2002, under the direct supervision of Prof. N. W. Pammenter.

These studies represent original work by the author and have not otherwise been submitted in any form for any degree to any tertiary institution. Where use was made of the work of others, it has been duly acknowledged in the text

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ABBREVIATIONS

Symbol	units	Definition
GC550		<i>Eucalyptus grandis</i> x <i>camaldulensis</i> hybrid
GU210		<i>Eucalyptus grandis</i> x <i>urophylla</i> hybrid
TAG14		<i>Eucalyptus grandis</i> clone.
MAR	mm	Mean annual rainfall
SLA	m ² kg ⁻¹	Specific leaf area
ψ	MPa	Water potential
A	μmol m ⁻² s ⁻¹	Net photosynthetic rate
g _s	mol m ⁻² s ⁻¹	Stomatal conductance
E	mmol m ⁻² s ⁻¹	Transpiration rate
WUE		Intrinsic leaf water using efficiency
VPD	kPa	Vapour pressure deficit
PAR	μmol m ⁻² s ⁻¹	Photosynthetically active radiation
UAE		Ultrasonic acoustic emission
cUAE		Cumulated ultrasonic acoustic emission
EPH		Events per hour
ψ _L , EPH _{max}		Water potential of leaf at maximum events per hour
ψ _{CAV} , cUAE, %		Threshold water potential corresponding to the initiation of rapid cavitation
PLC ₅₀		Loss of 50% of maximum hydraulic conductivity
P _r	kPa	Root pressure
HPFM		High pressure flow meter
K _{s/LA}	kg s ⁻¹ MPa ⁻¹ m ⁻²	Shoot hydraulic conductance per leaf area
K _{r/LA}	kg s ⁻¹ MPa ⁻¹ m ⁻²	Root hydraulic conductance per leaf area
K _{p/LA}	kg s ⁻¹ MPa ⁻¹ m ⁻²	Whole plant hydraulic conductance per leaf area
R	kg ⁻¹ s MPa	Hydraulic resistance
R _L	kg ⁻¹ s MPa m ²	Resistance of shoot leaf
R _p	kg ⁻¹ s MPa m ²	Resistance petiole
R _{plant}	kg ⁻¹ s MPa m ²	Resistance plant
ANOVA		Analysis of variance
P		Probability

Chapter 1: Introduction and Literature Review

1.1 A short note on *Eucalyptus* spp. and their role in the South African forestry industry

1.1.1 Importance of *Eucalyptus* spp.

The genus *Eucalyptus* belongs to the family Myrtaceae and comprises a large number (about 800) species, which are indigenous to the Australia mainland, Tasmania and Papua New Guinea. The environmental adaptability within the genus is an important characteristic, which has permitted wide exotic transfers from the middle of 18th century to various tropical and subtropical parts of the world. *Eucalyptus* is one of the most widely planted valuable sources of hardwood trees in the world and emigrated as early as 1803, by way of Mauritius to South Africa (Zacharin, 1978) to meet the existing wood demand through the forestry industry.

The genus *Eucalyptus* has a wide range of potential uses, making it an ideal and valuable tree in world society. Trees are mainly grown for the potential uses of hardwood timber, essential oils extracted from leaves (Gupta and Mascarenhas, 1987), honey, pulpwood used in paper industry (van Wyk, 1990; Le Roux and Van Staden, 1991) and fiberboard, tanning materials, decoration, windbreaks and for many other miscellaneous uses. The *Eucalyptus* trees are producing most of the hardwood in the world. The current hardwood production, mostly bound for pulpwood, from the *Eucalyptus* plantations of South African forestry industry is about 8 million cubic meters per year and production is expected to rise to 14 million cubic meters per year by the year 2010 (Smith, 1996).

E. grandis is mainly used for mining timber, or chip production and pulpwood industries and accounts for 40% and 46% of the total market consumption, respectively (van Wyk, 1990). By 1973, one-third of the total commercial timber standing in the country consisted of *Eucalyptus* plantations, and *E. grandis* alone

comprised 80 % of that, grown mostly for pulpwood and for round mining timbers (Zacharin, 1978). The present South African total plantation resource of *Eucalyptus* constitutes approximately 40 % of the total commercial timber, and of this 29 % is *E. grandis*. This species is mainly used for pulpwood or mining timbers (Smith, 1996).

1.1.2 Forestry in South Africa

The fast growing *Eucalyptus* trees were being extensively cultivated in the second half of the 18th century to satisfy the demand for the timber as natural forests are inadequate to supply wood in South Africa (Zacharin, 1978). Because of its fast growth, *E. grandis* is widely planted in warmer and moister areas, particularly of Africa and South America. *Eucalyptus* spp. are regarded as one of the most productive plantation crops in the forestry industry. Plantation forestry of *Eucalyptus* and *Pinus* is aggressively pursued in almost all the provinces of South Africa since natural forests are endangered and indigenous forest species are very slow growers and do not meet the South African demand for wood and pulp (van der Zel, 1989; Anon, 1996). However, the growth and distribution of *Eucalyptus* species is limited by climatic constraints such as temperature and water availability (Sommer and Wetzstein, 1984). In South Africa, the main factor limiting forestry expansion is the inadequate rainfall (Denison and Quaile, 1987), considering that South Africa has an average rainfall of 560-mm (Olbrich *et al.*, 1993).

1.1.3 Problems in forestry

The fast growth of *Eucalyptus* spp. and the increased demand for wood and wood products in South Africa has meant a steady increase in the extent of these plantations despite the fact that *Eucalyptus* spp. are reputed to consume large quantities of water. Planting these trees in water catchment areas decreases water runoff to a wide area, thus reducing the agricultural viability in these areas (February *et al.*, 1995). Thus, in some countries there has been opposition by agricultural and water interests to the *Eucalyptus* plantings which are believed to reduce the water available to both farm crops and underground water supplies (Zacharin, 1978).

Demands by the forestry industry for improved productivity and the introduction of plantation trees into marginal areas have created a need for a detailed understanding of the water relations of commercially grown *Eucalyptus* clones (Mulin and Park, 1992). In South Africa, as in many other countries, future expansion of plantation sites, even for conservation of potentially valuable germplasm, is difficult because of strict legislation pertaining to land and water use, and maintenance cost (Watt *et al.*, 2000). The vast amounts of land owned by forestry companies are increasing each year (AMIC Annual report, 1991; SAPPI Forests Annual Report, 1992) for the expansion of *Eucalyptus* plantations to meet the required demand in South Africa. Much of the extensive lands used for the plantation was previously the grassland and the change in vegetation type is expected to have serious long-term effects on the hydrology of the region. After a recent drought during 1991/92 in South Africa, the government has effectively banned new plantation establishment permits. This is because hydrologists have suggested that expansion of *Eucalyptus* plantation has exacerbated the effects of drought because the trees use considerable quantities of ground water (Smith, 1996). Currently, afforestation permits are granted or refused based on an assessment of the expected impact of plantations on water yields from the catchment areas (Olbrich *et al.*, 1993). This restriction on issuing of new plantation permits would be expected to affect the forestry industry in terms of wood production, which is required to meet the demand in South Africa. Apart from this, during a recent drought in South Africa, the forestry industry experienced heavy losses in *Eucalyptus* plantations. The drought caused severe mortality of some clones, whereas other clones survived the drought (Vander Willigen and Pammenter, 1998). Such losses have raised concern for improving silvicultural practices by selecting water use efficient clones that would not only survive but also continue to be productive under restricted water conditions (Olbrich *et al.*, 1993). Such clones need to be assessed physiologically under different conditions or treatments to see if they meet expectations.

1.2 Water flow through the plant

The movement of water through the soil-plant-atmosphere continuum is driven by transpiration that supplies water and nutrients to various parts of the plant. Adhesive-cohesive xylem wall and water properties combined together permit translocation of an unbroken water column from roots to leaves (Devlin, 1966; Bidwell, 1979), with water potential gradients increasingly negatively among the plant components. The hydraulic architecture influences the magnitude of the water potential gradients and the flow rate in the xylem.

1.2.1 Ascent of sap is a sustainable mechanism: the cohesion-tension theory

The cohesion-tension theory of Dixon and Joly (1894) (*loc. cit.* Devlin, 1966) is the most widely accepted as the mechanism for the ascent of sap in higher plants, rather than mechanisms like root pressure and capillarity forces. The theory of cohesion-tension is often challenged by some researchers who question the ability of xylem water to sustain very negative pressures when the water column is being pulled (Zimmermann *et al.*, 1994; Smith, 1994; Zimmermann *et al.*, 1995; Canny, 1995; Milburn, 1996). Assessing such negative xylem pressures by using different tools is important in understanding the functioning of plant water status under different habitats. Measured xylem pressure either by indirect (Scholander-Hammel bomb) or direct (cell pressure probe) methods have not shown consistent values at high negative pressures, and this led to the controversy over the cohesion-tension theory. The limitations of the xylem pressure probe technique are such that it cannot be used in the range of pressures that can be measured by pressure chamber, the pressure probe measures only at less negative pressures (Wei *et al.*, 1999). However, comparative studies between the non-destructive method of thermocouple psychrometry and the destructive pressure chamber showed a good 1:1 relationship (Oosterhuis *et al.*, 1983), and this further validated the pressure chamber measurements. In addition, Dixon's water pulling theory has been reasonably well supported by various authors

(Holbrook *et al.*, 1995; Pockman *et al.*, 1995; Steudle, 1995; Sperry *et al.*, 1996; Tyree, 1997; Wei *et al.*, 1999).

1.2.2 Water movement and the consequence of xylem cavitation

Transpiration pulls the water column upward under tension through the xylem tissue of the roots to the leaves and transpires to atmosphere. The column of water to leaves from the soil often breaks and interrupts the water supply, as a result of excessive tensions. Under normal circumstances xylem functions at pressures below vacuum (Tyree and Dixon, 1986) and the cohesive properties between water molecules allows water to remain liquid under tension (Tyree and Ewers, 1991). Because the vapour pressure of water is slightly above vacuum, liquid water in the xylem at sub-vacuum pressures is in a metastable state (Zimmermann, 1983).

The high tensions in the xylem cause dissolved gases to come out of solution into the vapour phase. Because the water is under tension, this micro-void will expand explosively to fill the conduit, a process known as cavitation. The cavitated conduit is at a pressure close to vacuum, and air will come out of neighbouring wet tissue (and diffuse from the outside of the stem) to fill the conduit with air at atmospheric pressure, forming an embolism. An embolized conduit will not conduct water until ψ_p returns to near-atmospheric pressure and refills the conduit cavitation (Tyree and Sperry, 1989a; Tyree and Ewers, 1991; Tyree *et al.*, 1994a). There are many reports published by various authors that cavitation, predominantly induced in the xylem as a result of drought (Zimmermann, 1983; Sperry and Tyree 1988; Sperry *et al.*, 1996; Cochard, 2002) and freezing (Sperry, 1993; Utsumi, 1999; Jaquish and Ewers, 2001), temporarily reduces xylem water transport. Also, embolisms could be caused by pathogens and regular mechanical damage in plant segments throughout the life span (Tyree and Sperry, 1989a).

Four mechanisms leading to cavitations in plant xylem tissue have been proposed by Pickard (1981) and Zimmermann (1983). The first mechanism suggests spontaneous homogenous nucleation of voids that occurs in the center of a water column; this

process ignores the adhesive properties of water to the walls. The second proposal is that cavitation may occur at the interface of water and hydrophobic patches on the walls in the absence of bubbles. The third proposed mechanism is that of nucleation at a hydrophobic crack (Pickard, 1981). Here, an air bubble trapped in a hydrophobic crack is suggested to pull out of the crack, based on its radius of curvature, by existing tension. The fourth proposal, the air-seeding hypothesis (Zimmermann, 1983) is probably the best understood (Tyree *et al.*, 1994a). This hypothesis suggests that air is sucked through the pit pores from an embolized xylem vessel into the adjacent full xylem vessel under the negative xylem pressure differences (Zimmermann, 1983). This is widely regarded as the primary mechanism by which cavitations spread throughout the conducting system (Tyree and Sperry, 1989a; Grace, 1993), and has been well demonstrated by Crombie *et al.* (1985); Cochard *et al.* (1992b); Sperry and Tyree (1988, 1990); Sperry and Saliendra (1994); Alder *et al.* (1996); Hacke and Sauter (1996); Sperry *et al.* (1996); and Sperry and Ikeda (1997).

van den Honert (1948) explained the long distance water movement in plants through the plant components as a catenary process from soil to atmosphere, using an Ohm's law analogy. Concurrently, transpiration in response to evaporative demand and the hydraulic resistance of xylem structures generates the low xylem water potential, which can decline below a threshold value for xylem cavitation (Sperry and Tyree, 1988). The hydraulic design of trees influences the movement of water from roots to leaves, thus different designs could have different consequences for diverse species of trees (Tyree and Ewers, 1991). According to Zimmermann's (1983) plant segmentation hypothesis, the presence of hydraulic constrictions at branch junctions and the base of petioles, maintains hydraulic sufficiency in the main part of the plant body. The leaves and twigs constitute small expendable segments that can be sacrificed to allow trees to survive extreme droughts. The plant segmentation hypothesis has been supported by models of woody plants that demonstrate a patchy pattern to the catastrophic loss of minor branches, and a concomitant improved water balance for the rest of minor branches which allows them to survive (Tyree and Sperry, 1988). Tyree *et al.* (1991) has generalized Zimmermann's plant segmentation hypothesis and generated a concept of vulnerability segmentation in which

expendable distal plant segments are more vulnerable to cavitation compared with the larger segments, and this has been clearly demonstrated in the field studies of Tyree *et al.* (1993b); Sperry and Saliendera (1994); Alder *et al.* (1996); Hacke and Sauter (1996); Sperry and Ikeda (1997); Linton and Nobel (1999); and Salleo *et al.* (2000).

1.2.3 Water deficit and its affect on growth

The transpirational path of the whole plant can be regarded as a hydraulic resistor (Koide *et al.*, 1989), which reduces the hydraulic supply. The success and even the survival of land plants depend on sufficient water moving upward from the roots to replace that lost from the shoots by transpiration (Kramer and Boyer, 1995). Water and its supply are essentially important for plant growth and development in order to maintain growth and cellular activities. A water deficit in plant cells leads to the concentration of solutes, changes in cell volume and membrane shape, disruption of membrane integrity, and denaturation of protein (Bray, 1997). A reduced water potential can cause reduced cell expansion, wall synthesis, protein synthesis, stomatal conductance and photosynthesis and an increased xylem dysfunction by cavitation events (Tyree *et al.*, 1993a). Boyer (1968) has proposed that growth-induced water potentials arise from the enlargement of the cell walls, which prevents turgor pressure, and low pressure would result in a low water potential that would favour further water uptake by the cells, which could provide for additional growth. Plant productivity requires photosynthetic CO₂ fixation and water is a major limiting factor for photosynthesis in many environments (Kramer and Boyer, 1995). In response to xylem embolisms, steeper water potentials in the xylem exist which can lead to stomatal closure. Such closure reduces both loss of water and uptake of carbon dioxide so that carbon assimilation by leaves decreases in the water stressed plants (Becker *et al.*, 2000). Embolism formation may be a common occurrence and play an important role in the inhibition of shoot growth at moderate water deficits (Schultz and Mathews, 1988). In response to water deficits, leaf growth rate decreased (Stoneman *et al.*, 1994). Reductions in lateral branching, and in leaf production and expansion were the leading contributors to the large differences observed in biomass production between well watered and water stressed plants (Osorio *et al.*, 1998; Fort

et al., 1997). Lower growth rates (Pereira *et al.*, 1992; Arneith *et al.*, 1998) and changes in leaf area and root tissue (Farrell *et al.*, 1996) have also been observed in response to water stress.

1.3 Plant hydraulic architecture

The hydraulic architecture of a plant can potentially limit water flow to the leaves and thus it can limit leaf water potential, stomatal opening and gas exchange (Tyree and Ewers, 1996). The transpirational path of the plant from roots to leaves can be regarded as a hydraulic resistor, with different plant components having different tissue hydraulic constrictions (Zimmermann, 1983; Koide *et al.*, 1989). Except in trees with strong apical dominance, a leaf specific conductivity strongly associated with low root to shoot hydraulic resistances and a controlled transpiration rate keeps the xylem water potential above a threshold point causing cavitation. Thus, hydraulic architecture is a determinant of xylem pressure. The vulnerability of xylem to cavitation may change the efficiency of the hydraulic architecture because cavitation in the xylem increases the hydraulic resistances (Tyree and Sperry, 1989a; Sperry and Pockman, 1993).

1.3.1 Techniques used to quantify hydraulic characteristics

Several techniques have been described to measure the hydraulic conductances. In the early 1980's most of the work was focused on determining the hydraulic conductances at the branch level. Sperry *et al.* (1988a), developed a low-pressure hydraulic conductivity apparatus, and used it to measure percent loss of hydraulic conductivities by embolism in the aerial plant components. Particularly branches were studied by Tyree *et al.* (1991); Tyree *et al.* (1993b); Cochard *et al.* (1992a); Vander Willigen and Pammenter (1998); Jaquish and Ewers (2001). Using a similar principle, Brodribb and Hill (2000) measured the whole root conductivity under hydrostatic suctions in saplings, whereas Jaquish and Ewers (2001) measured segmented root conductivity using the same method. Root hydraulic conductivity of seedlings, expressed per unit root surface area, has been estimated using the root pressure probe

(Rudinger *et al.*, 1994; Stedule and Mescheryakov, 1996; Steudle and Heydt, 1997). However, Tyree *et al.* (1994b) cautioned that this approach was appropriate only if flow is uniform over the whole root surface area and the main barrier to flow is at the outer surface. The conventional evaporative flux (EF) method of measuring whole plant resistances, involves the measurement of steady state evaporative flux densities from total canopy leaves (values of E calculated gravimetrically), and leaf to soil water potential gradients (Running, 1980; Tsuda and Tyree, 1997; Tsuda and Tyree, 2000; Brodribb and Hill, 2000). The resistance of the whole plant hydraulic pathway has also been calculated from the measurements of midday leaf transpiration rate and the difference between soil and leaf xylem water potential (Saliendra *et al.*, 1995; Kolb and Sperry, 1999) but these measurements were unrepresentative (Mencuccini and Grace, 1996), because the values of both transpiration and water potential will vary from leaf to leaf.

Whole-plant hydraulic conductances were measured from concurrent measurements of daily courses of leaf water potential and sap flux densities through the trunk (Cochard *et al.*, 1996; Lu *et al.*, 1996; Irvine *et al.*, 1998; Hubbard *et al.*, 1999), sap flow scaled to sapwood area (Andrade *et al.*, 1998) and using leaf water potential and leaf gas exchange (Hubbard *et al.*, 1999). Also, branch hydraulic resistances calculated from the measurements of xylem liquid specific permeability (Franks *et al.*, 1995).

Tyree *et al.* (1993a), has developed the high-pressure flow meter (HPFM) that measures and localizes whole plant shoot hydraulic resistance and its components, and it has been used by Tyree *et al.* (1993a); Tyree *et al.* (1993b); Yang and Tyree (1994); Cochard *et al.* (1997); Nardini and Pitt (1999); Nardini and Tyree (1999); Nardini and Salleo (2000). Interestingly, Zotz *et al.* (1998) have made a comparative study of shoots, using both the low-pressure flow meter (Sperry *et al.*, 1988a) and the HPFM, and the results obtained were consistent, although, using a low-pressure flow meter apparatus (Sperry *et al.*, 1988a) for root segment measurements (which are destructive) may be more sensitive. Tyree *et al.* (1995) has introduced a new version of the high-pressure flow meter, which more rapidly measures whole plant hydraulic

resistances, including roots, and which permits the localization of resistances to water flow in the plants. Recently, Zwieniecki *et al.* (2000) made a two-point flow meter, otherwise called a low-pressure steady state flow meter (SSFM) which operates on a similar principle to the HPFM. Tsuda and Tyree (1997, 2000) have shown that whole plant measurements made using both the HPFM and the EF methods, and following, Sack *et al.* (2002) using all three methods of HPFM, EF method and vacuum pump method on whole leaves, all yielded consistent results. However, drawbacks to the HPFM are that it is destructive and measures maximum conductances only.

1.3.2 Hydraulic architecture parameters

Since 1978, various researchers have described the hydraulic architecture parameters of woody plants by measuring hydraulic conductances at the aerial shoots, roots and whole plant levels (Zimmermann, 1978; Ewers and Zimmermann, 1983; Zimmermann, 1983; Tyree and Ewers, 1991; Tyree and Ewers, 1996; Tyree *et al.*, 1998). Hydraulic conductivity per unit pressure gradient (k_h) is equal to the ratio between water flux (F , kg s^{-1}) through the whole plant or plant segment and the pressure gradient (dp/dx , MPa m^{-1}). The relationship of hydraulic conductivity to the hydraulic architecture of woody plants determines specific conductivity (k_s), which is the ratio of k_h and xylem cross-sectional area. It is a measure of the porosity of the wood that supplies water through the plant segment. Leaf specific conductivity (k_l) is the ratio of k_h to the leaf area distal to the segment being measured. It measures the hydraulic sufficiency of the plant segment to supply water to leaves distal to that segment. The water storage capacitance (Q) is defined as water storage capacity (C) per unit tissue volume (V) or dry mass or, for leaves, per pressure change in water potential per unit leaf area (A_L). Huber value (HV) is the ratio the cross-sectional area to leaf area supplied. It measures the investment of stem tissue per unit leaf area fed. Very recently, the hydraulic architecture of root parameters has been described by Tyree and Ewers (1996) and Tyree *et al.* (1998) to understand the efficiency of root systems. The root hydraulic conductances normalized to leaf area estimates the sufficiency of the roots to provide water to leaves. Likewise, root conductances scaled to root surface area and root length measures the root water uptake efficiency via the

radial and axial path. The efficiency of hydraulic pathways measured in terms of carbon investment in roots and shoots, is assessed when conductances are normalized by total dry mass of the roots or shoots.

Water pressure gradients increase with tree height from the base to the apex, and water in branches at the tops is always under more tension than in trunks and lower branches. So resulting k_1 values in plant segments are strongly associated with pressure gradients (Zimmermann, 1978; Tyree and Ewers, 1991; Tyree and Ewers, 1996). Wherever k_1 values are low, pressure gradients must be steeper to maintain high flow rates (Zimmermann, 1978). There is also a rough correlation between k_1 values and evaporative flux density and perhaps, plant size (Tyree and Ewers, 1996).

Comparative hydraulic architecture studies by Tyree and Ewers (1991, 1996) on different growth forms showed that high k_1 values are due to high HV in most growth forms and k_s values of temperate hardwoods are greater than conifers. However, there is some overlap in the values of k_1 and k_s between gymnosperms and angiosperms (Tyree and Ewers, 1996). However, lianas have low HV, but high k_s compensates for this, and generates high values of k_1 (Ewers and Fisher, 1991; Ewers *et al.*, 1991).

1.3.3 Hydraulic architecture of stems and branches

The non-living complex xylem tissue has been recognized as the principal pathway for upward movement of soil water in plants since the time of Hales (1727) (*loc. cit.* Fahn, 1967) in the early 18th century. The xylem tissue, consisting of vessels and tracheids together called tracheary elements, are primarily concerned with the transport of water and also function as a supportive tissue. Fibres and sclereids in the xylem are concerned with strengthening of the plant body and parenchyma is involved in storage. Angiosperm xylem has both vessels and tracheids, but gymnosperms contain only tracheids. The vessel members are usually perforated to various shapes on the end walls and lateral walls through which water flows (Fahn, 1967 & 1990). Tracheids are composed of perforated cells, which have only bordered pits that are present at their end and lateral walls. The intervacular pits are commonly present

between two tracheary elements, with very few between tracheary elements and fibres or parenchyma cells (Fahn, 1967 & 1990). Excretion products coming from angiosperm living cells (called tyloses) occur as a response to wounding or parasite infection (Fahn, 1967; Zimmermann, 1983). Tylosoids protruding from epithelium cells in secondary xylem of gymnosperms, may partially or completely block the water conduction (Fahn, 1967; Cochard and Tyree, 1990).

*An outline of xylem architecture in *Eucalyptus* sp.*

Eucalyptus spp. wood is termed diffuse porous, where the vessels are more or less equal in diameter and uniformly distributed throughout the growth ring (Fahn, 1990). The pattern of distribution of the vessels in the wood is single. In *Eucalyptus* short irregularly shaped tracheids are present in the immediate proximity of the vessels. These tracheids do not form a separate continuous vertical system and they are termed vasicentric tracheids (Fahn, 1990). The vestured pits found in the xylem of species of the family Myrtaceae (e.g. *Eucalyptus* spp.) phylogenetically are considered an advanced form of pit (Fahn, 1990). The length of fibres, tracheids, and vessel members has been found to increase from the center of the trunk towards its periphery through at least a certain number of annual rings (Fahn, 1967). Fibre length also varies along the trunk: in *Eucalyptus gomphocephala*, for example, the longest fibres were found to occur at a height of about 6ft above ground level (Fahn, 1990). Tyloses are commonly found in the lumen of secondary xylem (Fahn, 1967 & 1990).

1.3.4 Leaf hydraulic architecture

A leaf is composed of predominantly an epidermis, mesophyll and vascular tissue. The epidermis of leaves of different plants varies in the number of layers that protect the internal leaf tissue. The mesophyll is distinguished into palisade parenchyma and spongy parenchyma. When the palisade parenchyma is present on both sides of the leaf, the leaf is said to be isobilateral, whereas if palisade tissue only occurs on one side of the leaf with the other side composed of spongy parenchyma, the leaf is termed dorsiventral. In certain species of *Eucalyptus* it is not possible to distinguish

between the two types of parenchyma, and the mesophyll is composed entirely of palisade cells that become specialized in such a way that the efficiency of photosynthesis has been increased (Fahn, 1990). Leaf vascular tissue occurs as either single or several closely associated vascular bundles that form the veins which sometimes include the vascular tissue together with surrounding non-vascular tissue (Fahn, 1990). The reticulate or closed arrangement of the veins, resulting from branching into secondary and tertiary veins, forming a network of veins in the leaf, is common in dicotyledonous leaves (Fahn, 1990; Roth-Nebelsick *et al.*, 2001). The smallest mesophyll areas, which are surrounded by the thinnest branches of the veins often contain only tracheids, are called areoles (Fahn, 1990), so the entire venation structure of a dicotyledonous leaf is usually not totally closed (Roth-Nebelsick *et al.*, 2001).

The venation system supplies the leaf lamina with water, nutrients, hormones, and other solutes via the xylem, and includes phloem for the export of carbohydrates. The vein system also provides a flat lightweight structure that mechanically stabilizes the leaf (Roth-Nebelsick *et al.*, 2001).

Leaf hydraulic resistances, comprising both vascular and non-vascular components, were initially measured with pressure chambers by measuring the initial flow rate, after a rapid increase in gas pressure from a previous balance pressure using leaves both infiltrated and non-infiltrated with water (Tyree and Cheung, 1977; Stroshine *et al.*, 1985). Recently, a HPFM has been used to measure hydraulic resistances of leaves infiltrated with water (Yang and Tyree, 1994; Nardini *et al.*, 2001; Sack *et al.*, 2002); with this technique, by severing veins of different sizes the resistances within the leaves can be mapped.

The main resistances to water flow in whole shoots resided in the leaves (Table 1.3.1) (Tyree *et al.*, 1993a; Tyree *et al.*, 1993b; Yang and Tyree, 1994; Cochard *et al.*, 1997; Nardini and Pitt, 1999; Sobrado, 2000). In some species, resistance of leaves exceeded 80 % of the total resistance of the shoots (Nardini and Salleo, 2000). Leaf-blade resistances are relevant to a better understanding of stomatal physiology

because they allow the estimation of gradient in water potential between minor veins and stomata. A large resistance would cause a large reduction in leaf water potential which could account for closure of stomata under adverse conditions (Tyree *et al.*, 1993a; Yang and Tyree, 1994).

A progressive, increasing resistance of the shoots, petioles and leaf blades has been observed (Tyree *et al.*, 1993a; Tyree *et al.*, 1993b; Yang and Tyree, 1994; Cochard *et al.*, 1997). The leaf resistance has resistance to water flow through vascular (minor vessels) and the non-vascular (mesophyll) tissue. Resistance to water flow in vascular or non-vascular tissue is measured by differences between initially measured total leaf resistance and that measured after removal of the corresponding components. The differences between the total leaf resistances and the resistance after cutting open the leaf margin (vessel ends) is the vascular resistance and the remainder is non-vascular resistance. In a single leaf, total non-vascular tissue hydraulic resistances are much higher than vascular resistances (Tyree and Cheung, 1977; Yang and Tyree, 1994). Although, the vascular component of leaf blade resistances were about double that of the petioles (Yang and Tyree, 1994), petiole resistance was about double that of shoots (Tyree *et al.*, 1993b). Stroshine *et al.* (1985) demonstrated that increased leaf length related to a high resistance to water flow through vascular bundles. In contrast, the highest hydraulic resistance was located in stems of the current years growth of *Quercus pubescens* and stems of increasing age showed progressively lower values of hydraulic resistances (Nardini and Pitt, 1999).

Table 1.3.1 Contributions (%) of the leaf blade to the total whole plant shoot hydraulic resistances measured by the HPFM.

Species	Leaf hydraulic resistances	References
<i>Juglans regia</i>	80%	Tyree <i>et al.</i> , 1993b
<i>Quercus</i> species	80-90%	Tyree <i>et al.</i> , 1993a
<i>Acer sacharum</i>	>50 %	Yang and Tyree, 1994
<i>Acer rubrum</i>	50%	Yang and Tyree, 1994
<i>Fraxinus excelsior</i> L.	90%	Cochard <i>et al.</i> , 1997
<i>Quercus pubescens</i>	90%	Nardini and Pitt, 1999
<i>Laurus nobilis</i> L.	92%	Nardini and Salleo, 2000

1.3.5 Hydraulic architecture of roots

The soil water absorbed by the root hairs and epidermis flows through the symplastic and apoplastic pathways of the cortex, finally across the osmotic barrier of endodermis and wets the tracheary elements, from which upward movement is driven by root pressure, capillarity or transpirational pull to the top of plants (Devlin, 1966; Salisbury and Ross, 1991). The axial resistances in the root xylem is relatively low compared with radial resistance which may thus limit soil water uptake into the root (Rowse and Goodman, 1981; Frensch and Steudle 1989; Steudle and Brinckmann, 1989; Lo Gullo *et al.*, 1998; Steudle and Peterson, 1998). Water stress changes the architecture of the non-vascular pathway and decreases the hydraulic conductance of the roots (Lo Gullo *et al.*, 1998). The changes in patterns of the flow of water into roots occurs mainly by development of the endodermis (Kramer, 1983; Lo Gullo *et al.*, 1998), the formation of an exodermis (Perumalla and Peterson, 1986; Lo Gullo *et al.*, 1998) and changes in the hydraulic conductivity of root cells (Radin and Mathews, 1989). The studies related to soil-plant resistances to water flow suggest that the resistance of the soil-root interface can be very high (Running, 1980; Kramer and Boyer, 1995; Faiz and Weatherley, 1977) and root shrinkage can reduce the contact surface area between soil and root (Blizzard, 1980; Faiz and Weatherly, 1982).

The resistance offered by the root-soil interface and by the radial pathway of water movement through the cortex of fine roots is more complex than that of axial movement through woody segments (Tyree and Ewers, 1996). The early studies on plant hydraulics were mostly confined to the shoot level, as the roots are underground and more difficult to examine. However, the development of the HPFM permits the measurement of root conductances without the necessity of excavating the root from the soil (Tyree *et al.*, 1995). If the plants are grown in pots, roots can be excavated after the measurement of conductances, so that data can be expressed per unit root dry mass.

1.3.6 Whole tree hydraulic architecture

The primary location of resistance to water flow could be at the soil or soil-root interface, in the root radial pathway, in the plant water conducting system, or in the non-vascular component of the leaves, and may limit the required water supply to leaf evaporating surfaces (Kramer and Boyer, 1995). The overall whole plant hydraulic conductance depends on path length, degree of branching, transverse area of xylem, number and size distribution of xylem conduits.

Recent studies have been extended from whole shoots to whole plant to localize the sites of major resistance to water flow from roots to leaves. There is evidence that shows that the majority of resistances are located in the roots compared to shoots or leaves (Tsuda and Tyree, 1997; Nardini and Tyree, 1999; Brodribb and Hill, 2000). By contrast, in some species, partitioned root-shoot hydraulic conductances were approximately equal (Tyree *et al.*, 1998; Becker *et al.*, 1999; Nardini and Pitt, 1999; Nardini and Tyree, 1999). In *Acer saccharinum*, root resistance was double that of shoot resistance, and within the shoot the stem resistance was larger than the resistance leaf or petiole (Tsuda and Tyree, 1997).

Ryan and Yoder (1997) proposed that increase in axial pathway limits hydraulic supply to the top leaves of trees as they aged and restricted their growth. There is support for their hypothesis such as in *Pinus sylvestris*, leaf area specific hydraulic conductance decreased with increasing path length (Mencuccini and Grace, 1996). In *Betula occidentalis*, measured conductances were relatively low in adults compared to juveniles and shorter length of xylem pathway in juveniles increased the conductances (Salindera *et al.*, 1995). In contrast, in five tree species of a lowland tropical forest, conductances increased exponentially with tree heights (Andrade *et al.*, 1998). Also, it has been suggested that an exchange of internal water storage from various sizes of capacitors may partially diminish the increase in axial hydraulic resistances in growing trees (Goldstein *et al.*, 1998; Andrade *et al.*, 1998).

The whole plant hydraulic resistance of *A. saccharinum* was low compared to other temperate species and it assures high transpiration rates in the presence of sufficient water (Tsuda and Tyree, 1997). In light demanding pioneer tree species whole plant hydraulic conductances scaled to leaf area, and their root and shoot conductances scaled to leaf area / dry weights of roots or shoots were higher than shade tolerant species (Tyree *et al.*, 1998). But both scaled by total root-shoot dry weights showed greater resistances in the shoots (Tyree *et al.*, 1998). A decrease in root-shoot resistances can maintain less negative water potential and it may promote both extensions of growth and high net assimilation rates. Also, less carbon may be needed to provide efficient hydraulic pathways compared to species with higher root and shoot resistances (Tyree *et al.*, 1998).

Substantially lower conductivity was observed in conifer shoots compared with angiosperm shoots (Zwieniecki and Holbrook, 1998). Most of the hydraulic resistances (inverse of conductance) of *Fraxinus excelsior* L. occurred in the oldest branch xylem (Cochard *et al.*, 1997). Despite less efficient hydraulic pathways in conifers, the whole plant hydraulic resistances of small angiosperms and adult conifers normalized to leaf areas were similar; this similarity was brought about by higher leaf and root resistances in small angiosperms (Becker *et al.*, 1999). Likewise, in three drought adapted *Quercus* species, root resistances scaled for leaf and root surface area, shoot resistances scaled for leaf area and leaf blade resistances were higher than for water demanding (mesophilous) *Quercus* species (Nardini and Tyree, 1999).

In addition, hydraulic conductances in the seedlings of *Eucalyptus camaldulensis* were lower in both stems and branches of plants growing in humid site than those in a semi-arid site (Franks *et al.*, 1995). However, leaf specific conductances of branches of some *Eucalyptus* spp. clones were higher in trees on mesic sites than in trees on xeric sites (Vander Willigen and Pammenter, 1998). An increase in hydraulic conductances in branches, trunks and crowns is closely associated with the increasing sapwood area (Andrade *et al.*, 1998; Cochard *et al.*, 1997). An adult tropical tree of

Schefflera morototoni has low stem resistance with high stem capacitance that may permit the species to survive during long dry seasons (Tyree *et al.*, 1991).

In spite of all the architectural features, hydraulic conductances are a function of the relationship between roots and shoots. Water evaporation must be replaced by absorption by the roots. As such, changes in leaf area leading to changes in evaporation must be accompanied by changes in the root system, promoting conductivity from roots to leaf. There is evidence in *Saccharum* spp. hybrids and *Pinus taeda* L. that partial defoliation caused rapid increases in stomatal conductance, re-establishing the original hydraulic conductance relationship with the functioning leaf area (Meinzer and Grantz, 1990; Pataki *et al.*, 1998). Also, in *Eucalyptus nitens* (Deane and Maiden), rates of net CO₂ assimilation (A) increased following green pruning (Pinkard and Beadle, 1998b) and the magnitude of the response was generally greater in ramets than trees, and increased with increasing severity of pruning (Pinkard *et al.*, 1998). However, excess thinning decreases both height and diameter of trees (Pinkard and Beadle, 1998a). However, these authors have not considered the hydraulic architecture parameters relating to water relations, to show the influences of hydraulic conductances on A and g_s.

1.4 Loss in hydraulic conductivity

Loss in hydraulic conductivity is frequently observed among the different growth forms (conifers versus angiosperms, shrubs, and lianas). This has been related to phenomena such as the influence of conducting tissues (Tyree and Ewers, 1991; Tyree and Ewers, 1996; Zwieniecki and Holbrook, 1998), species differences (Brodribb and Hill, 1999), diurnal variation in water demand (Zwieniecki and Holbrook, 1998), water stress (Schultz and Mathews, 1988; Alder *et al.*, 1996; Hacke and Sauter, 1996; Lo Gullo *et al.*, 1998), seasonal differences in water availability or freezing winter temperatures (Sperry *et al.*, 1987; Sperry *et al.*, 1988b; Cochard *et al.*, 1997; Brodribb and Hill, 2000; Brodribb *et al.*, 2002), development and ageing of branches (Cochard *et al.*, 1997) and the downward orientation in shoots of *Vitis vinifera* L. (Schubert *et*

al., 1999). This loss in conductivity is strongly related to cavitation events in the xylem, and subsequent embolism formation.

1.4.1 Xylem cavitation and its detection

Emboli in water conducting tissue arise from cavitations caused by water stress or winter freezing and block the passage of water through the tissue. (Zimmermann and Brown, 1971; Sperry, 1986; Jaquish and Ewers, 2001; Cochard, 2002). Cavitation events in the tissues can be monitored microscopically, anatomically, acoustically and hydraulically.

Renner and Ursprung (1915) (*loc. cit.* Zimmermann, 1983) first observed the phenomenon of cavitation in the annulus cells of fern sporangia under the microscope at tensions of ca. -30 MPa and Milburn (1970) (*loc. cit.* Zimmermann, 1983) observed cavitations in ascospores at tensions of -1.80 to -7.10 MPa. However, Pierce (1936) (*loc. cit.* Tyree and Sperry, 1989a) may have been the first person to demonstrate the occurrence of cavitation in the stem segments of trees *in situ* when transpiration was high, by freezing stem segments with liquid nitrogen and blowing air through the subsequently excised segment.

Milburn and Johnson (1966) introduced an acoustic technique to allow the detection of cavitation in whole organs. They clamped a microphone probe to the petiole of a *Ricinus* leaf suspended in air and detected amplified vibrations or clicks when the walls of xylem that had been strained inwards by high tensions in the water were released by cavitation events. The dominant frequency was around 500Hz (Milburn, 1979). However, they used low frequency equipment and so investigations were confined to the laboratory and only with certain plants (Milburn, 1973a, b). After their initial effort, various workers in the middle of the 1970s & 1980s used the acoustic detection technique in the audible range of 500Hz to detect cavitation events in xylem sap of many plants.

Considering the above, Tyree and Dixon (1983) developed a more powerful technique to detect acoustic emissions using ultrasonic frequencies to measure the energy and frequency range of the acoustic emissions. The advantage of ultrasonic acoustic emission (UAE) over the low frequencies acoustic technique is that the UAE technique selectively amplifies high frequency vibrations and simultaneously filters out the lower range frequencies associated with audible sound that can be produced by various experimental manipulations (Tyree and Sperry, 1989b). Ultrasound acoustic emissions associated with cavitation events (Tyree and Dixon, 1983; Tyree and Sperry, 1989 a, b) are presumably produced by the vibrating walls of cavitating xylem conduits (Tyree *et al.*, 1984). Ultrasound is propagated for shorter distances in air and the problem of extraneous noise is therefore minimized, and thus it became possible to use this technique in the natural environment (Jackson and Grace, 1996). In the field, UAE sensors were mounted on the exposed xylem of trunks and daily cavitation events measured at an adjusted 74 decibels (Jackson *et al.*, 1995a). Also, *in situ* measurements have been demonstrated, using an UAE with low amplification gains on plant material excised from gymnosperms (Tyree and Dixon, 1983; Tyree *et al.*, 1984; Sandford and Grace, 1985; Tyree and Dixon, 1986; Pena and Grace, 1986; Nardini and Salleo, 2000) and from angiosperms (Milburn, 1973a, b; Sanford and Grace, 1985; Ritman and Milburn, 1988; Lo Gullo and Salleo, 1991; Vander Willigen *et al.*, 2000). Detected UAE data have been available for various plant organs like internodes, and node to petiole junctions (Salleo and Lo Gullo 1989; Lo Gullo and Salleo, 1991) and leaf blades (Milburn, 1973a; Kikuta *et al.*, 1997; Salleo *et al.*, 2000; Nardini *et al.*, 2001).

Sperry *et al.* (1988a) introduced a direct but destructive method to quantified the extent of xylem embolisms using excised plant segments in a low-pressure hydraulic conductivity apparatus. The hydraulic conductivities are measured when the segments are perfused with perfusing solution at a low pressure before and after the removal of air embolism by a flush at a high pressure of 175 kPa. The hydraulic conductivity of the plant segment is computed as the quotient of mass flow rate and pressure gradient. The percentage difference in the initial and maximum conductivity is the percentage loss of hydraulic conductivity. Kolb *et al.* (1996) have modified the design of the

technique of Sperry *et al.* (1988a) to facilitate hydraulic studies of densely branched shoot or root systems, low conductances in small plant forms, by pulling water under a given vacuum pressure through the xylem. In a latest development, Nardini *et al.* (2001) used the same method and measured the loss of hydraulic conductivity by embolism in leaves.

Salleo and Lo Gullo (1989) quantified the xylem embolism by perfusing the wood with safranin dye under high pressure for several hours. Later, the dyed stem materials were sectioned and observed under the microscope for stained and unstained xylem conduits. The reduction in xylem hydraulic conductivity by embolisms was localized and quantified from the stained and unstained vessels with their diameters being taken into account.

Comparative studies made by Lo Gullo and Salleo (1991) showed similar result among the acoustic emission, low-pressure hydraulic conductivity and anatomical methods. Similarly, studies of Hacke and Sauter (1995 & 1996) demonstrated that cavitation quantified by acoustic emission, hydraulic measurements on air-dehydrated tissue and air pressure injected tissue showed similar results. Nevertheless, each of these methods has advantages and disadvantages, so they are not alternative to one another (Lo Gullo and Salleo, 1991). As pointed out by Salleo *et al.* (2000) an UAE technique does not provide information about the impact of xylem cavitation on wood hydraulic conductance. However, the acoustic emissions technique is nondestructive and allows for continuous monitoring (Lo Gullo and Salleo, 1991; Jackson and Grace, 1996; Salleo *et al.*, 2000).

Very specialized, nondestructive and costly methods have been developed by various researchers to detect xylem embolisms using a beam of gamma rays (Dixon *et al.*, 1984), X-rays (Habermeh, 1982) and NMR studies (Ratkovic and Bacic, 1993) with computer tomography (Raschi *et al.*, 1995). In addition, cryo-scanning electron microscopy has been used to visualize the occurrence of cavitation *in situ* (Utsumi *et al.*, 1996; Ohtani and Fujikawa, 1999; McCully, 1999; Utsumi *et al.*, 1999).

According to Tyree and Sperry (1989a) hydraulically measured vulnerability curves generate a useful gauge of the impact of embolism on plant water relations. But, such xylem vulnerability measurements are usually made in the laboratories, on either excised stems or root segments, and these segments cannot sense hydraulic or chemical signals from the roots, that would influence stomata and hence possibly avoid cavitation events. Therefore, little is known about daily cavitation events measured occurring in living plants in the field, except the measurements by Jackson *et al.* (1995 a, b); Jackson and Grace (1996) using an UAE sensor.

In this present study, the daily courses of xylem cavitation events were detected using an ultrasonic acoustic emission instrument concurrently with other leaf physiological parameters (ψ_x and leaf gaseous exchange). These are non-destructive methods, which has an advantage in time-based experiments with limited numbers of replicates.

1.5 Vulnerability of xylem to embolism

Cavitation is biologically important because embolized conduits reduce the hydraulic conductivity of xylem under water stress condition (Tyree and Sperry, 1989a); this occurs mostly in the more vulnerable secondary organs, thereby protecting the primary plant body. It has been suggested that xylem operates close to the water potential leading to catastrophic embolism cycles that lead to total xylem dysfunction (Tyree and Sperry, 1989a). The vulnerability of xylem to cavitation of various plant components has been revealed from the vulnerability curves using various methods by various researchers. It is apparent vulnerability differs among species, plant components and sites. There does appear to be a good correlation between vulnerability of xylem to cavitation in various plant components and their drought tolerance (Cochard *et al.*, 1992a; Alder *et al.*, 1996; Hacke and Sauter; 1996; Kavanagh *et al.*, 1999; Brodribb and Hill, 1999)

1.5.1 Segmentation: xylem vulnerability of plant components

Studies on four European oak species indicate that vulnerability to water stress induced cavitation of petioles and twigs were similar for each species, but significant differences in vulnerability between species were identified, the most vulnerable being *Quercus rubra* (Cochard *et al.*, 1992a). In *Acer saccharum*, leaf bearing smaller stems were more vulnerable than larger stems. The vulnerability of stems to cavitation in the tropical, tree *Schefflera morototoni* was greater than that of *Acer saccharum*, and it has been suggested that this is a drought avoidance mechanism in *S. morototoni* as the water released by cavitation events is available for transpiration (Tyree *et al.*, 1991). According to Salleo *et al.* (2000) leaves of *Laurus nobilis* were more susceptible to xylem cavitation than their supporting shoots, and these data support the segmentation hypothesis of Zimmermann (1983). According to Nardini *et al.* (2001), stems and leaf midribs of *Prunus laurocerasus* were equally vulnerable to xylem cavitation and the leaf-midrib hydraulic conductance data fitted to a model of hydraulic conductance of leaves suggested that water can flow around embolized regions in the leaf hydraulic pathways. The petioles of drought grown *Juglans regia* were more vulnerable to xylem cavitation than stems (Tyree *et al.*, 1993b). Likewise in *Alnus glutinosa*, petioles were more vulnerable to cavitation (Hacke and Sauter, 1996). In *Fraxinus excelsior*, vulnerability of leaf rachides within branches decreased along the sap pathway such that rachides of leaves from lower branches were less vulnerable than upper branches (Cochard *et al.*, 1997).

Most of the available data are confined to the shoots only. Even if measurements are partitioned into leaves, petioles, twigs, branches and main trunk they would not be able to identify the real site of xylem vulnerability, without studies extending up to the whole plant level, including roots. Indeed, recently published work on the vulnerability of the xylem of different plant components to cavitation show that roots are more vulnerable than other plant parts (Sperry and Saliendra, 1994; Hacke and Sauter, 1996; Alder *et al.*, 1996; Sperry and Ikeda, 1997; Kavanagh *et al.*, 1999).

In *Acer grandidentatum*, roots were more susceptible to embolism than stems, and furthermore, significant differences occurred between plants growing in riparian (wet) and slope (dry) sites, with riparian plants root being more vulnerable, whereas stem

vulnerability was not different between sites (Alder *et al.*, 1996). Similarly in *Populus balsamifera* roots were more vulnerable than twigs (Hacke and Sauter, 1996) and roots of *Psuedotsuga menziensii* (Mirb.) Franco and *Abies concolor* (Gord. & Glend.) are more vulnerable than their stem and smaller roots and stems are more susceptible than larger ones (Sperry and Ikeda, 1997). However, seedlings populations of *Psuedotsuga menziensii* (Mirb.) showed stems and roots were less vulnerable (Kavanagh *et al.*, 1999) than mature branches and roots (Sperry and Ikeda, 1997). In *Betula occidentalis*, xylem cavitation acropetally decreases from roots, trunks, twigs and to petioles, respectively and roots were more vulnerable to xylem cavitation among the other plant parts (Sperry and Saliendra, 1994). In contrast, whole plant measured vulnerability in *Acer saccharinum* showed petioles were most vulnerable at -0.5 MPa compared to roots (-2.2 MPa) and vulnerability of stems was intermediate between petioles and roots (Tsuda and Tyree, 1997). As *Acer saccharinum* is one of the most vulnerable species (Tyree *et al.*, 1994b) the difference in vulnerability of the segments may provide the ability to survive during droughts (Tsuda and Tyree, 1997).

1.5.2 Relationship between xylem vulnerability and response to drought

In woody dicotyledons, vessel diameters and lengths increase in a basipetal direction from twigs to branches, to stem and finally to roots and often continue to increase in the roots with increasing distance from the trunk, such hydraulic design leading to efficient flow (Zimmermann, 1983). The inner diameter of vessels are variable, depending upon age and location within the tree, and this is an hydraulically important parameter (Zimmermann, 1983). There is a general trend that large conduit diameters are more vulnerable to drought and freezing induced embolism (Lo Gullo and Salleo, 1991; Cochard and Tyree, 1990), and also may be less able to recover from embolism once it occurs (Tyree and Yang, 1990). There is a positive correlation between xylem anatomical characteristics and vulnerability as has been reported from various studies. Thus, large vessel diameter in *Betula occidentalis* (Sperry and Salindera, 1994), *Fraxinus excelsior* L. (Cochard *et al.*, 1997) and longer and wider vessels in *Populus balsamifera* (Hacke and Sauter, 1995) correlated with hydraulic vulnerability. Xylem vulnerability can also increase with decrease in rachis diameter

(Cochard *et al.*, 1997), stem and roots xylem diameter (Sperry and Ikeda, 1997) and root xylem diameter, but not the stem xylem diameter in douglas-fir-seedlings (Kavanagh *et al.*, 1999). In contrast, vulnerability to xylem cavitation in *Alnus glutinosa* (Hacke and Sauter, 1996), *Acer grandidentatum* (Alder *et al.*, 1996), *Psuedotsuga menziesii* and *Abies concolor* (Sperry and Ikeda, 1997) and two CAM succulents (Linton and Nobel, 1999) was not correlated with either root size or conduit diameter.

In addition, by Poiseuille's law, the hydraulic conductance per unit pressure gradient is proportional to the conduit diameter to the fourth power (Calkin *et al.*, 1986). So, smaller conduits induce higher xylem tensions and thus more water stress in the plants. However, narrow xylem conduits provide an interwoven auxiliary transport system when many of the wider conduits become embolized (Hargrave *et al.*, 1994). Nonetheless, embolism formation in those conduits is not directly related to conduit diameter (Tyree and Sperry, 1989a). It is the diameter of the pore in the pit membrane on the conduit walls that determines conduit vulnerability to embolism formation. The pit membrane pore depends on the availability of water, reserves carbohydrates and the specific genetic information for wall construction (Sperry and Tyree, 1988). Therefore, conduits large in diameter may have many pit pores (Hargrave *et al.*, 1994) and be easily embolized. Available evidence shows that the vulnerability to embolism is related to pit pore in the conduit (Alder *et al.*, 1996; Sperry and Ikeda, 1997; Harvey and Van Den Driessche, 1997). Diameters of root conduits are larger than those of stems with more pit membranes than stems and the pressure required to displace the torus from its sealing position over the pit aperture may be reduced (for gymnosperms only, as no torus in angiosperms), making roots more vulnerable than stems (Sperry and Ikeda, 1997). The smaller the pores in the pit membranes, the lower the water potential required to induce embolism (Sperry and Tyree, 1988; Cochard *et al.*, 1992b). However, the existence of pores will reduce the hydraulic conductivity (Sperry and Tyree, 1988; Tyree and Ewers, 1991) well below the theoretical maximum predicted by Poiseuille's law (Tyree *et al.*, 1994a; Tyree and Ewers, 1996).

Embolism vulnerability curves constructed in stem seedlings of *Eucalyptus camaldulensis* Dehnh., from two different climatic zones but grown together showed that threshold water potential for initiation of embolism was lower in semi-arid grown zone seedlings compared to humid zone ones (Franks *et al.*, 1995). The threshold xylem tension to induce cavitation was as high as -0.5 MPa in an evergreen stem of *Curatella americana* L and 50% of hydraulic conductivity was lost by embolism around -1.4 MPa (Sobrado, 1996). The branches of four different seven years old *Eucalyptus* spp. clones that were collected from mesic and xeric sites showed that vulnerability of xylem to cavitation differed among clones, but not the sites, and the most drought susceptible clone was the one with highest vulnerability (Vander Willigen and Pammenter, 1998). On the other hand, Kavanagh *et al.* (1999) found that vulnerability of shoot and root xylem of douglas-fir seedling populations varied genetically and with the climate of the area the seedlings came from and shoot and root xylem was most susceptible in seedlings grown in a coastal wet site. Xylem vulnerability to drought induced embolism in both tropical heath and mixed dipterocarp forest tree species were variable, and with such large differences within both forest type species there were no significant difference between forest types (Tyree *et al.*, 1998). A large range of xylem vulnerability to cavitation was found to occur among stem segments of conifer species, but plants from wet sites were found to be more prone to cavitation than those from arid zone (Brodribb and Hill, 1999).

The roots of *Artemisia tridentata* are more vulnerable than stems, and actual hydraulic conductances predicted from vulnerability data show that during drought roots limit the water supply such that transpiration is reduced to maintain water potentials within the safety margin (Kolb and Sperry, 1999). It has been suggested that an increase in hydraulic resistance of roots caused by xylem cavitation is a beneficial safety margin to protect the stem from low xylem pressure during extreme droughts (Sperry and Ikeda, 1997). On the other hand, Sperry and Saliendra (1994) and Alder *et al.* (1996) suggested that reduction in soil water extraction by high root resistances may prolong the water availability during a drought and prevent cavitation at the soil-root interface.

1.6 Stomata and photosynthetic capacity

1.6.1 What is sensed by stomata for their regulating role in plants?

Hydraulic architectural designs and xylem vulnerability to embolism can lead to stomatal closure and thus reduction in water loss, resulting in a decline in carbon uptake by the leaves. Stomata are synergistic gas regulators in the plant organs that keep the plant water status above the cavitation threshold by limiting rate of diffusion of water vapour out, but at the same time permitting diffusion of CO₂ into leaves. Stomata responses to both plant and environmental factors determines their movements. Stomatal responses that decrease water loss also decrease photosynthesis, and responses that increase photosynthesis also increase water loss (Christopher *et al.*, 1989).

Stomatal closure is one of the plants important defense mechanisms against further water loss and ultimate death by desiccation (Kirschbaum, 1987). Water stress brought about stomatal closure and caused photoinhibition in *Eucalyptus pauciflora* (Kirschbaum, 1987). Low stomatal conductances caused lower rates of carbon fixation in water- stressed than in well-irrigated *Eucalyptus globulus* (Labill.) (Pereira *et al.*, 1992), and so over- all plant productivity can be restricted by stomatal conductance.

There are various reports that stomata respond to hydraulic signals caused by a decrease in leaf water potential, whereas other reports revealed that chemical signals transmitted from either leaves or roots, to cause stomatal closure under unfavorable conditions.

Meinzer and Grantz (1990) suggested that changes in stomatal conductances positively related to plant hydraulic conductances of sugar cane were probably mediated by the level of root metabolites (abscisic acid, ABA). Fort *et al.* (1997) demonstrated that different watering regimes leading to soil drying reduced the whole plant biomass in oak seedlings. The effect was mediated through low stomatal

conductance, although ABA produced in the roots in response to drought did not reach the shoot. Stomatal closure in response to partial soil drying was not due to a direct hydraulic limitation but to some non-hydraulic, root-to-shoot signaling (Crocker *et al.*, 1998).

According to Saliendra *et al.* (1995) root-to-shoot non-hydraulic signaling is an inefficient mechanism for rapid stomatal control due to the lengthy transport time in large plants. Changes in leaf water potential can affect stomatal conductance independently of soil water potential (Saliendra *et al.*, 1995). In *Glycine max* [Merr.] L. cv. Clark., stomatal conductance is determined by a signal produced by local leaf water potential rather than root or soil water status (Bunce, 1999). Xylem cavitation in detached or well-watered whole plant shoots produces hydraulic signals responsible for rapid regulation of stomatal conductances, regardless of any root signals (Salleo *et al.*, 2000).

Short term root chilling caused an immediate decrease in leaf water potential and transpiration and such changes resulted from the decrease in root conductance due to increased fluid viscosity and this increased xylem cavitation in conifers and angiosperms (Brodrigg and Hill, 2000). In a similar method, in *Quercus robur*, transpiration decreased linearly with decreasing soil and root temperature, but without changing the leaf water potential, as the effect of chilling induced reduction in root hydraulic conductances (Cochard *et al.*, 2000). Cochard *et al.* (2000) speculated that the possible effect on stomatal conductances in *Quercus robur* might have been regulated by an hydraulic signal, as ABA was not detected in the xylem sap. However, the response of long term leaf physiology and plant growth to reduced root hydraulic conductance brought about by soil chilling has not been investigated to our knowledge.

Xylem cavitation may also act as a rapid hydraulic signal initiating the stomatal response (Tyree and Ewers, 1991). Jones and Sutherland (1991) suggested that plants might either reduce stomatal conductances to prevent xylem cavitation or maximize stomatal opening, even if at the cost of some cavitation. Also, Nardini and Salleo

(2000) suggested that some xylem cavitation-induced reduction in shoot hydraulic conductances is the signal for stomatal closure preventing runaway embolism.

On the other hand, Thomson *et al.* (1997) and Jones (1998) suggested that stomatal response to drought is the result of the integration of hydraulic and root-generated chemical signals. However, the mechanism linking xylem cavitations and stomatal response remains uncertain and requires further investigation.

1.6.2 Plant hydraulic supply and its influence on leaf photosynthetic capacity

It is a common concept that higher hydraulic conductances, both root and shoot, lead to less leaf water stress, a decrease the extensive amount of xylem cavitation, and an increase leaf gas exchange, which in turn promotes growth. But it is still unclear whether a high hydraulic conductance, due to wider conduits can be of advantage to plants under water stress conditions (Nardini and Tyree, 1999). Few studies have reported parallel measurements of both hydraulic conductances and the leaf level physiology to show the relationships between them.

Ryan and Yoder (1997) hypothesized the hydraulic limitation in old and tall trees, where increasing axial hydraulic resistances decreased the stomatal conductances, lead to lower net CO₂ uptake, resulting in the poor growth. However, Becker *et al.* (2000) have argued that the growth of tall trees is not necessarily limited by increasing axial resistances, and that it could be genetically programmed competitive factors, like resource allocation and reproduction, which essentially permit the survival of old trees but limit further growth.

However, several recent studies have revealed that differences in hydraulic conductances could affect photosynthesis through effects on gas exchange. There is a correspondence between decreasing hydraulic conductances in different levels of branches in ageing conifer trees, and a decline in stomatal conductances. (Mencuccini and Grace, 1996; Hubbard *et al.*, 1999). The hydraulic conductance of *Phaseolus vulgaris* L. leaves were positively correlated with steady state values of stomatal

conductances and photosynthetic rate in water deficient plants (Sober, 1997). Stomatal control of transpiration was often coordinated with leaf-area-based water transport capacity in sugar cane (Meinzer and Grantz, 1990). Both stomatal conductances and transpiration were positively correlated with the conductance of the hydraulic soil-leaf pathway in evergreen woody species, suggesting that limitations to transpiration associated with high atmospheric evaporative demand were attenuated in deeply rooting species (Meinzer *et al.*, 1999). Drought increasing hydraulic resistances in mature scots pine were associated with stomatal closure that prevented excessive embolisms, and with reduced growth associated with low CO₂ assimilation rate (Irvine *et al.*, 1998). The reduction in hydraulic conductance of the branch xylem of *Betula occidentalis* through induction of embolisms by air-injection (Sperry and Pockman, 1993), and notching (Sperry *et al.*, 1993), and the increase in whole plant hydraulic resistance in ponderosa pine seedlings by air-injection into the stem (Hubbard *et al.*, 2001) brought about immediate closure of stomata, which reduced transpiration to keep xylem pressure above the cavitation point. However, in experiments on branches of old and young *Pinus ponderosa* trees similar manipulations had no effect on the stomatal conductances and CO₂ assimilation rate (Hubbard *et al.*, 1999). Saliendra *et al.* (1995) demonstrated that pressurizing the soil reversed the closure of stomata in response to decreased soil-plant hydraulic conductances and increased evaporative driving forces. Following the partial removal of canopy leaf area, *Eucalyptus nitens* showed increased rates of CO₂ assimilation in the remaining leaves, as their stomatal conductances increased steeply following pruning (Pinkard *et al.*, 1998b). A group of rainforests conifers and angiosperms showed a close significant relationship between the leaf photosynthetic capacity (θ_{PSII}) measured by chlorophyll fluorescence and the stem hydraulic supply expressed per leaf area (Brodribb and Field, 2000). In contrast, leaf specific hydraulic conductivity in *Eucalyptus grandis* did not change, despite increases in leaf photosynthetic capacity under different fertilization regime (Clearwater and Meinzer, 2001).

These available data supporting the hydraulic limitation hypothesis come mostly from temperate woody plants, rather than tropical plants. As such, the validity of the

hydraulic limitation hypothesis in tropical canopy trees needs to be tested with trees representing a range of sizes and species (Goldstein *et al.*, 1998).

1.7 Xylem recovery

1.7.1 Xylem embolism repair by root pressure

The repair of xylem embolism following loss in hydraulic conductivity is important in maintaining the supply of water to evaporating surfaces. There are two well-known mechanisms for embolism recovery; replacement of embolized xylem by new xylem production, and refilling of embolized xylem by near-positive xylem pressure. Production of new xylem tissue is generally a seasonal pattern, and some species lack refilling mechanisms (Cochard *et al.*, 1994) and depend solely on newly forming xylem vessels (Cochard *et al.*, 1997, 2001). To dissolve xylem embolisms, adequate near-positive xylem pressure over time is required. The surface tension of water will raise the pressure of air in a bubble above the pressure in the surrounding water, this effect increasing with decreasing bubble size, and the high pressure of the air in the bubble will cause it to dissolve in the water, refilling the embolized conduit (Tyree and Ewers, 1996). Positive root pressure can increase the xylem water pressure sufficiently to bring about the dissolution of embolism. Root pressures are present in most plants, except in conifers, but only when ample moisture is present in the soil and when humidities are high, that is, when transpiration is low (Salisbury and Ross, 1991). The root pressure is considered an active process and is a consequence of pressure developed in the tracheary elements of the xylem as a result of metabolic activities of roots (Devlin, 1966).

However, the pressure produced by roots of trees is generally inadequate to dissolve embolism, and is able to refill xylem only in smaller plants (Devlin, 1966). Nonetheless, it has been well documented that high positive pressures rapidly dissolve xylem embolisms in the twigs of *Laurus nobilis* (Salleo *et al.*, 1996). Likewise, in grapevines and sugar maple, vessels embolized in winter have been filled by root pressures in the spring season (Sperry *et al.*, 1987; Sperry *et al.*, 1988b) and after rainfall in *Acer*

grandidentatum (Alder *et al.*, 1996). Recovery of full hydraulic conductivity associated with midday positive root pressures have been reported in *Betula cordifolia* (Sperry, 1993). In contrast, root pressure in many dicotyledonous vines were inadequate to refill embolized vessels in canopy stems and these vessels remain permanently non-conductive (Tyree and Ewers, 1996). Embolism in roots may be partially reversed when the soil is wet (Alder *et al.*, 1996).

However, positive root pressures do not develop in all species, and other processes leading to near-atmospheric xylem pressures must occur. Overnight root pressures were not detected in the clonal ring porous temperate tree species *Sassafras albidum* and *Rhus typhina* in frozen soil, and the reversal of embolisms must have occurred by a mechanism other than positive root pressures (Jaquish and Ewers, 2001). Furthermore, positive root pressures are not detected in coniferous species, and recovery mechanism are unknown. It is apparent, however, that embolism repair whilst xylem water is under tension does occur. Daily refilling of root xylem vessels of field-grown maize (McCully *et al.*, 1998) was observed at the time of highest transpiration rate (McCully, 1999). Similarly, diurnal repair of xylem embolism and a balance between cavitation occurrence and removal was observed by Zwieniecki and Holbrook (1998) in *Fraxinus americana*, *Acer rubrum* and *Picea rubens*, who suggested that this is an energy demanding process that involves the active secretion of solutes from adjacent phloem tissue. Salleo *et al.* (1996) suggested an auxin-induced loading of the embolized conduits with phloem solutes, thus decreasing their osmotic potential and promoting xylem refilling.

1.7.2 Water storage capacitance

The amount of water absorbed by the plant root system is not equal to the amount water transpired, as part of the transpirational water stream is routed into adjacent tissues, called capacitors. The tissue capacitance is defined as the relative change in tissue water volume for a given change in tissue water potential (Nobel, 1983). The change in plant size with age, and the existing water potential gradients between capacitors and transpirational path may determine the water storing capacity of plants.

Zimmermann (1983) explained water storage in plants by three mechanisms: a) elastic, which is water taken up or released as a consequence of change of tissue volume, b) capillary, water associated with air spaces adjacent to tracheary elements; this water is released at potentials about -0.5 MPa, and c) cavitation, this is water released when cavitation events occur in xylem conduits. The water storage capacity in plants might determine their ability to survive under adverse conditions (Tyree and Yang, 1990; Tyree *et al.*, 1991; Tyree and Ewers, 1996). The water conserved in capacitors postpones the onset of water deficits and the dehydration of cells (Kramer and Boyer, 1995). Water storage capacity may partially compensate for increases in axial hydraulic resistances under water shortage (Goldstein *et al.*, 1998). Water exchange from capacitors occurs on a diurnal and seasonal basis (Waring and Running, 1978) and may be tightly coupled to fluctuations in environmental conditions (Goldstein *et al.*, 1998). The recharge of internal sapwood storage water for transpiration has been demonstrated by Running (1980). In some cases high water storage capacity may maintain maximum rates of transpiration (Tyree *et al.*, 1991; Goldstein *et al.*, 1998), whereas in other instances capacitor water makes a negligible contribution to transpirational water loss (Koide *et al.*, 1989; Tyree and Yang, 1990).

1.8 Present study

To our knowledge, few studies have been undertaken into the hydraulic architecture of fast and tall growing *Eucalyptus* species or hybrid clones. Studies like those of Franks *et al.* (1995) and Vander Willigen and Pammenter (1998) measured hydraulic conductances in stems and branches only, using the xylem air-permeability method and the low pressure flow conductivity apparatus, respectively. Recently, branch level hydraulic architecture has been studied using the low-pressure steady state flow meter (SSFm) by Brodribb and Field (2000), and using the HPFM by Clearwater and Meinzer (2001). Some of these authors demonstrated a good relationship between some parts of above ground hydraulic architecture and the leaf photosynthetic capacity (Brodribb and Field, 2000). The relationships between root hydraulic architecture and leaf physiology or the role of roots as a component of whole plant hydraulic architecture are unknown. Becker *et al.* (2000) suggested that observations

at the whole plant level are more pertinent than those at the branch level for determining transport sufficiency of water and solutes to leaves.

The overall objectives of this study were to relate plant hydraulic architecture to leaf physiology and growth of three commercial *Eucalyptus* spp. clones, and to assess the influence of soil water on these characteristics.

The experiments consisted of growing three *Eucalyptus* spp. clones in pots, and subjecting them to either high or low watering regimes. During growth various physiological measurements were undertaken. Harvests were taken at ages of 14 and 21 months and growth responses assessed. Studies were undertaken on potted material for convenience: it is possible to manipulate water supply, and at the end of the experiment roots can be excavated relatively easily. In addition, some measurements were taken on young three months old saplings of two of the clones growing at a mesic site in the field. Unfortunately, similar aged plants of these clones growing on a drier site were not available.

The specific objectives of the study were:

(a) to measure the impact of high and low watering treatments on growth rate of potted plants three selected *Eucalyptus* spp. clones and relate these to leaf physiological properties such as water potential, stomatal conductance and gas exchange characteristics.

(b) to assess the effects of treatment and clone on hydraulic characteristics, including hydraulic architecture and vulnerability to cavitation. This involved destructive measurements of root pressure and of hydraulic conductances, using a high-pressure flow meter, at the end of the experimental growth period, and non-destructive detection of cavitation events using an ultrasonic acoustic emission detector.

(c) to assess whether there are any relationships among hydraulic characteristics, leaf physiological properties and growth, and whether these are influenced by water supply and clone.

(d) to assess whether any relationships identified in (c) above are causative: do low hydraulic conductivities or high vulnerability to cavitation lead directly to reduced gas exchange, low photosynthetic carbon assimilation, and low growth? To do this requires experimental long-term manipulation of hydraulic conductances; this was achieved by subjecting plants to root chilling (by chilling the soil) for a period of two months.

Chapter 2: Sapling Maintenance and General Physiological Techniques

This Chapter describes the process of selection, preparation and maintenance of the plant material, and the general physiological techniques common to all the studies, together with studies to validate the acoustic emission technique to assess cavitation events. Details of experimental design are given in the appropriate chapters.

2.1 Selection of material and maintenance of potted saplings

This section describes the establishment and maintenance of potted saplings of three selected *Eucalyptus* spp. clones grown in either 25 l or 85 l pots over a period of 14 and 21 months with two watering regimes, respectively.

2.1.1 Plant materials and study site

Three *Eucalyptus* spp. clones were selected on the basis of their drought susceptibilities, as assessed from the experiences of field foresters. The clones chosen were GC550, a *Eucalyptus grandis* x *camaldulensis* hybrid, GU210, a *Eucalyptus grandis* x *urophylla* hybrid, and TAG14, a pure *Eucalyptus grandis* clone. TAG14 is considered to be drought susceptible, GC550 relatively drought tolerant, whilst the drought response of GU210 which is recently developed hybrid, is not known. Planting material (rooted macrocuttings) was obtained from Mondi Forests, Tree Improvement Research Unit, Hilton, South Africa.

Potted material was grown outdoors in the greenhouse complex, School of Life and Environmental Sciences, University of Natal, Durban, South Africa, fully exposed to natural solar radiation.

2.1.2 Potting process and potting medium

Pots used were either 25 or 85 l capacity, for short- (14 month) and long- (21 month) term studies, respectively. Plastic sheeting was placed under the pots to restrict root penetration into the ground through the bottom drainage holes. Single holes, 7.7 cm in diameter, were drilled in the side of the pots at half-height to permit the insertion of a soil moisture probe. These holes were covered on the inside with a fine perforated plastic mesh to prevent soil falling from the pot, but permitting insertion of the soil moisture probes. On the outside the holes were covered with an aluminum foil and over that a double layer of plastic (which could be removed) to prevent evaporation of water from the soil.

The potting soil used was a mixture of four parts sand, four parts loam and three parts of compost (4:4:3), delivered in two separate loads. One load one was used to fill the 25 l pots, the other for the 85 l pots; thus within a pot size, the soil was homogeneous, but may have differed slightly between pot sizes. Pots were filled with soil according to a technique described by Bohm (1979). Wet soil was added layer by layer and uniform packing was obtained by adding water to the pot and permitting drainage after the addition of each layer. Thereafter, all pots were watered several times and allowed to stand for 2 weeks before planting. Ramets were potted on 24th February, 2000 and initially watered daily to field capacity ($0.38 \text{ m}^3 \cdot \text{m}^{-3}$) for a period of four weeks to permit establishment. At this stage, the pots were fitted with lids, with central holes for the stem and a slit from the central hole to the edge to allow removal of the lid. This arrangement was to exclude rain so that water supply could be controlled; during non-rainy days lids were removed to permit soil aeration. Prior to beginning the watering treatments, water supply was reduced for a few days so that the soil water content of the low watering treatment was reduced to a level below field capacity. At the start of watering treatment, initial mean plant height was 0.32 m, over-bark stem diameter 0.1m above soil was 2.6 mm, and soil water content of the low and high water treatments was $0.24 \text{ m}^3 \cdot \text{m}^{-3}$ and $0.35 \text{ m}^3 \cdot \text{m}^{-3}$, respectively. Watering treatments were initiated on 3rd of April 2000. Unfortunately, regular

monitoring of soil water was not possible as the soil probe used (ThetaProbe Type ML2x, Delta-T-Devices, Cambridge, England) had delicate probes which broke on several occasions.

2.1.3 Watering treatments

Two watering treatments were applied. The 'high water' treatment was designed to mimic the annual rainfall (1280-mm) in the region where the selected clones were grown (Fig. 2.1.1), and the low water treatment was 70% of this. The surface area of the pot was calculated, and the volume of a column of water this area and 1280 mm high gave the total water to be added to the high water treatment over a year. The rate of application was varied over the year according to mean monthly rainfall, and the water for one month was added in eight to nine equal amounts, twice every week (generally Mondays and Thursdays). It was subsequently realized that there was an incorrect assumption in this calculation: in the field, at an espacement of 3.1m x 2.1 m, the volume of soil (to a depth of 1.5 m = 9.76 m³), and hence the amount of water, available to each plant was considerably greater than that available to the plants in the pots (0.025 m³), and the potted plants, even under the high water treatment, became consistently water stressed as they became larger, particularly in the 25 l pots. Consequently, in May 2001 watering regimes were upgraded for 85 l potted plants by doubling the amount of water in the high water treatment (200%) and increasing the low water treatment to 140%.

2.1.4 Nutrient and Fungicide treatments of potted plants

Two months after planting soil samples were taken from both sets of pots (25 l and 85 l) to a depth of 150 mm. The subsamples from different pots were bulked and well mixed and sent to the Soil Analysis and Fertilizer Advisory Service of the KwaZulu-Natal Departmental of Agriculture, Pietermaritzburg, South Africa for analysis, and the results obtained are shown in Table 2.1.1.

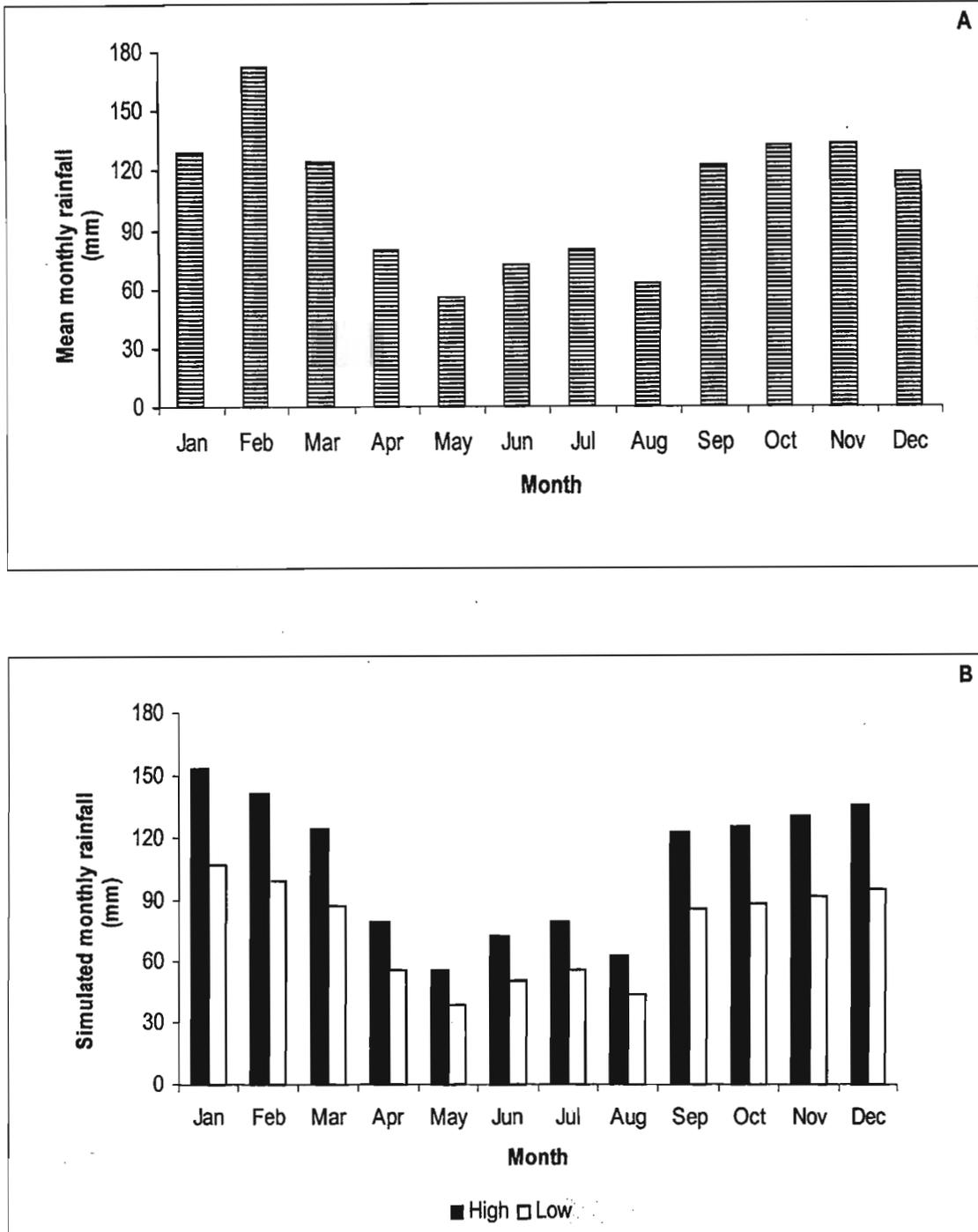


Fig. 2.1.1 Mean monthly rainfall for the past 16 y from the nursery at KwaMbonambi (A), and the simulated rainfall used as the high and low watering treatments for the 14 month potted trial (B). Data courtesy of Tree Improvement Research, Zululand Division, Mondi Forests, KwaMbonambi, South Africa.

Macronutrient were applied from six months after planting. Agrofert Mondi Orange (N: P: K ratio 13.50:18.00:11.40) was made up at a concentration of 1 g l^{-1} and applied as the irrigation water, once a week (thus nutrient application varied through the year as watering varied). Trace elements were applied as a foliar spray, once a month, as 2.5ml Trelmix (Hubers, South Africa) per litre. The composition of Trelmix is 18g Fe, 4g Cu, 2g Zn, 1g B and 0.4g Mo per litre. It was realized both nutrients and fungicides were important particularly when plants were grown in limited conditions like pots in terms of long term studies. As such, both soil and foliar fungicides and nutrients were sprayed once plants were watered frequently. Soil (per litre: 1g prochloraz manganese chloride (Sporgon; Hoechst Schering AgrErvo, South Africa): per litre: 1.25ml tebuconazole (Folicur, Bayer, South Africa)) and foliar fungicides (per litre: 2g mancozeb (Dithane; Efekto, South Africa):Sporgon : per litre:1ml chlorothalonil (Bravo; Shell, South Africa)) were sprayed on Thursdays and Mondays in the first week of each month. Six months after establishment (i.e: in end of August, 2000) soil macronutrients (per litre: 1g Agrofert Mondi Orange N: P: K 13.5:18:11.4) and foliar trace elements solutions (per litre: 2.5ml Trelmix (18g Fe, 4g Cu, 2g Zn, 1g B and 0.4g Mo; Hubers, South Africa)) were added. Soil nutrient was dissolved in irrigating water and added according to their monthly prescribed treatment levels on all Mondays, and foliar nutrients were sprayed on Thursdays after the plants were watered, once a month during the first week. Both fungicides and nutrients were applied throughout the experiment.

Table 2.1.1 Soil chemical characteristics of the potting medium (sampling depth 0-15cm).
Different lots were used for the two pot sizes, which differed slightly in composition.

Soil Characteristic	85 l potted medium	25 l potted medium
Sample density (g/L)	1360.00	1340.00
Soil Particle Fractionation:		
Clay (<0.002mm)%	9.00	9.00
Fine silt (0.02-0.002 mm)%	4.00	4.00
Sand & coarse silt (2.0-0.02 mm)%	87.00	87.00
Texture class	Sand	Sand
Total Nitrogen (%)	0.135	0.116
Phosphorus (mg/100g)	9.56	33.58
Potassium (mg/100g)	13.6	9.63
Calcium (mg/100g)	72.94	54.70
Magnesium (mg/100g)	19.04	15.15
Manganese ($\mu\text{g/g}$)	11.03	8.21
Zinc ($\mu\text{g/g}$)	26.41	18.60
Sodium (mg/100g)	1.12	0.91
EC ($\mu\text{S/cm}^{-1}$)	496.00	425.00
Exch. Acidity (mmol/100g)	0.037	0.045
Total cations (mmol/100g)	5.59	4.31
Acid saturation (%)	1.00	1.00
pH (KCl)	6.91	6.95
pH (H ₂ O)	6.83	6.83
NIRS organic carbon (%)	-	2.00
NIRS Clay (%)	15.00	18.00
Sodium absorption ratio	0.47	0.39
Field capacity (% by weight)	35.3	36.3
Moisture content ($\text{m}^3.\text{m}^{-3}$)	0.392	0.419

2.2 General physiological techniques

This section describes the physiological techniques common to most aspects of this study. Details of experimental design, which depended on the specific objectives, are provided in the following chapters dealing with each aspect of the study.

2.2.1 Leaf water potential (Ψ_l)

Thermocouple psychrometry: A non-destructive method

Leaf water potential was non-destructively measured on potted plants using L-51 leaf hygrometer chambers and an HR-33T Dew Point Microvoltmeter (Wescor Inc., Logan, Utah, U.S.A) in the dew point mode. This non-destructive method of measuring leaf water potential for the potted trials allowed the retention of total leaf area in the shoot. This ensured that maximum shoot hydraulic conductances, measured by a high-pressure flow meter on the same samples were not overestimated (removal of leaves for measurements with a pressure chamber would create lesions through which water would readily pass). Leaves were randomly clamped into leaf chambers at two different levels for each measurement point, one from the upper and one from the middle part of the canopy. Taking of measurements began at 06.00 h and continued at 60-min intervals until 12.00 h and thereafter at every 120-min until evening. The chambers were clamped onto the leaves the evening before the measurements were taken to ensure equilibration of more than 12 h, even though vapour equilibration had probably occurred before this time. Preliminary trial studies show a period of 2 h to be adequate to ensure complete vapor equilibration between the psychrometer chamber and the leaf.

Diffusive resistance to water vapour movement from the leaf to hygrometer leaf chambers was reduced by gentle abrasion of the adaxial leaf surface using aluminum oxide. Then the abraded leaf surface area was cleaned with deionized water, allowed to dry for ten minutes, and the psychrometer leaf chambers sealed against the abraded area (Savage *et al.*, 1984). Vertical supports from the ground were used to hold the psychrometers in position in the leaf canopy. The psychrometers aluminium housing

were insulated with small blocks of polystyrene and white plastic covers to minimize temperature fluctuations.

Leaf shading was minimized to allow adequate vapor pressure equilibration between leaf and the thermocouple psychrometer (Oosterhuis *et al.*, 1983). The leaf chambers were calibrated every after five sets of measurements with NaCl solutions at laboratory temperature (approximately 25°C).

Pressure chamber: A destructive method

A pressure chamber was used to measure leaf water potential whenever removal of leaves would not affect subsequent measurements of other physiological characteristics. For measurements of water potential leaves were not collected from plants used for hydraulic studies. Where appropriate, gas exchange studies were conducted on a selected leaf and then it was immediately covered with a polythene bag and cut under the bag at the petiole-stem junction and the leaf water potential was measured using a Scholander model pressure chamber (Scholander *et al.*, 1965). As the petioles were very short, the leaf blade was trimmed from the midrib for 1cm from the base to ensure that the petiole protruded through the pressure chamber seal.

Comparative measurements between thermocouple psychrometer and the pressure chamber

Comparative studies in measuring leaf water potential were made to ensure that the thermocouple psychrometer and the pressure chamber (Scholander *et al.*, 1965) produced values that were more or less same both in the laboratory and the greenhouse area. Water potentials of leaves of well-watered potted plants of *Eucalyptus* spp. clones were measured in the morning at 08.00 h and at 12.00 h on the same plant using both techniques. Leaves that had been clamped into thermocouple psychrometers were initially measured using the dew point mode. This was followed immediately by pressure chamber measurement of an adjacent leaf, and then on the original leaf after the psychrometer chamber had been removed, leaves being covered with polythene bag prior to excision. The two techniques gave similar values for

water potentials on the same leaf in the experimental area and the laboratory, where adjacent leaf values measured by the pressure chamber were slightly lower than thermocouple psychrometry values (Table 2.2.1).

Table 2.2.1 Comparative measurements between thermocouple psychrometer (TP) and the pressure chamber (PC) at two different experimental sites. Means are given \pm SEM (n=2).

Method	clone	Time	Experimental condition	$\Psi_{L \text{ adjacent}}$ (MPa)	$\Psi_{L \text{ original}}$ (MPa)	Technique
TP	GU210	08.00	Laboratory	-	-0.37 \pm 0.05	TC
PC	GU210	08.00	Laboratory	-0.39 \pm 0.03	-0.37 \pm 0.04	PC
TP	GU210	12.00	Laboratory	-	-0.87 \pm 0.10	TC
PC	GU210	12.00	Laboratory	-0.87 \pm 0.13	-0.87 \pm 0.18	PC
TP	GU210	08.00	Greenhouse area	-	-0.47 \pm 0.03	TC
PC	GU210	08.00	Greenhouse area	-0.52 \pm 0.05	-0.48 \pm 0.03	PC
TP	GU210	12.00	Greenhouse area	-	-1.65 \pm 0.07	TC
PC	GU210	12.00	Greenhouse area	-1.74 \pm 0.06	-1.66 \pm 0.04	PC

There are similar comparative studies that found a nearly 1:1 relationship between psychrometer and the pressure chamber measurements both in the field and laboratory (Oosterhuis *et al.*, 1983; Savage *et al.*, 1984). There were two main problems that were often faced during the measurement of leaf water potential using leaf thermocouple psychrometers in *ex-situ* studies like this. The first was tearing of the leaf clamped into the leaf chamber when high wind velocities occurred during the measurement time. The second problem was observed in some of the vigorously transpiring leaves; water droplets from vapour condensed in the cavity-wells of the thermocouple psychrometers which would lead to some underestimation of midday leaf water potential in the greenhouse area. To overcome these problems, three to five chambers were clamped on each plant and the values of either an undamaged leaf or from a chamber not contaminated by condensed water were taken.

2.2.2 Leaf gas exchange

Measurements of net CO₂ assimilation rate (A), transpiration rate (E) and stomatal conductance (g_s) were performed from morning (06.00 h) to evening (18.00 h or 16.00 h) at one hour intervals up to midday and then at two hours interval till evening with a portable infrared gas analyzer (model LI-6400, Li-Cor, Inc., Lincoln, NE, U.S.A). A healthy entire leaf from the center but outer edge of the canopy was labeled with white thread so that repeat measurements could be made on the same leaves throughout the day. These measurements were generally taken from a leaf adjacent to that attached to the leaf psychrometers. Early morning measurements were started after wiping off the dew (if any) that had condensed on the leaf surface and then it was left for 10 min for the surface to dry. The leaf to be measured was clamped inside the leaf chamber of the IRGA, and when the values displayed by the instrument had been stable for 40 to 60 s, five to ten records per leaf were taken. Measurements were made on two leaves per individual plant and the data pooled give an average value for that time per each plant. All the measurements were carried out on clear days under ambient conditions and concurrent incident photosynthetically active radiation (PAR, $\mu\text{mol m}^{-2} \text{s}^{-1}$), humidity and temperature of the air were measured from the sensors fitted on the top of the leaf chamber of the IRGA to calculate the diurnal vapour pressure deficit (VPD, kPa).

2.2.3 Vulnerability to xylem cavitation

Daily xylem cavitation events detected by ultrasonic acoustic emissions (UAE)

Daily courses of xylem cavitation events were counted in the main stem by the ultrasonic acoustic emission method, using a model I15I, ultrasonic sensor and preamplifier model 4615, Physical Acoustic Corp., Princeton, NJ, USA, sold as a drought stress monitor.

A 20mm-long strip of main stem bark was carefully removed from 0.2m above the soil surface by a sharp razor. A thin layer of water-soluble KY lubricant jelly was smeared between the UAE sensor head and the area of exposed xylem to facilitate the easy transmission of acoustic events to the clamped sensor as well as avoid surface evaporation. The sensor was mounted directly on the exposed tissue portion with masking tape and then using rubber bands to tie both sensor and stem. The portion of stem connected to the sensor was usually covered with polythene film to avoid any overnight rain wetting. The sensor and stem were firmly bound to a vertical support to reduce extraneous recording events caused by movement.

Daily xylem cavitation events were measured at 1-minute intervals from 18.00 h to 18.00 h or 16.00 h the following day. A dead time (SCETO) of 400 μ s with amplification gain of 72 decibels were selected as these settings reduced to a minimum background noise in the experimental area. The AE sensor was usually installed 30 min prior to commencement of measurements to ensure equilibration. An UAE sensor was suspended in the air for a few days at the pot trial site at different gain settings, and settings minimizing extraneous noise was chosen, as described by Jackson and Grace (1996). The adjusted amplification gain used recorded on average less than 0.02 background cavitation event per minute at the pot trial site, which converted to 31 events per day made up of 13 during the day and 18 overnight.

A software package written for use with a personal computer was used to download the record of cumulated events (CE) and events per hour (EPH) during the measured time intervals. EPH were plotted in relation to time, and as concurrent leaf water potentials were measured, EPH could be plotted as a function of leaf water potential. Also, cumulated UAE were expressed as a percentage of the first plateau maximum corresponding to the cumulative number of UAE recorded at the time, as described by Salleo *et al.* (2000). The percentage of cumulated UAE (cUAE,%) was also plotted against water potential recorded concurrently.

Low pressure flow meter: hydraulic vulnerability curve

The hydraulic conductivity and xylem vulnerability to embolisms were measured according to the methods as described by Sperry *et al.* (1988a) and later modified by Vander Willigen and Pammenter (1998). The hydraulic conductivity of the plant segment is computed as the quotient of mass flow rate and pressure gradient (Sperry *et al.*, 1988a). Vulnerability of xylem to cavitation and embolism was measured as the percentage loss of hydraulic conductivity with decreasing xylem water potential of the plant segments.

The whole plant main stems were cut at the soil surface and the shoot immediately brought to the laboratory. To prevent stem embolism and evaporation, the cut end surface was sealed with parafilm and the entire shoot wrapped in black plastic bags.

The proximal cut end of the stem was perpendicularly re-cut while submerged in water. The whole shoot was covered and the proximal end was connected with PVC tubing to a reservoir of filtered, distilled and degassed 0.01M HCL, which prevents long term declines of xylem conductivity caused by microbial growth within the xylem conduits (Sperry *et al.*, 1988a; Cochard *et al.*, 1992a). The distal end was then cut and clamped under water to PVC tubing that connected to a glass tube with the tip dipping into a beaker of water on a four-place analytical balance. Water was allowed to flow from the reservoir, through the shoot to the balance. The amount of water flowing through the branch at a given head pressure was recorded as a mass every 30 s for 5 min using a programmed computer. This measurement constituted the initial hydraulic conductivity.

After measurement of initial conductivity shoot xylem pressure was measured from two leaves, one each from near the distal and middle portions of the shoot, using a Scholander pressure chamber. The shoot was then dehydrated on the bench top over a period of three to four days while both distal and proximal ends were tightly sealed with parafilm. During this period 5 to 6 measurements of xylem water potential and

concurrent hydraulic conductivities were taken for each sample. Before taking each measurement the shoots were fully covered with black plastic bags and allowed about 70 min for equilibration of tissue water potential throughout the shoots. A single vulnerability curve was produced from a shoot that had been subjected to several measurements, instead of taking individual measurements from numerous shoots, as the required number of samples was not available, as described by Pammenter and Vander Willigen (1998).

Once the shoot flow had virtually ceased, a maximum conductivity was measured after the shoots were fully perfused with water at 375 kPa for 60 minutes until water dripped from the leaves.

Measurement of vessel length

Shoot vessel lengths were estimated according to the methods of Zimmermann and Jeje (1981) and Ewers and Fisher (1989). Proximal cut ends of stems were connected to an air pressure (150 kPa) and distal end of the stem was dipped into water and trimmed back until air bubbles could be first seen to emerge. This measurement was made because the stem segment taken for conductivity measurements must be longer than the longest vessel. With the potted plants used in this study, entire main stems had to be taken.

Hydraulic conductivity

The formula below was used to calculate the hydraulic conductivity (K_h) with units in $\text{kg m MPa}^{-1} \text{s}^{-1}$.

$$K_h = \frac{\text{Rate of flow (kg s}^{-1}\text{)} \times \text{Length of shoot (m)}}{\text{Pressure head (MPa)}}$$

Percentage loss in conductivity

The percentage loss in conductivity (PLC) was calculated as,

$$PLC = \frac{(K_h \text{ max} - K_h)}{K_h \text{ max}} * 100$$

Where K_h and $K_h \text{ max}$ are the actual and maximum hydraulic conductivity of the shoots, respectively.

Vulnerability curves were plotted, as the percentage loss in hydraulic conductivity against the xylem water potential, as described by Sperry *et al.* (1988a).

2.2.4 Measurement of root, shoot and whole plant hydraulic conductances using a high-pressure flow meter (HPFM)

The high- pressure flow meter (HPFM) is the equipment that was designed to measure the hydraulic resistances, or inversely hydraulic conductances of whole plants and their components. The basic principles of the high-pressure flow meter are described in detail elsewhere (Tyree *et al.*, 1993a; Tyree *et al.*, 1993b; Yang and Tyree, 1994), and initially used to measure shoot hydraulic conductances under steady state conditions. But Tyree *et al.* (1995) briefly demonstrated the improved and rapid version of the HPFM using dynamic measurements of root hydraulic conductance in the field as well as laboratory. Here, pressurized water is perfused in the direction opposite to the transpiration stream into the base of a root stump while rapidly changing the delivery pressure and simultaneous measurements of flow rates are taken. Tyree's new version of the HPFM is being routinely used by various researchers to assess whole plant hydraulic conductances both in the field and laboratory (Tyree *et al.*, 1995; Tsuda. and Tyree, 1997; Tyree *et al.*, 1998; Becker *et*

al., 1999; Nardini and Pitt, 1999; Nardini *et al.*, 1999; Nardini and Tyree 1999; Clearwater and Meinzer, 2001).

The modified version of the HPFM is a powerful tool for measuring the hydraulic architecture of a whole plant rapidly (Tyree *et al.*, 1995; Tsuda and Tyree, 1997). However, it measures near maximum or potential hydraulic conductances because the high-pressure water perfusion rapidly compresses and dissolves the embolisms in conduits (Tsuda and Tyree 1997; Zotz *et al.*, 1998). Recent studies focused on the reliability of HPFM measurements on whole plants showed that values obtained by the HPFM generally agreed with those measured on segments by the traditional low flow conductivity apparatus (Zotz *et al.*, 1998), and on whole plants both woody and crop species, by the conventional evaporative flux technique (Tsuda and Tyree 1997; Tsuda and Tyree, 2000). This assumes the whole plants measured by the evaporative flux techniques were not suffering major embolism.

Description of the high-pressure flow meter

A portable HPFM similar to the one described by Tyree *et al.* (1995) was used in this study (Fig. 2.2.1). The captive air tank (CAT) (Model, Well-x-trol by Amtrol., USA), made from aluminium and with maximum working pressure of 1.5 MPa, had a water compartment of 3- l and an air compartment of the same volume separated by a rubber diaphragm as described by Tyree *et al.* (1995). The air compartment of the CAT was connected to a compressed air tank via a pressure regulator to a flow controller with a valve to pressurize and depressurize the CAT. On the side of the water compartment of the CAT an outlet of 6mm ID nylon pressure resistant tubing extended for 0.3m, via a 0.22 µm filter, to a secondary reducer connected to 3 mm OD, 1.5 mm ID FEP TEFLON tubing, which then connected to a valve of an Omnifit 8-way manifold (the inlet manifold). A pressure transducer (P1) was connected to one of the valves of this manifold. A second 8-way Omnifit manifold (the outlet manifold) was mounted close to the first, and a second pressure transducer (P2) was connected to one of the valves of this outlet manifold. The pressure transducers (P1 and P2) used

were small elements, relatively inexpensive, having a range of 1.3 MPa and a resolution of ± 0.1 kPa. Another of the valves of the outlet manifold was connected via appropriate tubing, to a compression fitting to which the plant material to be studied could be attached. In the case of roots and shoots, the outlet tubing was 1.9 m of FEP TEFLON, 3 mm OD, 1.5 mm ID, and in the case of leaves, a 1.4 m length of 1.5 mm OD, 0.13 mm ID. The other six pairs of valves between the inlet and outlet manifolds were connected by narrow bore peek capillary tubes (CT) of 1.5 mm OD and 0.16 m long, with IDs of 0.1 mm, 0.13 mm, 0.18 mm, 0.25 mm, 0.51 mm or 0.8 mm giving a range of resistances. The flow of water through a CT connecting the manifolds generated a pressure differential that was recorded by the pressure transducers. As the resistance of the CT was known, the flow rate could be calculated from the resistance and the pressure differential. During measurements, when plant material was attached to the outlet manifold, a CT was selected to give a pressure differential between 20 and 120 kPa. From the flow rate and inlet pressure of the tissue (pressure at the outlet manifold), the resistance of the plant material could be calculated. The resistances of shoots was measured in the quasi steady-state mode (flow rate measured at constant pressure at the outlet manifold); root resistances were measured during 'transients' with flow rate measured as the pressure at the outlet manifold was increased linearly over time. The HPFM used in this study, together with the computer programme to operate it, were kindly supplied by Prof. M. T. Tyree (United States Department of Agriculture Forest Service, North-Eastern Experiment Station, 705 Spear Street, Burlington, Vermont 05402, USA).

Measurements of whole plant hydraulic conductances (K_p)

In the present studies, the whole plant hydraulic conductances (K_p) were measured as described by Tyree *et al.*, (1993a); Yang and Tyree (1994) and Tyree *et al.* (1995). The resistance of the whole plant was calculated as the sum of the resistances (inverse of conductances) of the roots and shoots measured independently. Differences in whole plant conductances, and the conductances of the individual components among the different clones, treatments, ages and field site were observed.



Fig. 2.2.1 A high -pressure flow meter (HPFM) version of Tyree *et al.* (1995) used in this study.

Measurements of root hydraulic conductance (K_r)

Shoots were excised and 9 cm from the soil surface, and the cut stump was shaved with a razor blade and a water- filled watertight compression fitting of the HPFM was

connected to the root stump. Root conductances (K_r) were immediately measured by the transient water flow method and three to four transients were taken for each measurement of root conductance.

During each measurement the needle valve (NV) was adjusted and pressure (P) increased using a pressure regulator on the compression tank at a rate of 6 to 8 kPa s⁻¹ from 0 to 0.6 MPa. The HPFM recorded the flow (F) with the increasing pressure (P) every 3 s, over a period of 90 s, the data were plotted and linear portion of the plot selected to calculate the root hydraulic conductances from linear regression of the data (Tyree *et al.*, 1995). The entire set of transient flow measurements (4) was achieved in about 6 to 7 min. In the first transient, flow measured was marginally different from the following transients for per each point. The first transient slightly overestimated conductance compared with other transients latterly measured on the same root sample. This is because of inelasticity of the tubing due to initial building up of low pressure both in the tubing and the compression fitting when its measured for first time after the first connection of compression fitting to the root stump. However, subsequent transients were measured under stable hydrostatic pressure both in the HPFM tubing and the compression fitting and the data points were non-linear between 0 MPa and 0.225 MPa and then became linear up to 0.6 MPa. The observed non-linear portion was because of inadvertently trapped air-bubbles in the compression fitting between the HPFM and the root (Tyree *et al.*, 1995), and those points were excluded from linear regression used to estimate root conductance. The first transient flow measurement was also rejected in this study and 3 to 4 subsequent transients were taken for one conductance point per each sample.

Once the root conductances were measured, the pots were re-saturated with water and roots were excavated carefully by washing with gentle stream of water and by hand. Broken fine root from the washing water and soil were sieved through a 2 mm-sieve and added to main root system. The collected roots were later oven dried at 60°C for 48 hours for dry mass.

Measurement of shoot hydraulic conductance (K_s)

The whole shoot hydraulic conductance and its components, was measured as described by Tyree *et al.* (1993a) and Yang and Tyree (1994). The shoots were excised and the proximal end re-shaved perpendicularly and connected to the water-filled, airtight compression fitting of the HPFM. Shoots connected to the HPFM were kept under shade and covered with black plastic bags to avoid excessive evaporation from the shoots during measurement (temperatures ranged from 25°C to 29°C). Distilled, degassed and water filtered to 0.22 μm for 60 min was perfused through the shoot at a pressures of 0.525 to 0.575 MPa until water began to drip from leaf blades and water flow (F) entering the branch became constant. At this stage, the leaf air spaces are filled with water and leaf water potential is presumed to be zero, correspond to a time when the flow rate shoot conductance and hence become relatively stable (quasi-steady-state). The computer linked to the HPFM monitored flow rate and pressure from the time shoot perfusion started and calculated hydraulic resistance and the corresponding time at pre set intervals. When the shoots were fully infiltrated with perfused water and calculated resistance remained constant, that point was recorded for the shoot resistance.

Perfusion of water under pressure could plug open conduits with fragments from the original cut, increasing measured resistance, and so it is recommended that the stem is re-cut after a perfusion measurement. However, preliminary studies with the *Eucalyptus* spp. clones used in this study indicated that this plugging effect did not occur, and so the re-cutting precautions were not required.

Measurements of the components shoot conductance (K_s)

Resistances of the individual shoot components were calculated from the differences between resistances before and after removal of each component after measuring the whole shoot resistances. Here, components of whole shoots (R_{+S}) are regularly removed, such as leaf blades (R_L), petioles (R_P), leaf bearing branches (R_{LBB}), non-

leaf bearing branches (R_{NLBB}) and the main stem resistance (R_{MS}). The measurements were taken by the HPFM using quasi- steady state measurements to calculate the whole plant resistances and each of the plant component resistances (units of $\text{kg s}^{-1} \text{MPa}$), as described below.

Resistance of whole shoots (R_{+S}), leaves (R_L), petioles (R_P), leaf bearing branches (R_{LBB}), non-leaf bearing branches (R_{NLBB}) and the main stem resistances (R_{MS})

The resistance to water flow was measured first on a whole primary shoot with secondary lateral branches and leaves present. The resistances to water flow of the whole shoot with lateral branches and leaves is given by

$$R_{+S} = P / F_{+L}$$

Where R_{+S} is the resistance to water flow of the whole shoot with secondary lateral branches and leaves present, F_{+L} is the flow rate through the branches and leaves and P is the water perfusing pressure at the base of the shoot.

Leaves (R_L)

After measuring the R_{+S} , the leaves were clipped with a pair of scissors by cutting them at the base of petioles and new resistance of leaves were measured calculated from,

$$R_{-L} = P / F_{-L}$$

Where the subscript $-L$ on a value means the value with leaves removed from the shoots.

Leaf resistances of shoots (R_L) was calculated by differences.

$$R_L = R_{+S} - R_{-L}$$

Petioles (R_p)

The petioles, which are attached to the twigs were excised at their bases and changes in resistance give the resistance of petioles per each shoot,

$$R_p = P / F_p$$

Where the subscript $_p$ on a value means the value without petioles

Petiole resistances (R_p) were calculated by differences.

$$R_p = R_L - R_{-p}$$

Leaf bearing branches (R_{LBB})

The leaf bearing branches were next excised from the shoot and resistances of leaf bearing branches (R_{LBB}) were measured.

$$R_{LBB} = P / F_{-LBB}$$

Where the subscript $_{-LBB}$ on a value means the value with leaf-bearing branches removed from the rest of the shoot. Resistance of leaf bearing branches was calculated by differences.

$$R_{LBB} = R_{-p} - R_{-LBB}$$

Non-leaf bearing branches (R_{NLBB})

The non-leaf bearing twigs (R_{NLBB}) were successively excised from the main stem of the shoot and changes in resistance gave the resistances of non-leaf bearing branches.

$$R_{NLBB} = P / F_{-NLBB}$$

Where the subscript $_{-NLBB}$ on a value mean the value with non-leaf-bearing branches removed from the main stem.

$$R_{NLBB} = R_{LBB} - R_{-NLBB}$$

Main stem (R_{MS})

Following this, the main stem shoot resistance ($_{-MS}$) was measured.

Calculation of conductance values and its units

Hydraulic resistances for whole shoots (R_S), leaves (R_L), petioles (R_P), leaf bearing branches (R_{LBB}), non-leaf bearing branches (R_{NLBB}) and the main stem (R_{MS}) were converted to their conductances using the relations:

Whole plant shoot conductance (K_S) is, $K_S = 1/R_{+L}$ (R_S), and its components $1/R_L$ (K_L), $1/R_P$ (K_P), $1/R_{LBB}$ (K_{LBB}), $1/R_{NLBB}$ (K_{NLBB}) and $1/R_{MS}$ (K_{MS}).

Where as root conductance (K_r) is:

$$K_r = 1/R_r.$$

So, whole plant conductances (K_P) is calculated as the reciprocal of ($1/K_r + 1/K_S$), that is $R_P = R_s + R_r$.

Normalized hydraulic conductances

Hydraulic conductances of the whole plant, the root, the shoot and its components were normalized by leaf area ($\text{kg s}^{-1} \text{MPa}^{-1} \text{m}^{-2}$) or by dry weight ($\text{kg s}^{-1} \text{MPa}^{-1} \text{g}^{-1}$). On completion of each set of experiment, all excised leaves (without petioles) were measured on a model of CI-251 leaf area meter (CID, inc., Vancouver, Washington

State, USA). The leaves and rest of the shoots were oven dried at 60°C for 48 hours and the dry mass was obtained.

Whole shoot conductance normalised by leaf area ($K_{S/LA}$) is K_S ($\text{kg s}^{-1}\text{MPa}^{-1}$)/ LA (m^2) and so has units $\text{kg s}^{-1}\text{MPa}^{-1}\text{m}^{-2}$.

Similarly whole root conductance normalised by leaf area ($K_{r/LA}$) = $K_{r/LA}$ with units $\text{kg s}^{-1}\text{MPa}^{-1}\text{m}^{-2}$.

Hydraulic conductances can also be normalised by the dry weight of shoots (tsdw) and roots (trdw). Scaling conductances this way reveals the carbon investment to provide efficient hydraulic pathways (Tyree *et al.*, 1998). Thus root conductance per unit root dry weight is $K_{r/trdw}$ with units $\text{kg s}^{-1}\text{MPa}^{-1}\text{g}^{-1}$, and shoot conductance per unit shoot dry weight is $K_{S/tsdw}$, with same units.

2.2.5 Measurements of positive root xylem pressure (P_r)

Shoots were excised, the root stumps 9 cm from the soil surface were shaved by a sharp blade and the manometer was connected to the stump.

The manometer was connected via a water filled compression fitting, with other side of the fitting connected to a 1.8 m long air-filled transparent tube with an ID of 3 mm. The distal end was sealed tightly, the tube supported vertically over the root stump. The basal compression fitting was covered with aluminum foil to shade from sunlight that could raise the water temperature.

The manometer was connected around 17.00 h and any rise in the water column in the erect tube was observed at 06.00 h in the subsequent morning and again at midday. The rising water column decreased the length of the air bubble in the manometer tube, and the proportion of the tube occupied by the air bubble is inversely related to root pressure.

The root pressure (P_r) is calculated as,

$$P_r = \frac{P \text{ (atm)} \times T_L}{B_L}$$

Where P (atm), T_L and B_L are atmospheric pressure (0.1 MPa), total length of tube (m) and length of the bubble (m), respectively.

2.3 Validation of the UAE method of cavitation detection: a comparison between UA emissions and hydraulically measured loss of conductance

2.3.1 Introduction

An advantage of the UAE method of detecting cavitation events is that it is non-destructive. However, UA emissions do not measure the influence that cavitation events have on hydraulic conductance, and hydraulic quantification of xylem embolism is a better gauge (Tyree and Sperry, 1989a). In the studies described in this thesis it was simply not possible to grow a sufficient number of plants to directly measure effects of cavitation on hydraulic conductances, as these techniques are destructive. Thus preliminary studies were undertaken to compare the UAE and hydraulic techniques, and to assess whether the data provided by the UAE technique could be used as a measure of the effect of cavitation events on hydraulic conductance. Measurements of acoustic emissions and corresponding water potentials made on a plant on one day were compared with vulnerability to cavitation measured hydraulically on the same plant the following day.

2.3.2 Material and methods

Xylem cavitation detected by UAEs

Six replicate plants of the clone GU210 maintained in 25 l pots and subjected to high water treatment for 12 months were used for this study. Measurements were made on clear days only, when transpiration rates and xylem tensions would be high. An UAE sensor was clamped on the main stem at the height of 0.2m above the soil surface around 17.30 h and the DSM collected cavitation events until 14.00 h the following day. During the day, xylem water potential was assessed by measuring the water potential of on two to three matured leaves from the middle and upper layers of the canopy that were well exposed to environmental conditions, using L-51 leaf chambers. Leaf chambers were clamped onto the leaves at the same time that the UAE sensor was clamped to the stem, giving adequate time to equilibrate overnight before the measurements were taken. Leaf water potential was measured at 20-minute intervals from 06.00 h to 14.00 h or until xylem recovery was observed from the increasing water potential. Cumulated xylem cavitation events (cUAE) and events per minute (EPM) were collected by the DSM at 20 minute intervals during this period. Cumulative emissions were expressed as a percentage of the plateau or maximum number of events detected (cUAE,%), and plotted against the water potential measured at the corresponding time.

Hydraulic vulnerability curve

The hydraulic conductivity and xylem vulnerability to embolisms were measured according to the methods described by Sperry *et al.* (1988a) and later modified by Vander Willigen and Pammenter (1998).

After the UAE measurements on a plant had been completed at the end of the day, the same plant was watered to field capacity and harvested on the following morning. The whole shoot was cut close to the soil surface and immediately brought to the

laboratory. The terminal portion of the shoot was removed and, using a low-pressure flow meter, an initial hydraulic conductivity was measured and the stem was allowed to dehydrate, with conductivity and corresponding water potential being measured at intervals, as described above (Section 2.2.3). Five to six points for individual plant measurements of conductivity and corresponding water potential were taken on each plant. Finally, the stem was flushed with water at high pressure and maximum conductivity measured, and percent loss of conductivity calculated for each point.

2.3.3 Results

The accumulation of acoustic emissions, the rate of emission detection, and the corresponding decline in water potential over the course of the day are given in Figs. 2.3.1.A – C, respectively (the data are for all plants combined). Acoustic emissions were still being detected when the experiment was terminated at 14.00 h, and so a plateau value was not observed. However, maximum cUAE was estimated by fitting an exponential sigmoid curve to the data (Fig. 2.3.2), and the fitted value of maximum cUAE was used to calculate, individually for each plant, the value of cUAE,% every 20 minutes. UAEs accumulated in a sigmoidal manner, and the rate of emissions peaked (EPM_{max}) at about 11.00 h. cUAE,% was plotted against leaf water potential (Fig. 2.3.3.A) to yield a ‘vulnerability’ curve based on acoustic data. The vulnerability curve determined using the conventional hydraulic method is shown in Fig. 2.3.3.B (data for all plants individually are presented). Unlike the accumulation of emissions over time (Fig. 2.3.1. A) and the plot of cUAE,% against water potential, the hydraulic vulnerability curve was not sigmoidal in nature; rather it showed an increase in stem conductance loss with decreasing leaf water potential that could be described by a second order polynomial.

Chapter 2
Saplings maintenance and general physiological techniques

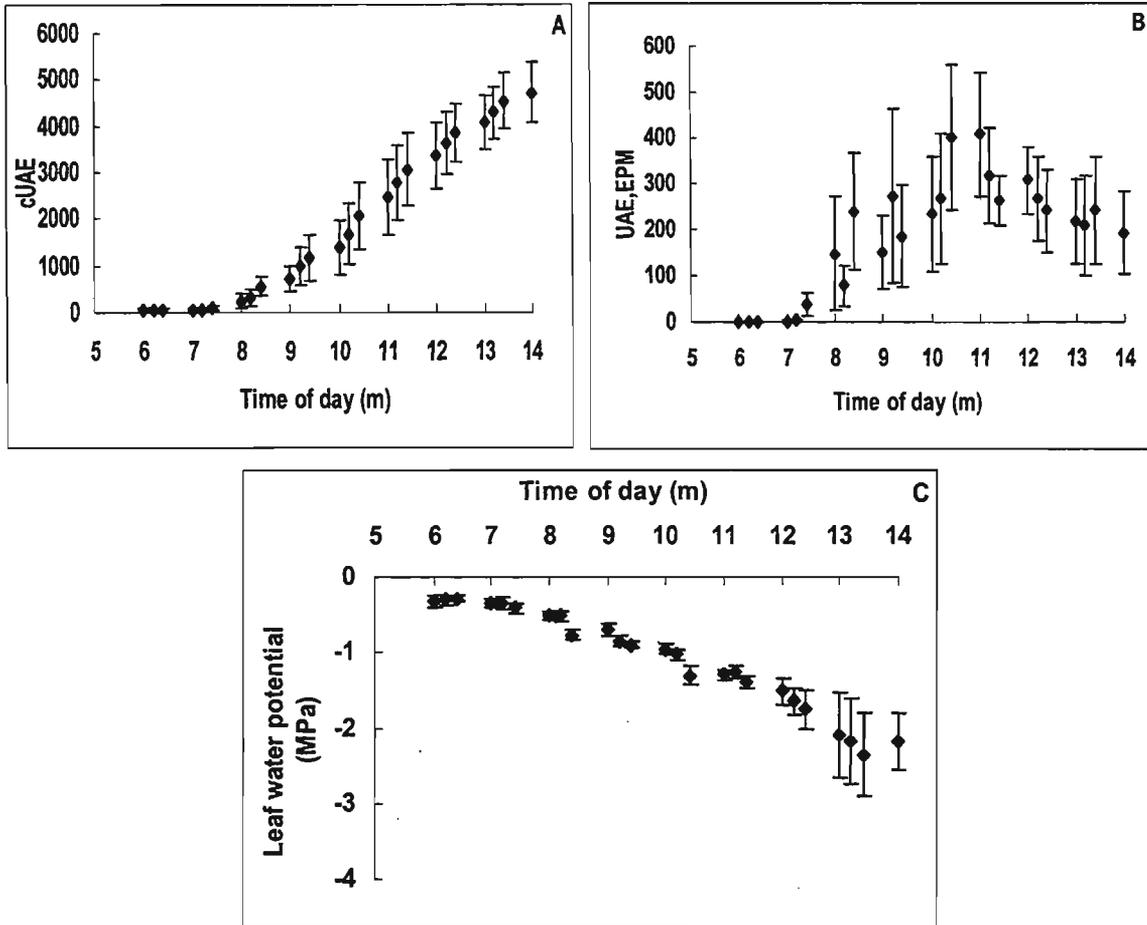


Fig. 2.3.1 cUAE (A), EPM (B) from the main stem, and concurrently measured leaf water potential (C) for 12 months old plants of clone GU210 subjected for high watering treatment. Error bars represent the \pm SEM of the mean (n=6). Note that the six replicates were measured on separate days.

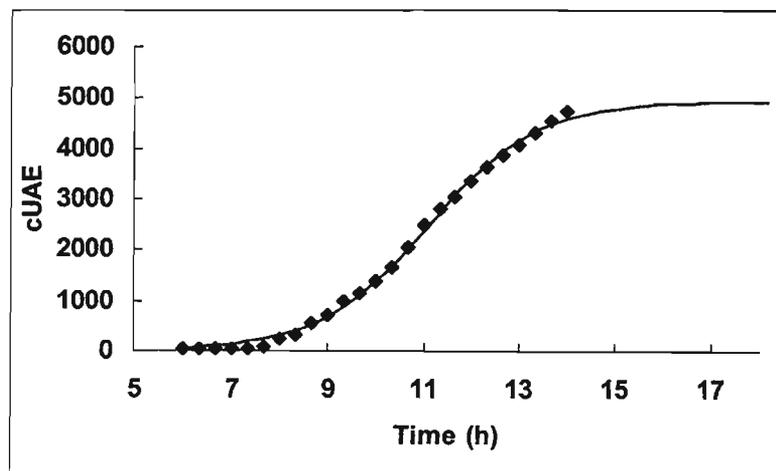


Fig. 2.3.2 Maximum cUAE estimated by fitting an exponential sigmoid curve to the cUAE data. The sigmoidal equation is $cUAE = 4944 / (1 + \exp(-0.87 * (\text{time} - 11.1)))$.

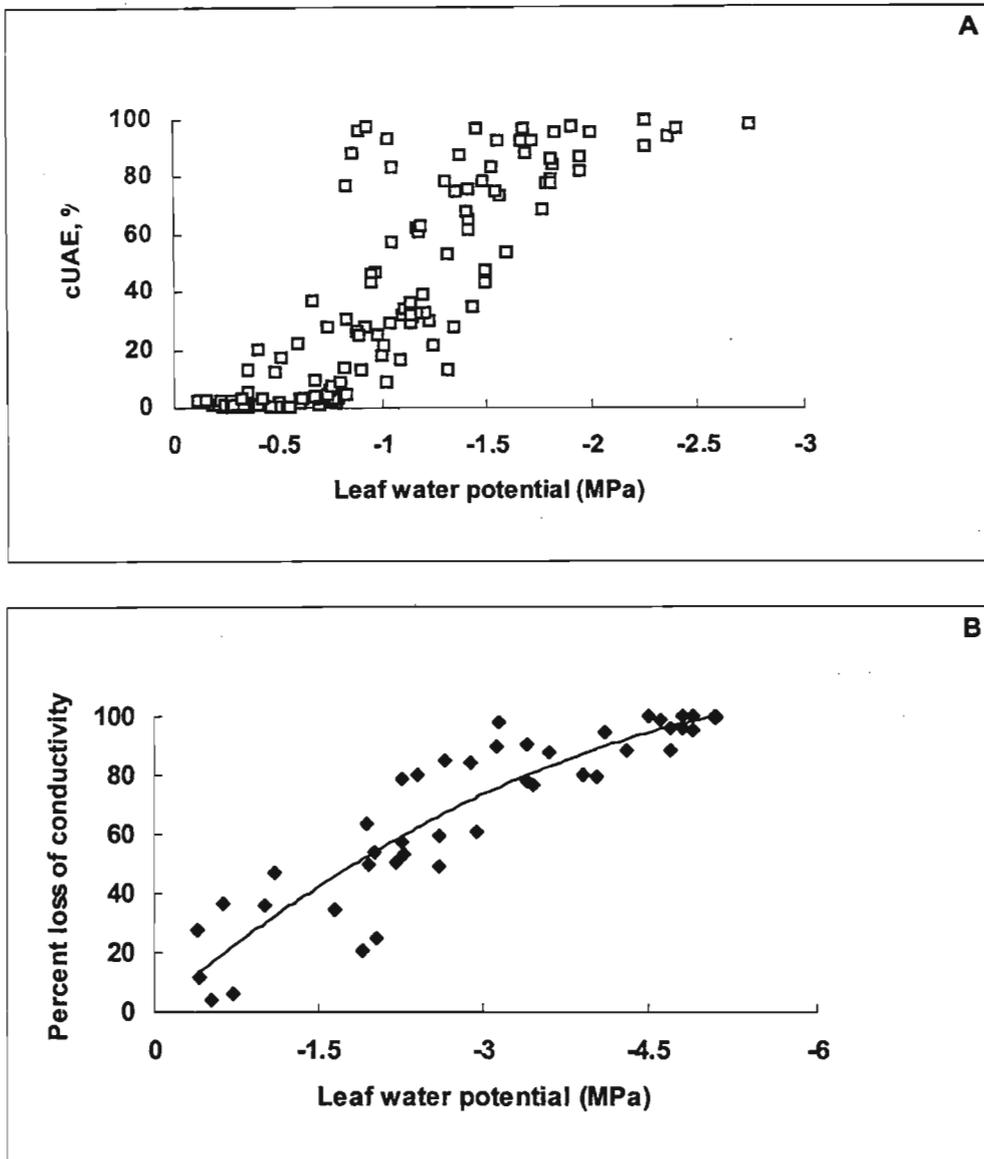


Fig. 2.3.3 (A) Cumulated UAEs from the main stem, expressed as percentage of the maximum (cUAE,%), as a function of leaf water potential in the main stem of 12 months old plants of clone GU210 subjected for high watering treatment. (B) vulnerability of xylem to cavitation illustrated as the percentage loss of hydraulic conductivity with decreasing water potential for the same plants. All data points from each replicate are shown (n=6).

The relationship between PLC and cUAE

The vulnerability of xylem to cavitation can be assessed by a number of criteria. When measured hydraulically the water potential corresponding to 50% loss of conductivity (PLC₅₀), or the water potential corresponding to the initiation of

conductivity loss can be used. Because acoustic methods do not directly measure the influence of cavitation events on hydraulic conductance, assessment of vulnerability using these methods is more subjective. The water potential corresponding to the accumulation of 50% of total acoustic emissions (cUAE,%₅₀) would not necessarily be a good measure, as cUAE will depend on the water potential developed by the transpiring plant. For example, the minimum water potential developed by whole plants in pots in this study was higher (about -2.5 MPa, Figs. 2.3.1.C and 2.3.3.A) than those achieved by dehydration of excised shoots during hydraulic measurements (about -5 MPa, Fig. 2.3.3.B). Consequently, the curves of cUAE,% vs. water potential and of PLC vs. water potential do not overlay, and the water potentials corresponding to PLC₅₀ and cUAE,%₅₀ differed. The same argument would apply to using the maximum rate of acoustic emissions (EPM_{max}) as a measure of vulnerability. A method that has been proposed (Salleo *et al.*, 2000) is to determine the threshold water potential corresponding to the initiation of rapid cavitation events, the 'initiation' being judged as the mean of the water potentials for all data points lying between 5% and 7.5% of total cumulative emissions ($\psi_{CAV,cUAE,\%}$). In this study the data points between 5 and 10% were used.

In this study the water potentials corresponding to different assessments of 'vulnerability' were as follows:

hydraulic method;	PLC ₅₀	-1.95 MPa
acoustic method;	cUAE,% ₅₀	-1.30 MPa
	EPM _{max}	-1.30 MPa
	$\psi_{CAV,cUAE,\%}$	-0.73 MPa

From these values it is clear that the acoustic data cannot be used directly to assess the degree of conductivity loss. Similar lack of correspondence of the two methods has been reported by Cochard (1992) and Jackson *et al.* (1995a), at two different studies in *Pinus sylvestris* L. and Hacke and Sauter (1995); Nardini *et al.* (2001), with hydraulically measured PLC₅₀ generally occurring at lower water potentials than estimates from acoustic methods. One possible reason for this is that the acoustic

method may sense emissions from tracheids and fibres, which would have no influence on conductivity (Cochard and Tyree, 1990).

However, it can be demonstrated that the hydraulic and acoustic techniques are measuring the same phenomenon. The data sets of PLC vs. water potential and cUAE vs. water potential were searched to find points of the same water potential in the two sets. The PLC and cUAE values corresponding to these common water potentials were then used to relate PLC to cUAEs. A plot of these data pairs showed a linear relationship between PLC and cUAEs (Fig. 2.3.4; $r^2 = 0.62$), with the total cUAEs corresponded to a PLC of about 80%. These data suggest that acoustic emissions were in fact an expression of cavitation events occurring in the xylem conduits. Similar conclusions have been drawn by Lo Gullo and Salleo (1992); Salleo and Lo gullo (1993) and Salleo *et al.* (2000).

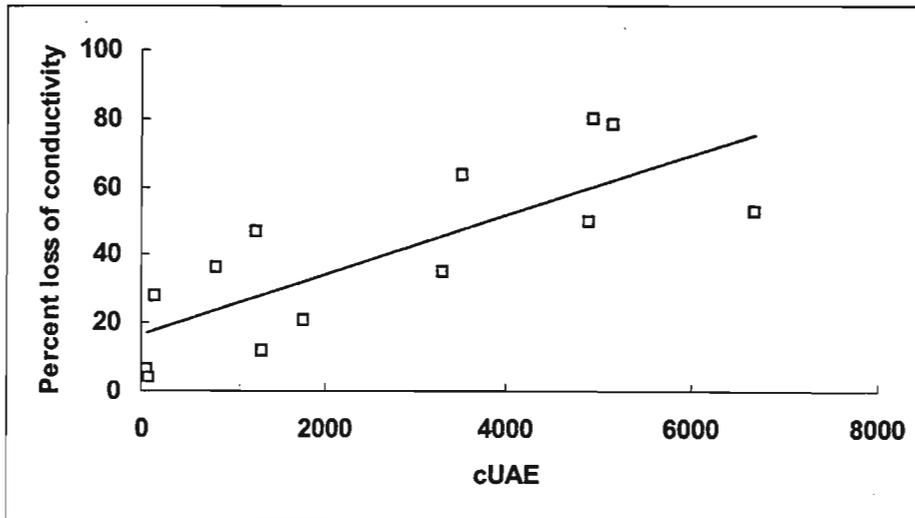


Fig. 2.3.4 The relationship between cUAEs and PLC of stems of 12 months old plants of the clone GU210 subjected to high watering. See text for details of how the data were derived.

The slopes of vulnerability curves determined using the hydraulic technique are generally sigmoidal in shape (Tyree and Sperry, 1988; Lo Gullo and Salleo, 1992; Pammenter and Vander Willigen, 1998; Kavanagh *et al.*, 1999). However, the clone GU210 yielded a more gradual increase in conductivity loss with decreasing water

potential, and similar shaped curves were reported in three coffee cultivars (Tausend *et al.*, 2000). Interestingly, the cUAE,% curve for GU210 was almost sigmoidal in shape. The water potential corresponding to PLC₅₀ for GU210 (-1.95 MPa) was lower than that of field grown *Eucalyptus* spp. clones plants (-1.50 MPa), one of which was also a GU clone (Vander Willigen and Pammenter 1998), but higher than that *E. calmuldulensis* (-3.0 MPa) (Franks *et al.*, 1995).

2.4 Statistical analyses

Analyses of growth characteristics

All statistical analysis were conducted with the Statistical Package for Social Sciences (SPSS) for Windows. For each set of experiments, both potted and field trials, six to eight replicates were used throughout the experiment for each physiological parameter studied. In the pot experiments, for clonal and treatment effects, two-way analyses of variance (ANOVA) were performed, followed by Scheffe`s multiple range test to assess significance of differences among the clones and between treatments. In the case of the field studies two *Eucalyptus* spp. clones 3 months of age were compared by one-way analysis of variance. The slopes of the relationship between transpiration and the leaf water potential for the treatments and the clones were statistically compared using the GraphPad Prism, version 3.02 package.

Chapter 3: 3 Month Old Field Saplings

Chapter: 3.1 Introduction

3.1.1 Objectives

The physiological characteristics of two *Eucalyptus* spp. clones in the field were studied on 3-month-old plants at a mesic site. An advantage of studying young field grown saplings is that root restrictions will not affect physiological parameters and growth, as may occur in potted material.

The main objective was to assess the interrelations between hydraulic architecture and plant water relations, including xylem cavitation, and leaf gas exchange.

Additionally, as most of the experimental work described in this thesis was carried out on potted plants, the field-grown material could act as a bench-mark to assess whether root restriction in pots influenced the hydraulic and physiological characteristics of this material.

3.1.2 Site description

Field studies were conducted on a *Eucalyptus grandis* x *urophylla* clone (GU210) and a pure *Eucalyptus grandis* clone (TAG14). Material was growing on mesic sites with a mean annual rainfall of 1280 mm. Saplings were planted at an espacement of 2.1 x 3.1 m. Site characteristics are presented in Table 3.1.1. Measurements were made in August 2001. No rainfall fell during the study period. The location of the sites (compartments) were 28° 35' 32"S, 32° 04' 21"E (Ratrays) and (28° 41' 11"S, 32° 03' 25"E (Nseleni).

Table 3.1.1 Details of site characteristics of *Eucalyptus* spp. clones planted in two compartments in the commercial mesic plantation site, Zululand, South Africa. Data courtesy of Paul Viero, Mondi Forest, Zululand division, South Africa. (ERD-Effective root depth)

<i>Eucalyptus</i> clone	Planted date	Compartment	Soil type	ERD(m)
GU210	12-04-2001	Ratrays (RG39)	Hutton (Hu 2200)	1.5+
TAG14	30-03-2001	Nseleni (NK26)	Fernwood (Fw121)	1.5+



Fig. 3.1.1 Three months old field grown *Eucalyptus grandis* (TAG14) at a mesic field site.

3.1.3 Height and stem over-bark diameter

The height from the stem base at the soil surface to the apical bud, and over-bark stem diameter at 0.1m above from soil surface, were measured on 15 randomly chosen samples of each clone.

3.1.4 Leaf water potential (Ψ_L)

Leaf water potentials (Ψ_L) were measured from pre-dawn to 14.00 h using a Scholander pressure chamber. At each measurement time eight to 14 leaves were sampled, each leaf from a separate plant.

3.1.5 Leaf gas exchange

Leaf gaseous exchange measurements (A , E , g_s) were carried out using a portable photosynthesis system and after these measurements the leaves were excised for water potential measurements with a pressure chamber. This was repeated on four plants, with one leaf per plant, with ten measurements being taken through the course of the day from 07.00 h to 14.00 h., and all measurements were repeated the following day (giving a total of eight replicates per time of day).

3.1.6 Stem xylem cavitation detected by an UAE

Xylem cavitation events in main stems were measured on three replicates of each clone using an ultrasonic acoustic emission (UAE) detector (as only one detector was available, replicates were measured on consecutive days). The sensor was clamped to the main stem, 0.1m above ground and below the branches bearing the canopy. The sensor was attached to the stem the evening prior to measurement, and the following morning was connected to the drought stress monitor (adjusted to a setting of 72 decibels) and emissions were recorded from 07.00 h to 15.00 h. Concurrent with recording of xylem cavitation events, gas exchange and water potentials were

measured on a single leaf on ten occasions during the course of the day (giving a total of three replicates).

3.1.7 Root pressures (P_r)

Root pressure was measured on eight to ten replicates by attaching manometers to decapitated root stump the evening before measurements. The length of the air column was recorded at 06.00 h and 12.00 h and root pressure expressed in unit of kPa above atmospheric pressure.

3.1.8 Root and shoot hydraulic conductances

Root and shoot hydraulic conductances were measured on eight replicates using a high pressure flow meter (HPFM) in the transient and quasi-steady state modes, respectively, as described by Tyree *et al.* (1994c; 1995) and Yang and Tyree (1994). In the field studies, root conductances were not measured on plants previously sampled for root pressure as adequate material was available. For each measurement of root conductance (K_r) the average of three transients were taken. In the shoots, total shoot conductance (K_s), leaf resistance (R_L), petiole resistance (R_P), resistance of leaf bearing branches (R_{LBB}), and main stem resistance (R_{MS}) were measured after sequentially excising all the plant components respectively. In the field studies, the resistance of non-leaf bearing branches (R_{NLBB}) was not measured, as only leaf bearing branches were present at that time. Total leaf area from each shoot was measured using a leaf area meter and the dry weight of leaves, petioles and stems were measured after oven drying at 60°C for 48 hours.

Chapter: 3.2 Results

3.2.1 Clonal differences in above-ground growth and biomass allocation

The above-ground parameters measured for the two clones are shown in Table 3.2.1. Shoot growth over the three months since planting, measured as height, stem over-bark diameter and total shoot biomass, as well as the biomass of the individual components, was significantly higher in TAG14 than GU210 ($P < 0.05$ in all cases). The leaf area ratio (LAR, leaf area/shoot dry mass) and specific leaf area (SLA, leaf area/leaf dry mass) were lower in TAG14 than GU210 ($P < 0.05$), and consequently, total leaf area did not differ between the clones.

Table 3.2.1 Growth parameters measured on two 3 month-old *Eucalyptus* spp. clones growing in commercial plantations at mesic sites. Means \pm SEM (n= 8). For each parameter different superscript letters indicate significant differences between the clones at $P < 0.05$ (one-way ANOVA).

Growth parameters	GU210	TAG14
Heights, (m)	0.53 \pm 0.01 ^a	0.68 \pm 0.02 ^b
Stem over-bark diameter, (mm)	9.58 \pm 0.47 ^a	10.94 \pm 0.34 ^b
Leaf area, (m ²)	0.61 \pm 0.07 ^a	0.68 \pm 0.09 ^a
Specific leaf area, (m ² kg ⁻¹)	19.52 \pm 0.64 ^a	15.04 \pm 0.67 ^b
Leaf weight ratio	0.62 \pm 0.03 ^a	0.55 \pm 0.01 ^b
Leaf area ratio, (m ² kg ⁻¹)	12.17 \pm 0.70 ^a	8.25 \pm 0.52 ^b
Leaf dry mass, (g)	31.27 \pm 3.59 ^a	44.38 \pm 4.89 ^b
Stem, petiole dry mass, (g)	19.02 \pm 2.62 ^a	36.37 \pm 3.45 ^b
Total shoot dry mass, (g)	50.30 \pm 5.50 ^a	80.75 \pm 8.18 ^b

3.2.2 Predawn leaf water potential (Ψ_{PDL})

If predawn leaf water potential (Ψ_{PDL}) can be taken as a measure of soil water potential (Ψ_s), the data in Table 3.2.2 showed that the compartment supporting clone TAG14 (Nseleni) had a higher soil water potential than the compartment supporting clone GU210 (Rattrays) ($F=7.50$, $P=0.011$, one-way ANOVA), although the rainfall pattern was similar for both compartments throughout the period. However, the soil types differed (Table 3.1.1) which could have given rise to slightly higher water retention in the compartment supporting the TAG14 clone.

Table 3.2.2 Predawn and midday leaf water potential of two 3 month-old *Eucalyptus* spp. clones in commercial plantations at mesic sites. Means \pm SEM (n=13-16 samples for predawn and 8 for middays). For each parameter different superscript letters indicate significant differences between the clones at $P<0.05$ (one-way ANOVA).

Clones	Predawn ψ_L (MPa)	Midday ψ_L (MPa)
GU210	-0.20 ± 0.03^a	-1.69 ± 0.04^a
TAG14	-0.12 ± 0.01^b	-1.56 ± 0.03^b

Midday values of ψ_L were higher in TAG14 than GU210 (Table 3.2.2, $F=6.14$, $P=0.027$, one-way ANOVA). These values of ψ_L were lower than those reported by Vander Willigen and Pammenter (1998) for 7 year-old trees, suggesting that establishing saplings may be more prone to water deficits than mature trees.

3.2.3 Diurnal patterns of leaf water potential and gas exchange

Leaf water potentials declined from predawn to about 11.00 h. The values in TAG14 were consistently higher than those in GU210 (Fig. 3.2.1. A), and these differences were significant from 09.00 h to 12.00 h ($P < 0.05$). This may reflect the slighter higher predawn values for TAG14. After about 11.00 h, water potentials stabilised, probably because transpiration was no longer increasing.

During the morning rates of net CO_2 assimilation were marginally higher in TAG14 than GU210 (Fig. 3.2.1. B), although by midday differences were not significant ($F = 2.60$, $P = 0.130$). However, midday stomatal conductances and transpiration rates were higher in TAG14 than GU210 (Fig. 3.2.1.C and D, $F = 12.10$, $P = 0.004$ and $F = 7.80$, $P = 0.015$, respectively). The exceptionally high stomatal conductances measured in the early morning are probably a consequence of dew deposition and not meaningful. Stomatal conductances reached maximum values at about 10.00 h, before maximum radiation intensities and temperatures, suggesting stomatal limitation of transpiration, reducing the onset of water stress. A weak relationship between diurnal net CO_2 assimilation rate and stomatal conductances in GU210 (Fig. 3.2.2. A, $r^2 = 0.39$, $P = 0.000$), suggesting a little stomatal limitation of photosynthesis, compared with a very weak relationship in TAG14 clone ($r^2 = 0.12$, $P = 0.002$). Calculated leaf level water use efficiency (A / E) shows that only morning (10.00 h) water use efficiency of TAG14 clone was higher than GU210 (Fig. 3.2.2. B, $F = 7.26$, $P = 0.018$) and other calculated values were remained same between the clones ($P > 0.05$).

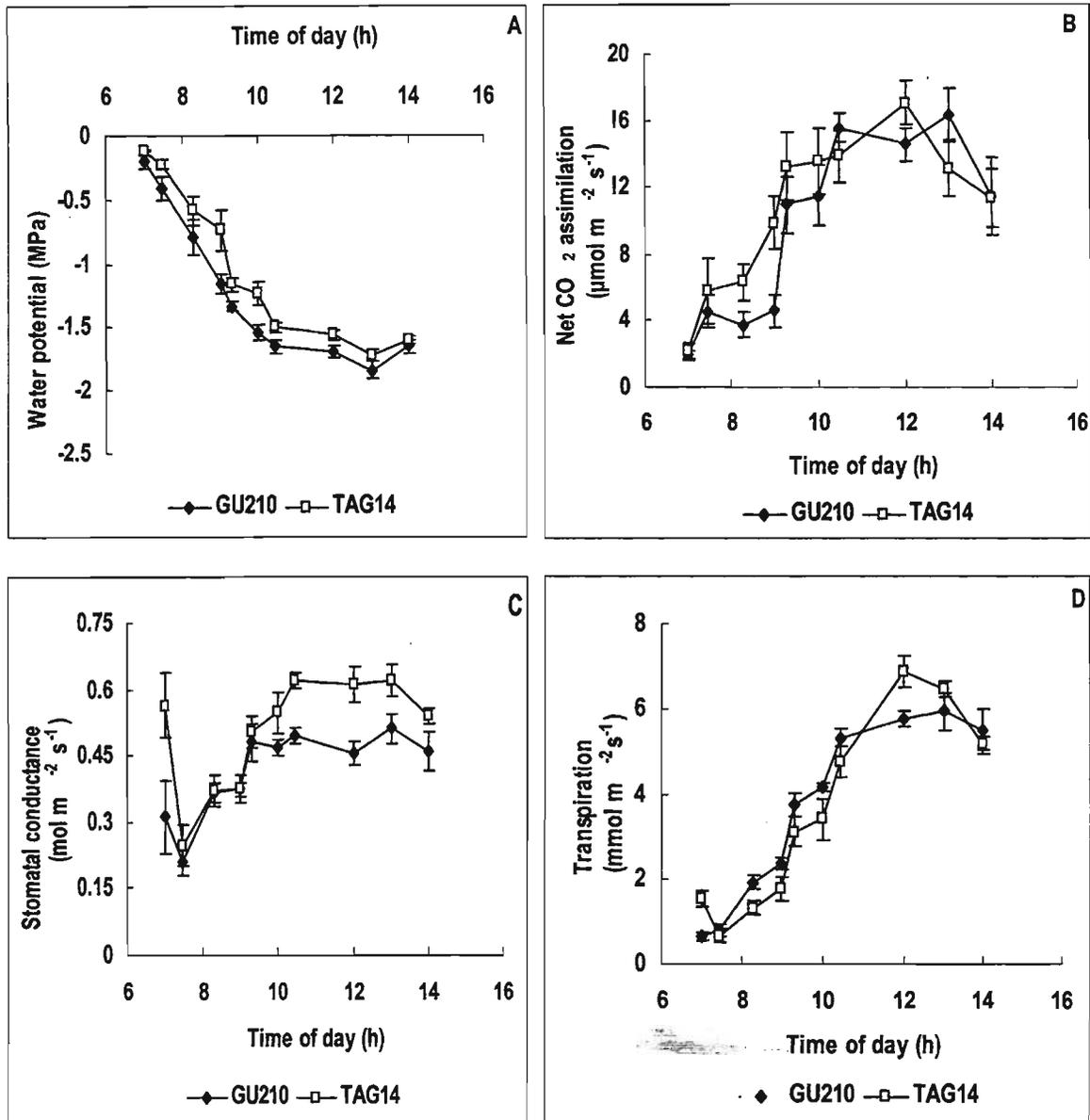


Fig. 3.2.1 Diurnal variations in instantaneous rates of leaf water potential (A), net CO₂ assimilation rate (B), stomatal conductances (C) and the transpiration (D) in two, 3 month old *Eucalyptus* spp. clones grown in the field at a mesic site. Error bars represent the \pm SEM (n=8).

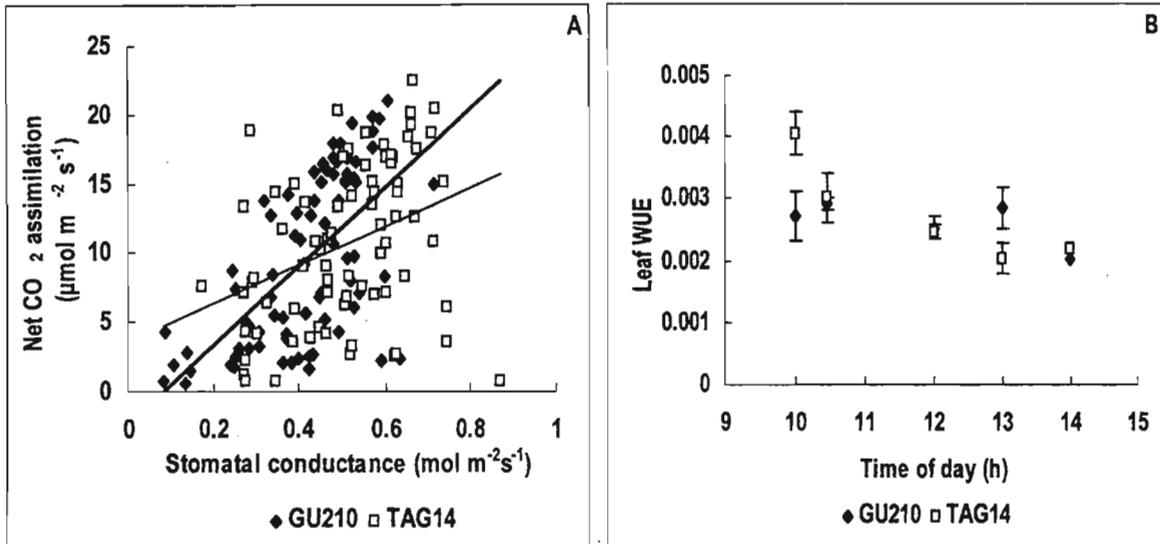


Fig. 3.2.2 The relationship between diurnal net CO₂ assimilation rate and stomatal conductances (A) and diurnal variations in instantaneous calculated mean leaf level water use efficiency (A/E) from 10.00 h to 14.00 h (B) for 3 months old saplings of the clones GU210 and TAG14 grown at mesic sites in the field. Error bars represent the \pm SEM (n=8). The fitted linear regressions represent for GU210 (bold line) and TAG14 clone (thin line).

Both clones showed rapidly increasing net CO₂ assimilation rate in the morning, corresponding to increasing incident photosynthetically active radiation. For comparison purposes, incidence PAR was log transformed (Fig. 3.2.3) and linear regression lines established the positive relationship between net CO₂ assimilation rate and the incidence PAR for GU210 (P=0.000) and TAG14 (P=0.000) clone. However, the difference in slope between the two clones was not statistically significant (F=0.40, P=0.527).

The higher values of g_s and E in TAG14 were associated with higher, not lower leaf water potentials, suggesting differences in hydraulic characteristics between the clones, and so leaf water potentials were plotted as a function of transpiration rates, up to the point of maximum transpiration rates (Fig. 3.2.4). The relationship in TAG14 was not really linear, suggesting that hydraulic resistance in this clone declined with increasing transpiration rates. When linear regressions were fitted to the data, the slope of the regression for GU210 was slightly steeper than that for TAG14. Although this difference in slopes is not statistically significant (F=2.30, P=0.126), the results

do indicate that the transpiration pathway in TAG14 may offer somewhat less hydraulic resistance than in GU210.

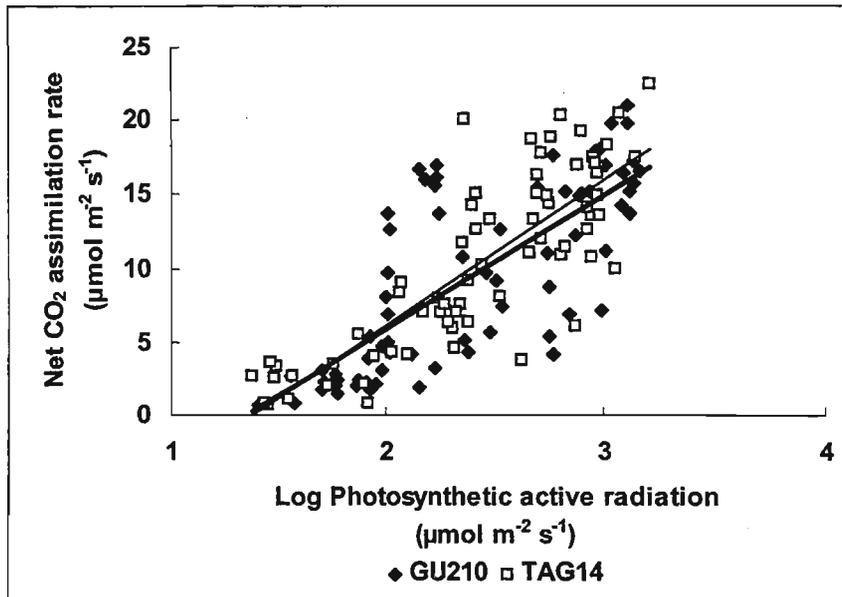


Fig. 3.2.3 The relationship between net CO₂ assimilation rate and log transformed incident photosynthetic active radiation for 3 months old saplings of the clones GU210 and TAG14 grown at a mesic site in the field. The equations of the fitted linear regressions are $y = 9.02x - 12.15$, $r^2 = 0.57$ for GU210 (bold line) and $y = 9.81x - 13.44$, $r^2 = 0.68$ for TAG14 clone (thin line).

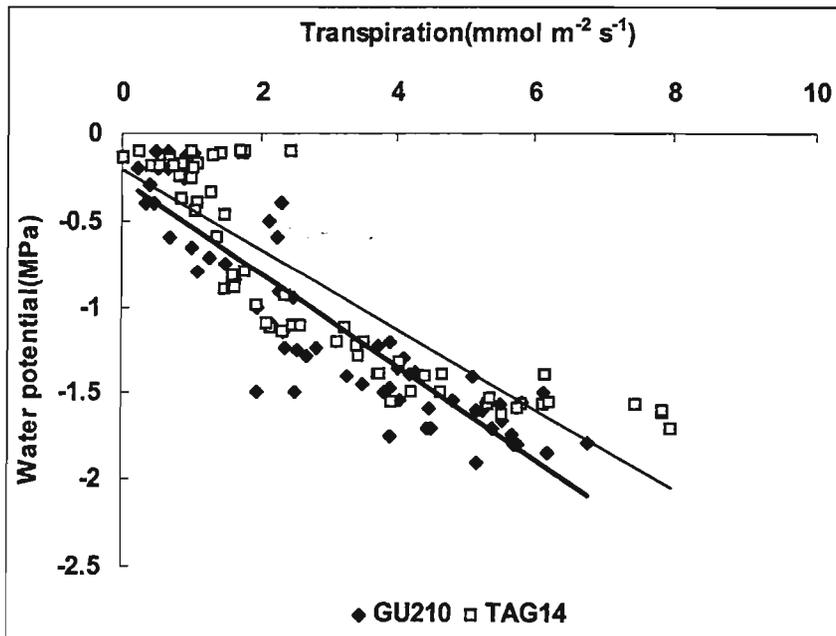


Fig. 3.2.4 The relationship between leaf water potential and transpiration rate for 3 months old saplings of the clones GU210 and TAG14 grown at a mesic site in the field. The equations of the fitted linear regressions are $y = -0.2691x - 0.2655$, $r^2 = 0.81$ ($P = 0.000$) for GU210 (bold line) and $y = -0.2319x - 0.201$, $r^2 = 0.75$ ($P = 0.000$) for TAG14 clone (thin line).

3.2.4 Ultrasonic acoustic emission detection of xylem cavitation

Only a small number of UAE events accumulated in both clones in the early morning, with acoustic events accumulating rapidly during mid-morning, levelling off after midday (Fig. 3.2.5). Total accumulated events in GU210 was more than twice that in TAG14. The data are expressed as events per hour, together with corresponding stomatal conductances and water potentials in Fig. 3.2.6. Cavitation increased progressively in both clones with decreasing xylem water potential from morning to midday. Maximum rates of cavitation in both clones occurred at mid-morning, with higher rates in GU210 (4216 EPH) than in TAG14 (1382 EPH). The water potentials corresponding to these maximum rates (-1.55 MPa and -1.49 MPa, respectively) were not significantly different ($F=0.45$, $P=0.540$).

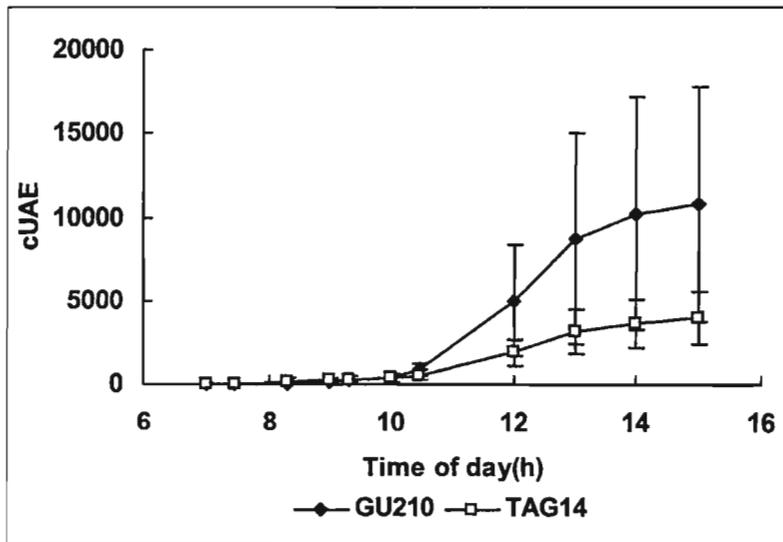


Fig. 3.2.5 Xylem cavitation events measured as cumulative ultrasonic acoustic emissions (cUAE) from 07.00 h to 15.00 h in 3 months old saplings of the clones GU210 and TAG14 grown at a mesic site in the field. Error bars represent the \pm SEM ($n=3$).

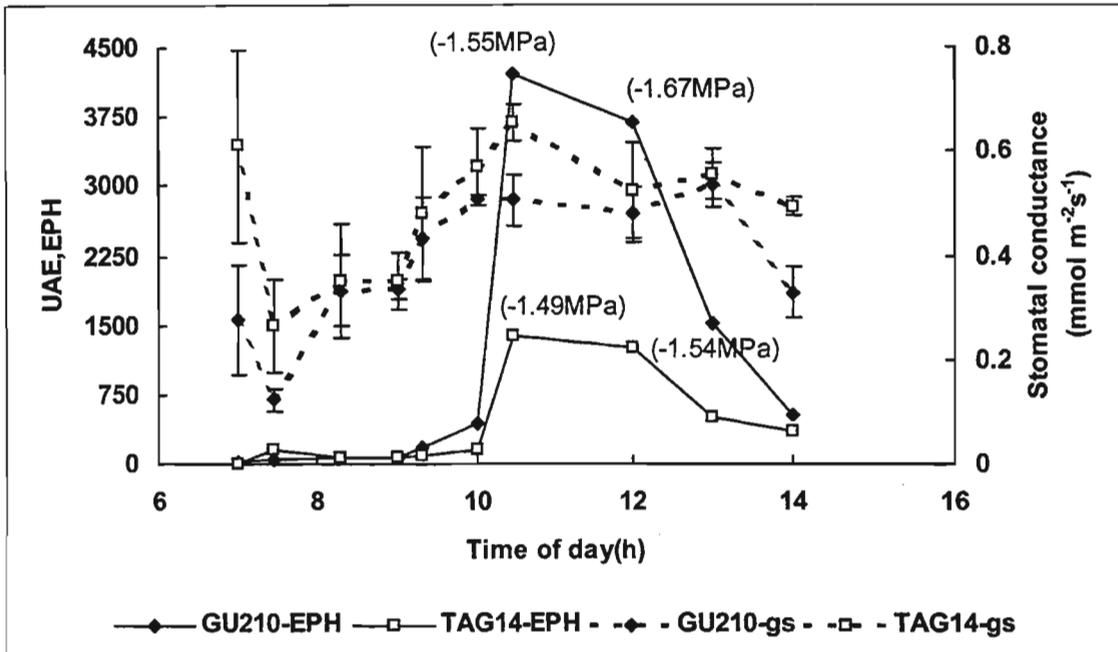


Fig. 3.2.6 Concurrently measured ultrasonic emission events per hour (UAE, EPH), with leaf water potentials (indicated in parentheses) and stomatal conductances (g_s) are shown for the two field grown clones. Error bars represent the \pm SEM ($n=3$).

In both clones there was a decrease in stomatal conductance from 10.00 h to 12.00 h, but the proportional decline was greater in TAG14 (20%) than in GU210 (7%). Although water potential continued to decline between 10.00 h and 12.00 h, and cavitation events continued, the decline in water potential was less in TAG14 (3%) than in GU210 (8%); this was probably a consequence of the greater proportional decline in g_s in TAG14. The decline in the rate of acoustic emissions was similar in the two clones (10% for TAG14 and 12% for GU210). The data suggest that the stomata of TAG14 are more sensitive to water potential than those of GU210: the decrease in g_s in TAG14 was greater than in GU210, although the corresponding water potential were similar (-1.49 MPa in TAG14, and -1.55 MPa in GU210).

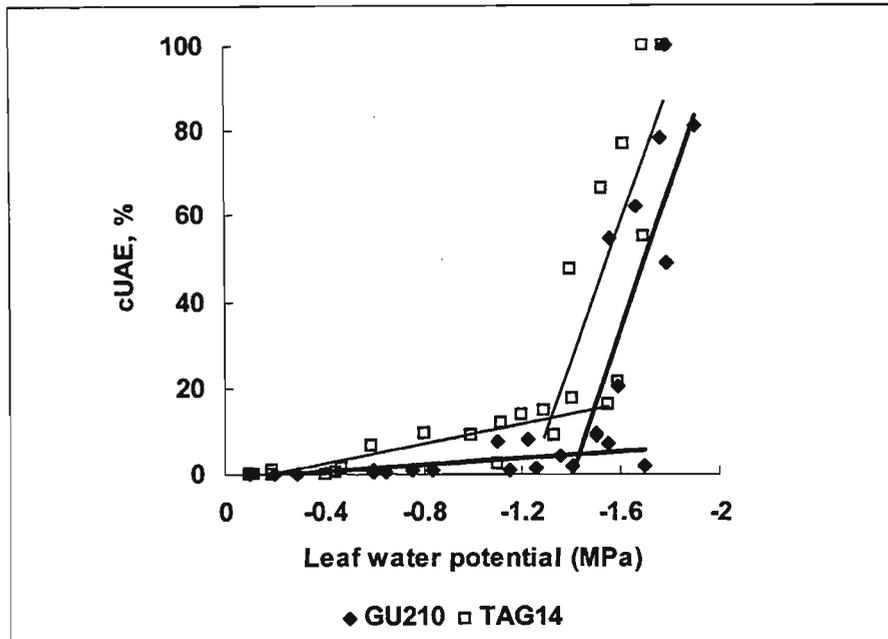


Fig. 3.2.7 Cumulated ultrasound acoustic emission expressed as percentage of the maximum (cUAE,%) in the main stems of 3 months old saplings of the clones GU210 and TAG14 grown at a mesic site in the field, plotted as a function of leaf water potential. See text for the meaning of the regression lines.

Cumulated acoustic events were expressed as percentages of the maximum cUAE and plotted against water potential (Fig. 3.2.7). If one accepts an arbitrary cavitation threshold as the water potential corresponding to the accumulation of 5 to 10% of the maximum acoustic emissions (Salleo *et al.*, 2000; Nardini *et al.*, 2001), the initiation of critical water potential triggering xylem cavitation ($\psi_{CAV,cUAE,\%}$) was -1.38 ± 0.10 MPa (SEM) for GU210 and -0.93 ± 0.18 MPa (SEM) for TAG14 (calculated as the mean of the water potentials corresponding to acoustic emissions between 5 and 10% of the total: $F=6.88$, $P=0.034$).

However, visual inspection of the data indicate that a marked increase in accumulation of acoustic events occurred at water potentials lower than these. Regressions lines were fitted to the data points corresponding to low cumulative events (<20%), and to the data points corresponding to high cumulative events (>40%), but also including the four to five points at the lowest water potentials with cumulative events below 20%. The intersection of these two lines gives the water potential at which a marked accumulation of events occurred, and could be taken as a

less arbitrary indicator of the threshold water potential generating substantial cavitation events. The values of the critical thresholds estimated this way were -1.42 MPa for GU210 and -1.32 MPa for TAG14, indicating that the stem xylem of TAG14 is more vulnerable to cavitation than that of GU210.

3.2.5 Root pressure (P_r)

De-topped stumps of both clones shows positive root pressures both overnight and at midday (Fig. 3.2.8). GU210 had a higher overnight root pressure of 41 kPa (n =10) than TAG14 at 28 kPa (n = 8), this difference being marginally significant (F = 4.30, P =0.055). Root pressure at midday was higher than the overnight value in both clones, respectively 62 kPa (n = 9) in GU210 and 53 kPa (n = 8) in TAG14. The difference between the clones was not significant (F=0.54, P=0.475), whilst that between overnight and midday was for TAG14 (F=10.76, P=0.005), but not for GU210 (F=3.48, P=0.080).

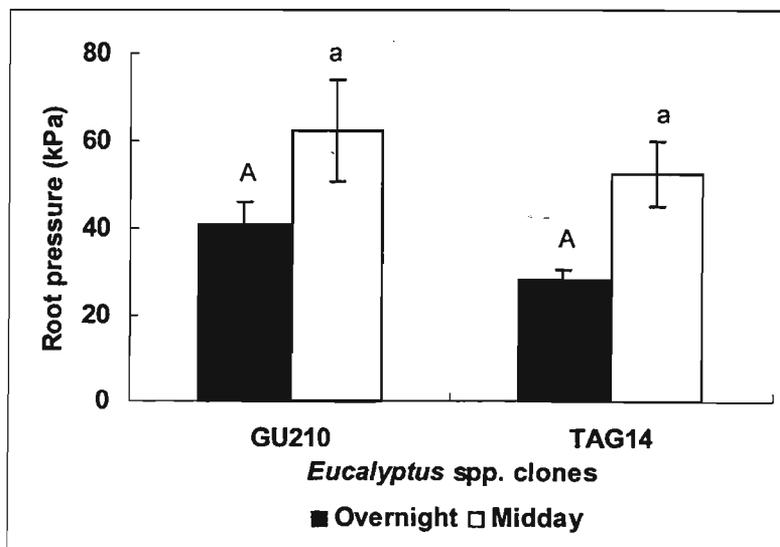


Fig. 3.2.8 Overnight and midday xylem root pressure measured in 3 months old saplings of the clones GU210 and TAG14 grown at a mesic site in the field. Error bars represent the \pm SEM (n=8 to 10). Different capital and small letters indicate significant differences at $P < 0.05$ between overnight and the midday (one-way ANOVA).

3.2.6 Root, shoot and whole plant hydraulic conductances

Absolute root and shoot hydraulic conductances measured by the HPFM are shown in Table 3.2.3. K_r values in TAG14 were significantly higher than those of GU210 ($F=18.47$, $P=0.001$), whereas the K_s of TAG14 was significantly lower than those of GU210 ($F=8.58$, $P=0.011$).

Table 3.2.3 Absolute hydraulic conductances (raw data from the HPFM) measured in 3 months old saplings of the clones GU210 and TAG14 grown at a mesic site in the field; means \pm SEM ($n = 8$). For each parameter, different letters indicate significantly different values at $P<0.05$ (one way- ANOVA within each parameter)

Absolute Hydraulic Conductances ($\text{kg s}^{-1}\text{MPa}^{-1} \times 10^{-5}$)		
Clones	K_r	K_s
GU210	5.21 ± 0.57^A	22.50 ± 2.29^A
TAG14	10.30 ± 1.12^B	14.80 ± 1.68^B

However, hydraulic conductances are more meaningful if normalised to some measure of plant size. Thus the data were expressed as shoot conductance per unit leaf area ($K_{s/LA}$), root conductance per unit leaf area ($K_{r/LA}$), shoot conductance per unit total shoot dry weight ($K_{s/tsdw}$) and whole plant conductance per unit leaf area ($K_{p/LA}$). Whole plant conductance was calculated as $K_p = 1/(1/K_s + 1/K_r)$. Root conductances were not normalised to the amount of root because of the difficulty of excavating roots of numerous individuals in the field.

$K_{r/LA}$ (Fig. 3.2.9. A) of GU210 was significantly lower than that of TAG14 ($F=16.30$, $P=0.001$), but $K_{s/LA}$ (Fig. 3.2.9. A) of GU210 was significantly higher than that of TAG14 ($F=9.90$, $P=0.007$). Similarly, $K_{s/tsdw}$ (Fig. 3.2.9. B) value was higher in GU210 than in TAG14 ($F=79.40$, $P=0.000$). The pattern of differences between the clones (higher shoot but lower root conductances in GU210, and the converse in TAG14) was such that whole plant hydraulic conductances ($K_{p/LA}$, Fig. 3.2.9. C) did

not differ significantly at the 5% probability level, although the difference was significant at the 10% level (GU210, $7.25 \times 10^{-5} \text{ kg s}^{-1} \text{ MPa}^{-1} \text{ m}^{-2}$; TAG14, $9.39 \times 10^{-5} \text{ kg s}^{-1} \text{ MPa}^{-1} \text{ m}^{-2}$; $F=3.30$, $P=0.089$).

Because the leaf areas of the clones were similar (Table 3.2.1), normalising shoot and root conductances by the leaf area supplied yielded values in the same relationship as the absolute values. This could be taken to suggest that the clones were not adjusting their leaf areas in relation to their hydraulic sufficiency, possibly because they were growing in mesic sites. However, despite the marked differences at the root and shoot level, whole plant conductances were similar, indicating that adjustment of leaf area was not necessary.

3.2.7 The components of whole plant resistances

The hydraulic resistances, normalised to leaf area, of the shoots (Table 3.2.4) of TAG14 were significantly higher than those of GU210 ($F=11.81$, $P=0.004$). Most of the shoot resistance was contributed by the leaves, which includes both vascular and non-vascular components, and leaf resistance of TAG14 was significantly higher than that of GU210 ($F=6.02$, $P=0.028$). The resistances of the other component of the stem were generally small and, except for R_{MS} , were not different between the clones. Root resistances were larger than stem resistances in both clones, and differed significantly between the clones ($F=13.61$, $P=0.002$).

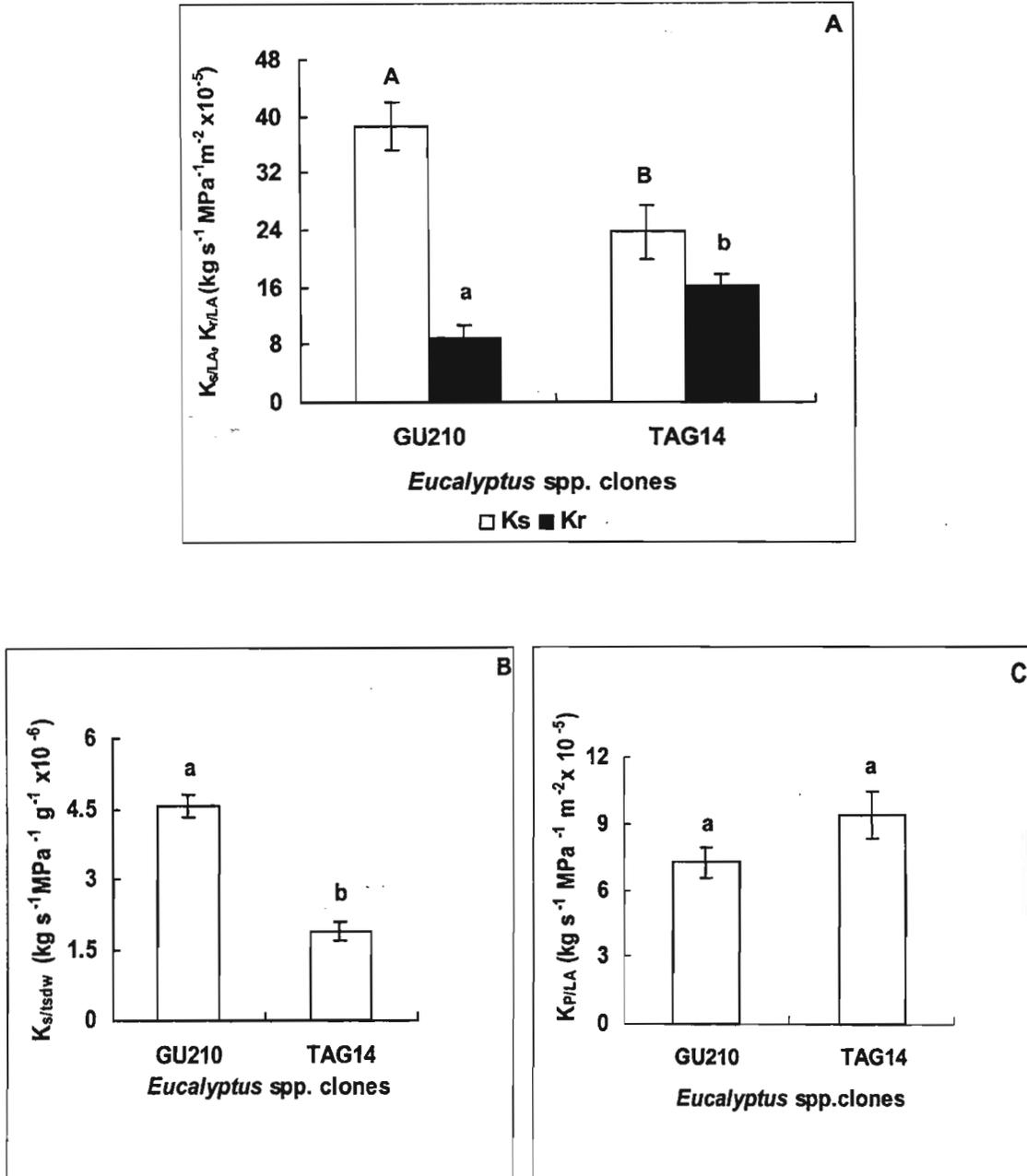


Fig. 3.2.9 Hydraulic conductances measured for 3 month old saplings of the clones GU210 and TAG14 grown at a mesic site in the field. (A) K_r and K_s expressed per unit leaf area, (B) K_s expressed per unit total shoot dry mass basis, (C) K_p expressed per unit leaf area basis. Error bars represent the \pm SEM (n=8). Different capital letters ($K_{s/LA}$), and small letters ($K_{r/LA}$) in (A), and small letters in B and C indicate significantly different values at $P < 0.05$ for clones (one way- ANOVA).

Table 3.2.4 Contributions to whole plant hydraulic resistances of the individual plant components of 3 month old saplings of the clones GU210 and TAG14 grown at a mesic site in the field; means \pm SEM (n = 8). For each component, different letters indicate significant differences between clones at $P < 0.05$ (one- way ANOVA).

Hydraulic resistances expressed per unit leaf area ($\text{kg}^{-1} \text{ s MPa m}^2 \times 10^3$)						
Clones	R_L	R_P	R_{LBB}	R_{MS}	R_S	R_r
GU210	1.22 \pm 0.15 ^A	0.042 \pm 0.01 ^A	0.81 \pm 0.11 ^A	0.37 \pm 0.05 ^A	2.75 \pm 0.30 ^A	12.00 \pm 1.35 ^A
TAG14	2.46 \pm 0.37 ^B	0.084 \pm 0.03 ^A	1.08 \pm 0.13 ^A	1.07 \pm 0.22 ^B	4.70 \pm 0.53 ^B	6.67 \pm 0.74 ^B

The proportional contribution of the shoots and roots differed between the clones (Table 3.2.5). In particular, in GU210, the roots contributed over 80% of the total resistance, while in TAG14, this contribution was less than 60%.

Table 3.2.5 Proportional contributions of individual components to the total plant hydraulic resistances of 3 months old saplings of the clones GU210 and TAG14 grown at a mesic site in the field; means \pm SEM (n = 8).

Contribution of hydraulic resistances (%) expressed per unit leaf area						
Clones	R_L	R_P	R_{LBB}	R_{MS}	R_S	R_r
GU210	10.50 \pm 0.68	0.30 \pm 0.07	5.47 \pm 0.22	2.50 \pm 0.13	18.80 \pm 0.74	81.20 \pm 0.74
TAG14	21.80 \pm 2.59	0.68 \pm 0.23	9.51 \pm 0.62	9.31 \pm 1.59	41.30 \pm 2.58	58.70 \pm 2.58

Chapter: 3.3 Discussion

3.3.1 Comparative growth

Total leaf area produced by plants of the two clones was the same (Table 3.2.1). This is at variance with reports that leaf area differed between three clonal lines of *Eucalyptus globulus* (Labill.) (Pita and Pardos, 2001), and a six clonal lines of *Eucalyptus camaldulensis* Dehnh. (Farrell *et al.*, 1996). However, both total leaf area and specific leaf area was the same in saplings of Scots pine and Sitka spruce (Jackson *et al.*, 1995a). Specific leaf area (SLA) can be brought about by a change in several leaf characteristics, both chemical and anatomical (Lambers and Poorter, 1992). The specific leaf area of GU210 was greater than that of TAG14, but despite the relationship that has been shown to exist between this parameter and photosynthetic rates (Pereira *et al.*, 1992), there was no difference in maximum net CO₂ assimilation rates obtained in the field between the clones. Similar results have been found for clones of *E. globulus* (Pita and Pardos, 2001). Changes in SLA may also be brought about by differences in the leaf veinal transport system (Lambers and Poorter, 1992), and leaf hydraulic resistance of GU210 was lower than that of TAG14, possibly suggesting less allocation of biomass to transport tissue in GU210 which could lead to higher specific leaf area.

Total shoot mass, plant height, and stem over-bark diameter of TAG14 were greater than GU210. This is consistent with a report of Pita and Pardos (2001) who found significant differences in these growth parameters between potted two and three month old specimens of three clones of *E. globulus*. In contrast, no significant differences among six month old potted specimens of three different clones of *E. globulus* were observed in dry matter accumulation (Osorio *et al.*, 1998). In another study, in contrast to our data, February *et al.* (1995) found there were no differences in total whole plant dry weight, and stems diameter between 16 month old *E. grandis* and the *E. grandis* x *camaldulensis* in large pots. It appears that inter-clonal differences apparent in young specimens disappear with age.

3.3.2 Leaf gas exchange

The measured values of g_s and A were high, but similar values have been found by other workers in *Eucalyptus* species (Pereira *et al.*, 1986; Dye and Olbrich 1993; Mielke *et al.*, 2000; Clearwater and Meinzer, 2001). The highest values of g_s and A usually were observed between 10.00 h and 12.00 h of day, with accordance with the report of Mielke *et al.* (1999) on *Eucalyptus* sp.

Although g_s , E and leaf water potential differed slightly between the clones, the lower stomatal conductance in GU210 did not appear to limit CO₂ assimilation, as there were no differences between the clones. Despite this lack of difference in photosynthetic rates, TAG14 produced higher above ground biomass than GU210. A similar lack of relationship between photosynthesis rate and growth was reported on field grown *E. globulus*, when two watering treatments with fertilizers were implemented, although higher water availability resulted in higher growth (Pereira *et al.*, 1989, 1992). In potted seedlings of *E. pauciflora*, water stress at first caused partial stomatal closure without any decline in the net CO₂ assimilation rate or intercellular CO₂ partial pressure, which was affected only under severe water stress (Kirschbaum, 1987). This apparent lack of stomatal limitation of CO₂ assimilation is supported by the observation of Clearwater and Meinzer (2001) that an increase in leaf nitrogen concentration and photosynthetic capacity in field grown *E. grandis* resulted from fertilizer treatment, although there was no effect on stomatal behaviour. Similarly, an increase in foliar nitrogen concentration may have increased the photosynthetic capacity of field grown *E. globulus* (Pereira *et al.*, 1992). This aspect of the influence of nutrients was not examined in this present study. According to Osorio *et al.* (1998), water deficits rather than leaf nitrogen levels are more important in delaying growth. Both Pereira *et al.* (1992) and Sheriff (1992) have suggested that the increase in biomass production as a result of fertilizer addition resulted from an increase in total leaf area and radiation interception and hence CO₂ assimilation by the canopy, rather than from an increase in photosynthesis rate per unit leaf area. However, in the present study, the total leaf area of the two clones was statistically similar. According to Sheriff (1992), very small differences in rates of carbon

assimilation have the potential to produce large differences in biomass over time. It is possible was that the slightly higher midday net CO₂ assimilation rate of TAG14 was adequate to accumulate more carbon than in GU210. As the clones had different stomatal conductances, their water using efficiency was expected to be different. According to Jones (1993) high water use efficiency is often associated with low productivity because efficiency in water use is acquired through stomatal closure which partially limits carbon assimilation rate. Instantaneous water use efficiency (A/E) did not differ between the clones, except on one occasion before midday, where TAG14 water use efficiency was higher. Although TAG14 showed greater above ground biomass, and GU210 had lower stomatal conductances, and presumably used less water, it was not possible to measure total water utilization by the plants, and so not possible to measure water use efficiency. However, there are reports where increasing water using efficiency was associated with poor growth in *E. grandis* and *E. grandis* x *camaldulensis* (February *et al.*, 1995; estimated from biomass production and amount of water use) and *E. microtheca* (Li, 2000; estimated as instantaneous leaf level A/E, and Li *et al.*, 2000a; estimated from ratio of biomass to transpiration).

3.3.3 Hydraulic architecture

Root and shoot hydraulic resistances

The root hydraulic resistances normalised by leaf area (R_{r*LA} , absolute root hydraulic resistance multiplied by total leaf area) were higher than shoot resistances normalised by leaf area (R_{s*LA} , absolute shoot hydraulic resistance multiplied by total leaf area), comprising 59% of total plant resistance in TAG14 and 81% in GU210. Commonly, root and shoot resistances are approximately equal, or root resistances are somewhat lower than shoot resistances (Tyree *et al.*, 1998; Becker *et al.*, 1999; Nardini and Tyree, 1999; Brodribb and Hill, 2000). However, there are a few reported occurrences of R_r constituting a large proportion of R_{Plant} : 66% in *Acer saccharinum* (Tsuda and Tyree, 1997), 55 to 65% in some *Quercus* species (Nardini and Tyree, 1999; Nardini and Pitt, 1999), 77% in *Toona australis* and 59 to 66% in a few conifer species (Brodribb and Hill, 2000). The value for GU210 measured in this study appears to be

the highest reported. In a preliminary field study at a different, but also mesic site, three month old samples of a different *Eucalyptus grandis* x *urophylla* clone (GU21) showed a very similar high proportion of (82%) of whole plant resistance contributed by the roots (unpublished data of the author). This suggests that high root resistances may be a genetic characteristic in *E. grandis* x *urophylla* hybrids.

The values of R_{r+LA} obtained in this study ranged from 6.67×10^3 to $12.00 \times 10^3 \text{ kg}^{-1} \text{ s MPa m}^2$ are very similar to values reported for *Eucalyptus regnans* ($7.87 \times 10^3 \text{ kg}^{-1} \text{ s MPa m}^2$) by Brodrribb and Hill (2000). Root hydraulic resistances measured in seven *Quercus* species ranged from 8.86×10^3 to $23.60 \times 10^3 \text{ kg}^{-1} \text{ s MPa m}^2$ (Nardini and Tyree, 1999), were reported as $20.6 \times 10^3 \text{ kg}^{-1} \text{ s MPa m}^2$ in *Acer sacharinum* (Tsuda and Tyree, 1997) and conifers had slightly higher values around $50 \times 10^3 \text{ kg}^{-1} \text{ s MPa m}^2$ (Brodrribb and Hill, 2000). The values reported here for the GU210 and TAG14 tended to be at the low end of the range reported for other species, suggesting that the roots of these clones were able to conduct water efficiently

The shoot hydraulic resistances measured in GU210 and TAG14 were 2.75×10^3 and $4.70 \times 10^3 \text{ kg}^{-1} \text{ s MPa m}^2$, respectively. Most of the shoot resistance resided in the leaves (R_L , including both vascular and non-vascular components), and ranged from 1.22×10^3 to $2.46 \times 10^3 \text{ kg}^{-1} \text{ s MPa m}^2$, comparable to the value of $4.69 \times 10^3 \text{ kg}^{-1} \text{ s MPa m}^2$ for *Eucalyptus regnans* (Brodrribb and Hill, 2000). However, compared with *Quercus* species (Tyree *et al.*, 1993a; Nardini and Pitt, 1999), *Juglans regia* (Tyree *et al.*, 1993b), *Acer saccharum* and *Acer rubrum* (Yang and Tyree, 1994), leaf resistances in the *Eucalyptus* clones in this study are low. Resistances of leaves as a proportion of shoot resistances were 56% and 52% in GU210 and TAG14, respectively, comparable to the 55% for *Eucalyptus regnans* (Brodrribb and Hill, 2000) and 50% in *Acer rubrum* (Yang and Tyree, 1994). Leaf resistances as a proportion of shoot resistances range from 20% in *Acer sacharinum* (Tsuda and Tyree, 1997) to 90% in some *Quercus* and *Fraxinus* species (Tyree *et al.*, 1993a; Cochard *et al.*, 1997; Nardini and Pitt, 1999). Petiole resistances (R_P) were very low compared with R_L , R_{LBB} and R_{MS} , contributing only 2 to 9% of total hydraulic resistances of the shoots. Contributions of leaf bearing branches was slightly higher in

GU210 with 29% than in TAG14 with 24%. The main stem contribution to total shoot resistance was greater in TAG14 with 23% compared with GU210 with 13%. The contribution of R_P and R_{MS} to total shoot resistance was less in *Eucalyptus* clones than reported for *Acer saccharinum*, with R_P contributing 15% and R_{stem} 63% (Tsuda and Tyree, 1997).

Root and shoot hydraulic conductances and its association with leaf physiology

Scaling of shoot hydraulic conductances to leaf area ($K_{S/LA}$) and to total shoot dry mass ($K_{S/tsdw}$) gave the same trend for both clones. Tyree *et al.* (1998) have recently discussed alternative scaling methods; the fact that GU210 had the higher normalised shoot hydraulic conductance ($K_{S/tsdw}$) suggests that this clone spends less carbon to provide an efficient pathway than does TAG14, which is in accordance with their different shoot dry masses.

A high shoot conductance should ensure rapid equilibration of the shoot with soil water at night, which would promote rapid growth by maintaining high values of meristem water potential (Tyree *et al.*, 1993a; Tyree *et al.*, 1998; Nardini and Tyree, 1999; Nardini and Salleo, 2000). In this present field study, the higher shoot conductances recorded in GU210 were in fact associated with lower leaf water potential, stomatal conductances, and above ground growth. The higher shoot conductance of GU210 is possibly a trait associated with strong apical control as it is thought to hydraulically favour the faster growing leader (Tyree and Ewers, 1996). However, a high shoot conductance alone will not ensure rapid equilibration with the soil if root conductance is low and the water cannot get into the stem. This was evident from the results that the higher $K_{r/LA}$ of TAG14 permitted sufficient water uptake from the soil to maintain lower leaf level water stress with higher g_s than in GU210. Tyree *et al.* (1993a), also suggested that low water potentials can cause reduced cell expansion, wall synthesis, protein synthesis, stomatal conductance and photosynthesis and an increase in xylem dysfunction by cavitation events. As such, the relationships between high and low shoot conductances, and the low and high above

ground biomass, respectively, was actually the inverse of this in the two clones if only shoot conductances were considered.

Whole plant hydraulic conductances ($K_{P/LA}$)

Whole plant hydraulic conductances will depend to some extent on xylem water potential, in that when xylem water potential falls below the cavitation threshold, whole plant hydraulic conductances will decrease as a consequence of the xylem embolism formed (Tyree and Sperry, 1989a). Although the difference between the clones in maximum whole plant hydraulic conductances measured using the HPFM ($K_{P/LA} = 1/(1/K_S + 1/K_T)$) was not significant ($P=0.089$; Fig. 3.2.9, C), there was a tendency for leaf physiology and above ground biomass production to correlate with this difference. Whole plant hydraulic conductance estimated from the relationship between transpiration rate and leaf water potential similarly did not show a significant difference between the clones (Fig. 3.2.4), but both methods indicated a higher conductance for TAG14 than GU210. In keeping with this, midday leaf water potentials, stomatal conductances and transpiration rates were higher in TAG14 than GU210 (Fig. 3.2.1), and total above-ground biomass of TAG14 was higher (Table 3.2.1). This is in accordance with the data of Vander Willigen and Pammenter (1998), who showed that 7 year old trees on mesic sites had higher biomass and branch leaf specific conductivity than those growing on xeric sites. The relationship between transpiration and leaf water potential for TAG14 was not really linear, particularly at high transpiration rates. This suggests the possible involvement of an hydraulic capacitance somewhere in the water transport system from soil to leaf that releases stored water at low water potentials, giving rise to an apparent increase in hydraulic conductance at high transpiration rates. Similarly, Koide (1985) and Tsuda and Tyree (2000) observed that hydraulic conductances estimated by the evaporative flux (EF) method (slope of the relationship between leaf water potential and transpiration rate) increased with increase of transpiration in sunflower plant. In this method transpiration and leaf water potential were measured almost instantaneously on the same leaf, so a 1:1 relationship between these two values is obtained. However, this

method cannot give a complete measure of whole plant conductance as the values of both transpiration and water potential will vary from leaf to leaf.

3.3.4 Hydraulic segmentation

According to the hydraulic segmentation hypothesis (Zimmermann, 1983; Tyree and Ewers, 1991), a plant may sacrifice small, expendable distal parts and safeguard the primary body from xylem cavitation events during severe water stress. There are reports that the distal parts of both shoots (Tyree *et al.*, 1993b; Kikuta *et al.*, 1997; Salleo *et al.*, 2000; Nardini *et al.*, 2001) and roots (Sperry and Saliendra, 1994; Alder *et al.*, 1996; Hacke and Sauter, 1996; Sperry and Ikeda, 1997; Kavanagh *et al.*, 1999) are more vulnerable to xylem cavitation. Further, combined studies of both hydraulic resistances and xylem vulnerability showed that distal parts like petioles, which had lower hydraulic resistances, were also more vulnerable to xylem cavitation (Tyree *et al.*, 1993b and Tsuda and Tyree, 1997), supporting the vulnerability segmentation hypothesis.

Hydraulic segmentation, in terms of increasing resistances, have been reported in the shoots of various species (Tyree *et al.*, 1993a; Tyree *et al.*, 1993b; Nardini and Pitt, 1999; Nardini and Salleo, 2000; Sobrado, 2000). Similar effects were found in the two *Eucalyptus* spp. clones studied here, in that resistances increased from the main stem to leaf bearing branches and then to leaves. Values of R_L and R_{MS} were higher for TAG14 than GU210. This suggests the existence of different hydraulic segmentation patterns in the shoots of the two clones. The higher leaf resistances in TAG14 suggest that leaves in this clone may be sacrificed earlier as a consequence of a water deficit, compared with GU210, giving a better safeguard to the primary body of TAG14. In support of this, it was observed that leaf shedding in TAG14 occurred before that in GU210 when three months old potted saplings were droughted for five days in the greenhouse area. Similar evidence for vulnerability segmentation was reported in *Juglans regia* L. where the association of higher R_L with lowest petiole resistances and subsequent leaf shedding was found (Tyree *et al.*, 1993b).

Further to the above discussion on hydraulic segmentation, the recently established patterns of vulnerability segmentation show that the most vulnerable plant segments do not necessarily have to be the distal plant segment with highest hydraulic resistances. The patterns that have emerged from combining hydraulic resistances and vulnerability curves have been reported by Sperry and Saliendra (1994), Tsuda and Tyree (1997) and Cochard *et al.* (1997). According to this the components of the plant with the highest hydraulic resistances (roots and leaf rachids) are less vulnerable to xylem cavitation than other plant components in *Acer saccharinum* (Tsuda and Tyree, 1997) and in *Fraxinus excelsior* (Cochard *et al.*, 1997). On the other hand, Sperry and Saliendra (1994) found that in *Betula occidentalis* roots had high conductances, as a consequence of xylem vessels that were wider than those in the twig, but also had high vulnerability. Similarly, it is possible that the roots of TAG14, with higher hydraulic conductances, might be at a higher risk of xylem cavitation, compared with those of GU210, although the high conductances in TAG14 had the advantage of maintaining good hydraulic sufficiency of the whole plant. Low vulnerability in plants with high hydraulic resistance might be favoured to prevent xylem cavitation over normal ranges of E (Tsuda and Tyree, 1997). However, to assess whether vulnerability segmentation occurs in the present study on the *Eucalyptus* spp. clones requires combined studies like those of Tyree *et al.* (1993b) and Tsuda and Tyree (1997).

If the high root resistance of GU210 is associated with narrow xylem conduits, as is the case in *Betula occidentalis* (Sperry and Saliendra, 1994), this high resistance might be associated with low vulnerability to cavitation. The high resistance would lead to lower water leaf potentials and stomatal conductances, reducing transpiration rates thus maintaining leaf water potential above the value triggering xylem cavitations. On the other hand the low resistance of TAG14 could be associated with high vulnerability (the stems are more vulnerable than those of GU210). Steudle and Heydt (1997) and Sperry and Ikeda (1997) have suggested that cavitation in finer roots could transiently limit water supply to the leaves; this could contribute to plant survival by maintaining soil water availability during a drought and preventing cavitation at the root-soil interface. Theoretically this phenomenon could occur in

TAG14 subjected to a soil water stress, but the experience of field foresters is that this clone is actually drought sensitive, not tolerant.

3.3.5 Stem xylem vulnerability to cavitation

The xylem vulnerability to cavitation, in terms of leaf water potential triggering xylem cavitation, was estimated by two methods; firstly, as explained by Nardini and Salleo (2000), Salleo *et al.* (2000) and Nardini *et al.* (2001), as the mean of the water potentials corresponding to acoustic emissions between 5 and 10% of the total ($\psi_{CAV, cUAE, \%}$), and secondly, as the intersection of lines fitted to data at low and at high cumulative emissions (Fig. 3.2.7). Both methods showed that leaf water potential initial triggering xylem cavitation was higher for TAG14 than GU210. Also, EPH_{max} occurred at a higher leaf water potential for TAG14 than GU210, although these values were not significantly different. The values calculated using the intersection method (-1.32 MPa for TAG14, and -1.42 MPa for GU210), were similar to the values of water potential corresponding to 50% loss of conductivity measured hydraulically on branches of seven year old trees of related clones (-1.31 MPa for TAG5 and -1.50 MPa for GU21) by Vander Willigen and Pammenter (1998); the patterns between clonal families was consistent between the two studies, indicating the importance of genotype in determining vulnerability to cavitation.

The concept of safety versus efficiency of the hydraulic supply system has been discussed by a variety of authors. The general trend is that the more efficient wide conduits are more vulnerable to cavitation (Zimmermann, 1983; Tyree *et al.*, 1994a; Lo Gullo *et al.*, 1995), and are more difficult to refill (Jaquish and Ewers, 2001), although, according to the 'air-seeding' hypothesis, it is the size of the pit membrane pore that determines vulnerability to cavitation. However, the reverse pattern was apparent in the *Eucalyptus* spp. clones investigated here: the hydraulic conductivity of the stem (where the UAE detector was placed) was lower in TAG14, but the vulnerability to cavitation was higher, relative to GU210. Similar negative correlations have been reported in some *Quercus* species (Cochard *et al.*, 1992a) and in seedlings of Douglas fir grown with different water availabilities (Kavanagh *et al.*,

1999). These authors attributed the effect to the different proportions of early and late wood in the seedlings; however, such an effect is unlikely in this *Eucalyptus* spp. clones, as *Eucalyptus* is diffuse porous, and the plants studied were only three months old. TAG14 showed more profuse branching than GU210, and the junction constrictions could have reduced shoot conductivity. It has been suggested that vessel vulnerability is coupled to the prevailing vessel water potential, and the data presented here are in accord with that; GU210 operates at lower water potentials (Fig. 3.2.1) and is less vulnerable to cavitation (Fig. 3.2.7) than TAG14.

Linking of stomatal conductances and xylem cavitation

A function of stomata is to reduce transpiration to levels that prevent leaf water potentials being reduced to the extent such that massive cavitation events and runaway xylem embolism cycles do not occur (Jones and Sutherland, 1991; Alder *et al.*, 1996; Sparks and Black, 1999; Salleo *et al.*, 2000). For both clones studied here, maximum stomatal conductance ($g_{s \max}$) and maximum rate of cavitation (EPH_{\max}) occurred at the same time (mid morning), but at different leaf water potentials (Fig. 3.2.6). Thereafter g_s decreased, as did the rate of cavitation, although leaf water potential continued to decline. The water potentials corresponding to the initiation of stomatal closure were in fact lower than those corresponding to the initiation of cavitation events (Fig. 3.2.7). Thus, stomatal control was not adequate to prevent substantial cavitations; this could indicate considerable redundancy in the hydraulic supply system in the main stem, where cavitations were measured. Interestingly, TAG14 had lower shoot hydraulic conductivity than GU210, and the decrease g_s in TAG14 following EPH_{\max} was 20%, compared with only 7% in GU210. This perhaps indicates that redundancy in the stem was less in TAG14 than GU210, and that consequently TAG14 is more sensitive to any mild change in leaf water potential, and may be more drought sensitive than GU210.

Stomata have been reported to maintain leaf water potential above the value of the water potential triggering xylem cavitation in a number of species (Saliendra *et al.*, 1995; Hacke and Sauter, 1996; Irvine *et al.*, 1998; Bond and Kavanagh, 1999;

Brodribb and Hill, 2000). In contrast, the midday leaf water potentials measured in this study were lower than the water potentials triggering cavitations ($\psi_{\text{CAV,cUAE}}$, %), and considerable cavitation events did occur (Fig. 3.2.6). Similarly, there are other reports of midday water potentials being lower than those triggering cavitations (Jackson *et al.*, 1995a, b; Hacke and Sauter, 1995; Sparks and Black, 1999).

An important question was whether stomata functioned to prevent xylem cavitations. Low stomatal conductances would prevent high tensions in the xylem water column, and reduce cavitations, as documented by Jones and Sutherland (1991), Alder *et al.* (1996), Sparks and Black (1999) and Salleo *et al.* (2000). In fact, the higher stomatal conductances in TAG14, relative to GU210, were associated with higher vulnerability, the reverse of predictions. However, cavitation events will release water into the transpiration stream, and if refilling can occur overnight, this effect could avoid low leaf water potentials during times of high transpiration rates. In accordance with this, the relationship between transpiration rate and leaf water potential in TAG14 was not truly linear (Fig. 3.2.4), suggesting a possible capacitance effect. According to Salleo *et al.* (2000), conduits of plants growing in wet soil and undergoing diurnal embolisms, can completely recover at night so that shoot cavitation remains a transient and completely reversible phenomenon.

3.3.6 Xylem recovery by positive root pressure

Positive root pressure is a mechanism by which embolized xylem conduits of small plants can be refilled (Devlin, 1966, 1990; Salisbury and Ross, 1991). Positive root pressures in woody species were reported by Sperry *et al.* (1987), Sperry *et al.* (1988b), Sperry (1993), Fisher *et al.* (1997) and Lopez-Portillo *et al.* (2000), with measured values ranging from 3kPa in *Fagus grandifolia* (Sperry, 1993) to 100 kPa in some vines (Sperry *et al.*, 1987), with a maximum recorded value of 225 kPa in a hemiepiphyte (Lopez-Portillo *et al.*, 2000). Van Der Meer *et al.* (1999) reported the presence of root pressure in *Eucalyptus regnans*. Both *Eucalyptus* spp. clones in this study exhibited positive root pressures, both overnight and at midday; midday positive pressures were also reported by Sperry (1993). Theoretically, a positive pressure of 10

kPa is required to raise a water column by 1 m, and so the pressures developed by the two clones in this study (41 to 62 kPa) were more than adequate to lift a water column the height of the plants (0.7 m), and this could be the processes by which embolisms are refilled.

It has been conventionally accepted that refilling of embolized vessels by root pressure occurs overnight, when transpiration is virtually absent. However, recently, Canny *et al.* (1997), McCully *et al.* (1998), and McCully (1999), using cryo-scanning electron microscopy, observed embolism repair in vessels of sunflower petioles and maize roots, concurrently with maximum transpiration rate. In a recent study, Zwieniecki *et al.* (2000) showed that the specific hydraulic conductivity of maple and tulip tree petiole was increased while the plant was actively transpiring and the xylem sap was under tension. They also indicated that living cells within a tissue specialized for long-distance transport were probably involved in the recovery in xylem conductance. Thus xylem recovery in any part of the plant concurrently with maximum transpiration rate may not be ruled out. Although significant only with overnight values, GU210 tended to have higher root pressures than TAG14, perhaps indicating that GU210 could refill embolized vessels more efficiently. Coupled with the lower vulnerability of GU210, this could contribute to a greater water stress tolerance in GU210 (as opposed to the possibility of TAG14 avoiding low leaf water potentials by releasing capacitance water through cavitation events).

Chapter 4: 14 Month Old Potted Saplings

Chapter: 4.1 Introduction

A major problem with working with mature tall *Eucalyptus* trees is the difficulty of access to the canopy to make physiological measurements on leaves, and to make estimates of root characteristics, because of the difficulty of excavating roots. Consequently, it was decided to undertake studies on saplings planted in pots large enough to prevent problems associated with restricted root growth. This permits detailed investigations of leaf physiology and hydraulic conductances under conditions of controlled water supply.

The objectives of the study on potted specimens was to answer the following questions:

- (a) Is there a relationship between hydraulic conductances and leaf physiological characteristics of *Eucalyptus* spp. clones subjected to different watering treatments?
- (b) Is there a relationship between the vulnerability of xylem to cavitation and watering treatment?
- (c) Are any differences in physiology reflected in differences in growth under the different water conditions?
- (d) Do any relationships identified in (a) to (c) above vary among clones? The question of whether any relationship between hydraulic conductances and productivity is causative (low hydraulic conductance leading directly to low productivity through effects on leaf physiology), or simply a case of co-variation of two parameters, is addressed in Chapter 7.

To answer these questions saplings of three clones considered to vary in drought tolerance were grown for 14 months in 25 l pots (this Chapter) and for 21 months in 85 l pots (Chapter 5; to assess any age-related changes) under different watering regimes. During the growth period extensive investigations of leaf physiological characteristics were undertaken, and a final harvest was made to measure growth and biomass allocation.

Chapter: 4.2 Materials and Methods

Details of clonal selection, planting and watering are given in Chapter 2, as this information concerning the details of the physiological measurements. This section provides information on experimental design and sampling.

4.2.1 Plant materials and samples

Twelve established cuttings of each of three *Eucalyptus* spp. clones (GC550, GU210 and TAG14) were planted in 25 l pots, and half were subjected to the 'high' watering treatment and the other half to 'low' water. An additional six cuttings of GU210 were included for comparative studies on ultrasonic acoustic emissions and low pressure flow meter measurements of vulnerability to cavitation (see Chapter 2). Planting date was the 24th of February 2000. The 42 pots were arranged in 4 rows (three rows of 12 and one row of ten pots). Within a row pots were 0.1m apart and rows were 0.3m apart. This spacing, which was considerably less than in commercial practice in the field (2 m x 3 m), was partially dictated by the space available.

4.2.2 Soil and plant water status measurements

Predawn leaf water potential (Ψ_{PDL})

Soil water potentials were estimated from predawn leaf water potentials on a monthly basis from July 2000 (when the plants were about 4 months old) until harvest at 14 months in March 2001. Water potentials were non-destructively measured with L-51 leaf psychrometers connected to an HR-33T Dew Point Microvoltmeter (Wescor, Inc., Logan, Utah U.S.A). Values of one to two leaves, depending on availability of leaf chambers, were measured each morning, and measurements repeated such that five to ten values were obtained for each clone under each watering treatment. To assess soil water potentials, predawn leaf water potentials were averaged across clones on a monthly basis, watering treatments being kept separate.

A



B



Fig. 4.2.1 Three different potted *Eucalyptus* spp. clones at the age of 14 months old (25 l volumes, A), and 21 months old (85 l volumes, B, see Chapter 5), at the trial site, greenhouse area, University of Natal, Durban, South Africa.

Diurnal measurement of leaf physiology and xylem cavitations

For many of the physiological parameters studied, because of equipment limitations, it was not possible to take measurements on all replicates at the same time. Thus measurements were spread over time, the specimen to be studied on the first day being determined randomly. A pattern was then established of measuring one specimen of each treatment, and then returning to the second replicate of each treatment and repeating the cycle. In this study, diurnal measurements of leaf physiology took place over the period from December 17th 2000 to March 31st 2001. Thus the 'replicate' values for any parameter are not strictly independent, measurements on different plants having been made on different days; however, the values were treated as independent replicates for analysis purposes, there being no other option.

Measurements were taken on two to three leaves per sample, from upper to mid canopy, at hourly intervals from 06.00 to 12.00, and then every two hours until 18.00. Water potentials were measured non-destructively, the leaf hygrometer chambers being attached to the leaves the afternoon prior to measurements, to ensure equilibration for more than 12 hrs. Measurements of net CO₂ assimilation rate (A), transpiration (E) and stomatal conductance (g_s), were made with a portable infrared gas analyzer (model LI-6400, Li-Cor Inc., Lincoln, NE, U.S.A), on leaves adjacent or opposite to the leaves concurrently being measured for leaf water potential.

The daily courses of xylem cavitation events were followed by detection of ultrasonic acoustic emissions (UAE) from the main stems of the plants being studied for water relations and gas exchange. An UAE sensor was mounted on the main stem 0.2 m above the soil surface at 18.00 h the day prior to measurements, the stem over-bark diameter being measured prior to mounting the sensor. Accumulated ultrasonic acoustic emissions (cUAE) and rate of emission (events per hour, EPH) were recorded from 06.00 h to 18.00 h. UAE were expressed as a percentage of the cumulative number of events occurring at the plateau (cUAE,%), as described by

Salleo *et al.* (2000). cUAE,% and EPH were plotted as a function of time of day, and as a function of the corresponding leaf water potential.

4.2.3 Morphological growth investigations

During the course of the experiment plant height from soil surface to the apical bud, and stem over-bark diameters at 0.1m above soil were measured (in the mornings, after being subjected to watering treatment) from the initiation of the watering treatments at 70 to 75 day intervals, until the harvest. After harvesting (and measurement of hydraulic characteristics) the plants were separated into leaves, stems and roots, and leaf area and biomass of each component measured.

4.2.4 Hydraulic characteristics

Collection of plant material

Whole shoots were excised 8 to 9 cm above soil level after the pots were fully watered to the field capacity that morning. Excised shoots were labelled, placed into a tub of water and transported to the laboratory covered in black polythene bags to reduce evaporation until hydraulic conductances were measured. The excised stumps were subsequently used to measure root pressure and then root hydraulic conductance.

Measurements of root pressure (P_r)

Once the excised shoots were severed, projecting root stumps (n=6) were shaved perpendicularly with a razor blade and the manometer was connected to the root stump using compression fittings. Thereafter, the rising water column in the manometer tube was observed the following day 06.00 h and 12.00 h. The proportion of the manometer tube occupied by the air-bubble in the morning and at noon gives the pressure in the bubble, and subtracting atmospheric pressure gives the root pressure above atmospheric.

Measurements of root hydraulic conductance (K_r)

The manometers measuring root pressure were disconnected from the stumps after the midday reading, the stumps re-shaved with a razor blade, and the compression fitting of the HPFM connected and filled with distilled, degassed and filtered water. The HPFM was connected to the other side of the compression fitting and root conductances (K_r) were immediately measured by transient water flow method, with three to four transients taken for value of root conductance (see Section 2.2.4). After these measurements, roots were excavated, carefully washed and biomass determined after oven drying at 60°C for 48 hours.

Shoot hydraulic conductances (K_s) and its components

Whole shoot hydraulic conductances (K_s) were measured by the quasi-steady state method using the HPFM. Thereafter, leaf resistance (R_L), petiole resistance (R_P), the resistance of leaf bearing branches (R_{LBB}), the resistance non-leaf bearing branches (R_{NLBB}), and main stem resistance (R_{MS}) were measured after sequentially excising the appropriate plant components. The total leaf area from the shoots was measured with a leaf area meter, and the biomass of the leaves, petioles and stems determined after oven drying at 60°C for 48 hours.

Chapter: 4.3 Results

4.3.1 Effects of watering treatments on growth and biomass allocation

Watering treatments had a significant effect on total plant biomass ($F=129.06$, $P<0.001$), with biomass being reduced by 44 to 49% by the low watering treatment, but there were no differences among clones ($F=0.87$, $P=0.429$) (Table 4.3.1). Significant reductions in biomass occurred in all plant components, but there were no differences among clones within a watering treatment. However, the extent of reduction in biomass of different plant components varied among clones, the most noticeable effect being that leaf mass was reduced by 32% in GC550, but by about 57% in the other two clones. Root: shoot ratios were not significantly influenced by watering treatment ($F=2.43$, $P=0.129$), as well as there being no differences among clones ($F=3.00$, $P=0.065$), although within each clone separately, reductions in root: shoot ratios with reduced watering occurred in GC550 and TAG14, with no effect in GU210.

Table 4.3.1 Growth parameters measured after harvest at 14 months of three *Eucalyptus* spp. clones subjected to high and low watering in 25 l pots. Means are given \pm SEM ($n = 6$). Letters represent (under each parameter) means separation by Scheffe's multiple range tests, showing comparison within the high (upper case lettering) and low (lower case lettering) watering treatments separately ($P<0.05$). Percentage reduction in growth parameters caused by watering treatment of each clone are also shown.

Clones	Treatments	Leaf DW,(g)	Stem DW,(g)	Root DW,(g)	Plant DW,(g)	R:S ratio
GC550	High	50.34 \pm 8.00 ^A	82.70 \pm 3.45 ^A	194.70 \pm 11.2 ^A	327.80 \pm 15.10 ^A	1.47 \pm 0.08 ^A
GC550	Low	34.55 \pm 4.86 ^a	50.20 \pm 2.31 ^a	97.55 \pm 5.50 ^a	182.30 \pm 6.11 ^a	1.20 \pm 0.15 ^a
% difference between treatments		31.36	39.31	49.91	44.40	
GU210	High	70.38 \pm 7.27 ^A	92.85 \pm 3.83 ^A	163.20 \pm 19.30 ^A	326.40 \pm 16.20 ^A	1.02 \pm 0.15 ^B
GU210	Low	28.83 \pm 4.18 ^a	50.83 \pm 1.63 ^a	87.98 \pm 5.89 ^a	167.65 \pm 8.43 ^a	1.11 \pm 0.08 ^a
% difference between treatments		59.03	45.25	46.08	49.10	
TAG14	High	49.73 \pm 10.50 ^A	87.17 \pm 2.59 ^A	171.05 \pm 25.90 ^A	307.94 \pm 34.90 ^A	1.25 \pm 0.14 ^{AB}
TAG14	Low	22.16 \pm 4.41 ^a	59.33 \pm 2.60 ^b	78.08 \pm 9.12 ^a	159.58 \pm 9.48 ^a	0.98 \pm 0.14 ^a
% difference between treatments		55.43	31.93	54.35	48.17	

Along with leaf biomass, total leaf area was also reduced by low water ($F=7.11$, $P<0.001$) (Table 4.3.2), although the extent varied among clones, being 23% in GC550 and 56% in the other clones. Under high water treatment the total leaf area of GC550 was less than that of the other two clones, but this difference was not apparent under reduced watering. Specific leaf area was not influenced by water treatment, but inter-clonal differences were such that, combined with differences in leaf area, there were no clonal differences in leaf weight ratio or leaf area ratio. Watering treatment had no effect on these two characteristics.

Table 4.3.2 Growth parameters measured after harvest at 14 months of three *Eucalyptus* spp. clones subjected to high and low watering in 25 l pots. Means are given \pm SEM ($n = 6$). Letters represent (under each parameter) the means separation by Scheffe's multiple range tests, showing comparison within the high (upper case lettering) and low (lower case lettering) watering treatments separately ($P<0.05$). Percentage reduction in growth parameters caused by watering treatment of each clone are also shown.

Clones	Treatments	LA, (m ²)	SLA, (m ² kg ⁻¹)	LWR	LAR, (m ² kg ⁻¹)
GC550	High	0.38 \pm 0.05 ^A	7.70 \pm 0.37 ^A	0.15 \pm 0.02 ^A	1.15 \pm 0.13 ^B
GC550	Low	0.29 \pm 0.03 ^a	8.70 \pm 0.43 ^a	0.19 \pm 0.02 ^a	1.61 \pm 0.14 ^a
% difference between treatments		22.53			
GU210	High	0.75 \pm 0.07 ^B	10.66 \pm 0.29 ^{AB}	0.22 \pm 0.03 ^A	2.32 \pm 0.25 ^A
GU210	Low	0.33 \pm 0.03 ^a	11.85 \pm 0.67 ^{ab}	0.17 \pm 0.02 ^a	1.88 \pm 0.12 ^a
% difference between treatments		55.83			
TAG14	High	0.66 \pm 0.10 ^B	14.82 \pm 2.16 ^B	0.16 \pm 0.03 ^A	2.14 \pm 0.18 ^A
TAG14	Low	0.29 \pm 0.04 ^a	14.03 \pm 1.44 ^b	0.14 \pm 0.03 ^a	1.86 \pm 0.26 ^a
% difference between treatments		55.97			

Growth in height and stem over-bark diameter (Fig. 4.3.1) differed such that by the end of the experiment, differences in these parameters between watering treatments was significant ($F=22.57$, $P<0.001$ and $F=61.53$, $P<0.001$, respectively). There were also clonal differences, with GC550 being taller than the other clones ($F=18.86$, $P<0.001$), and GU210 having greater over-bark diameter ($F=3.65$, $P<0.038$).

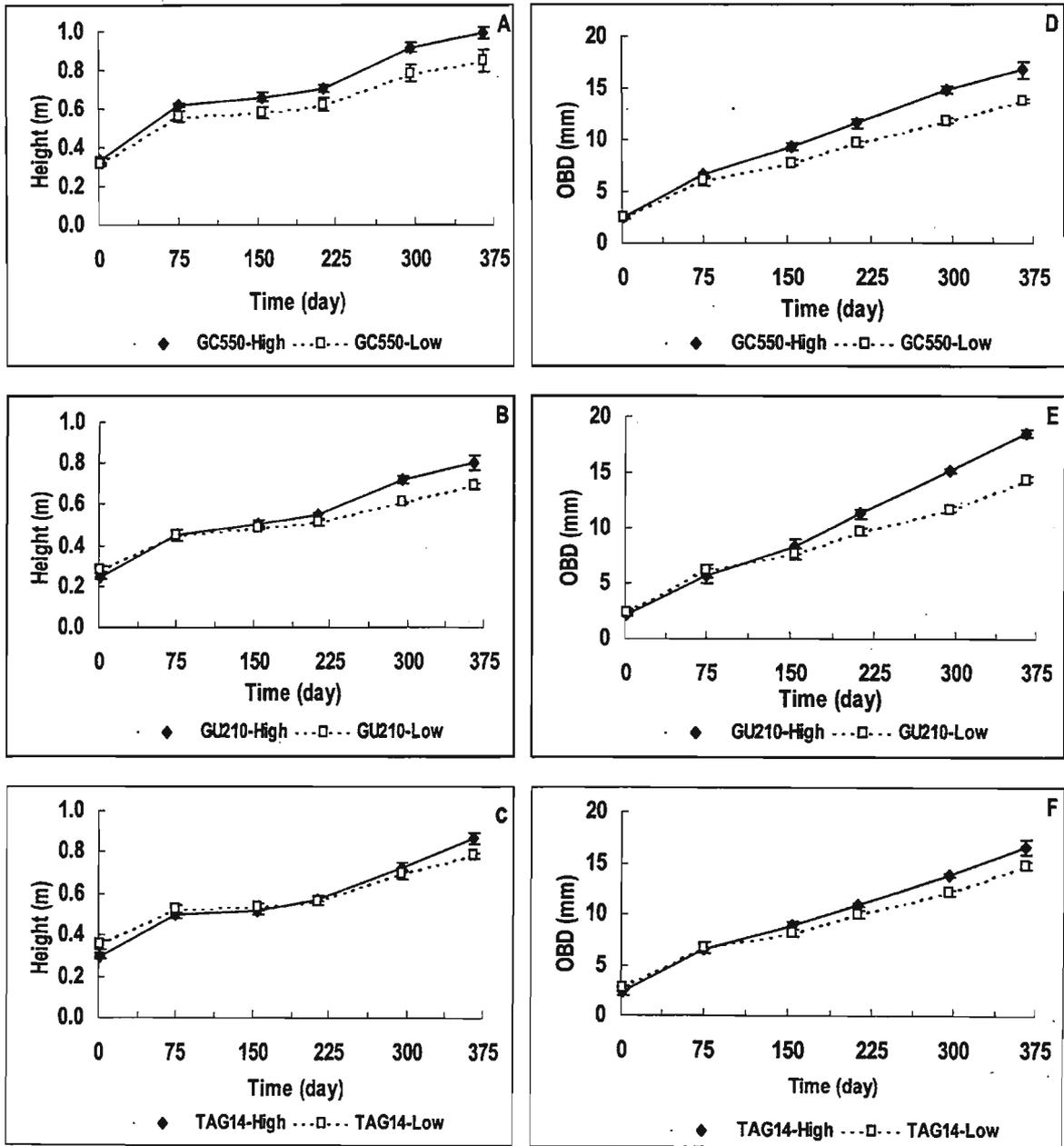


Fig. 4.3.1 Growth in terms of height (A, B & C) and stem over-bark diameter (D, E & F), as a function of time for 14 months from the implementation of watering treatments for three *Eucalyptus* spp clones grown in 25 l pots. Error bars represent the \pm SEM of the mean (n=6).

4.3.2 Predawn leaf water potential

Predawn leaf water potential (which can be used as a measure of soil water potential) was measured from 6th of July 2000 (when the plants were five months old) to 31st of March 2001 (except for November), and the results, averaged across the clones, are shown in Fig. 4.3.2. Although values were marginally higher for the plants receiving high water, these differences were not significant (except for the final reading, where, surprisingly, high watered plants had lower leaf water potentials).

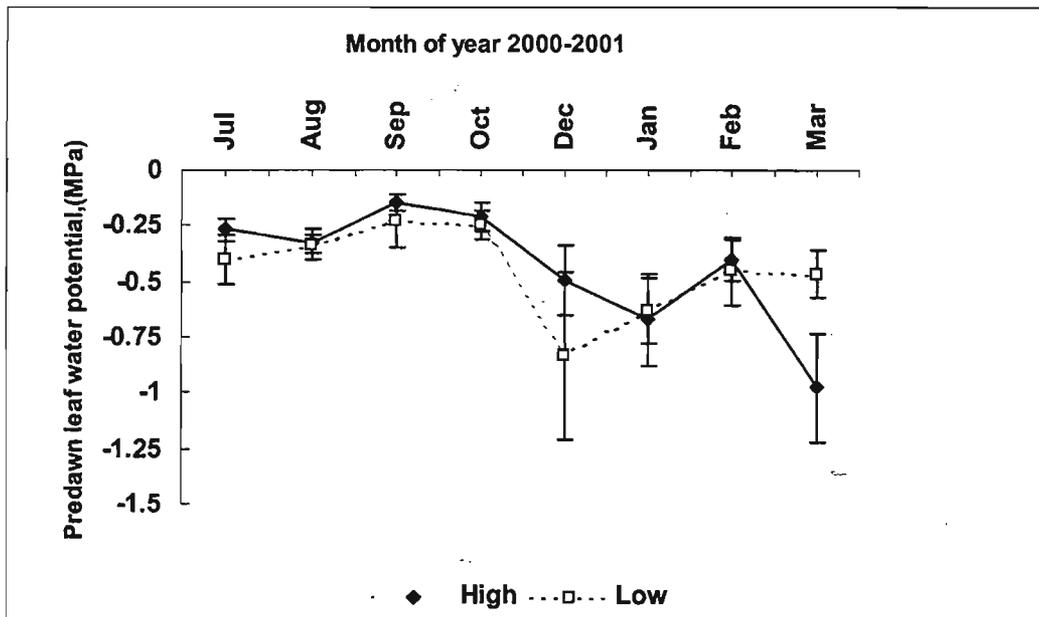


Fig. 4.3.2 Mean monthly predawn leaf water potential from July 2000 to March 2001 for the three *Eucalyptus* spp. clones grown in 25-l pots subjected to high or low watering treatment. Error bars represent the \pm SEM of the mean for each month (n=5 to 8).

The data for each clone within watering treatment were averaged across this measurement period (Table 4.3.3), but showed no differences between watering treatment ($F=0.04$, $P=0.847$) or among clones ($F=0.19$, $P=0.824$).

It was also noticed that, after watering, the soil surfaces of low watered plants were wet for a longer time than was the case for the high watered plants. It could be speculated that the better developed root systems of the high watered plants extracted

the applied water more rapidly than the less developed roots of the low watered plants.

Mild leaf wilting at midday was a common occurrence, indicating that plants of both treatments were suffering a water stress; recovery did take place after each watering. TAG14 showed necrosis at the tips as well as along the margins of a few leaves, which subsequently withered, suggesting that this clone is more susceptible to water stress.

Table 4.3.3 Mean predawn leaf water potential measured for three *Eucalyptus* spp. clones grown for 14 months in 25 l pots, and subjected to high or low watering treatment. Means are given \pm SEM (n=6). Letters represent (under each parameter) the means separation by Scheffe's multiple range tests, showing comparison within the high (upper case lettering) and low (lower case lettering) watering treatments separately (P<0.05).

Clones	Treatments	Ψ_{PDL} (MPa)
GC550	High	-0.44 \pm 0.11 ^A
GC550	Low	-0.42 \pm 0.06 ^a
GU210	High	-0.58 \pm 0.13 ^A
GU210	Low	-0.56 \pm 0.23 ^a
TAG14	High	-0.48 \pm 0.13 ^A
TAG14	Low	-0.54 \pm 0.10 ^a

4.3.3 Diurnal patterns of leaf physiological parameters

The mean values of PAR and VPD when leaf physiological parameters were measured are shown in Fig. 4.3.3. The low values prevalent when measurements were made on low watered TAG14 must have been co-incident, as there was no pattern to the sequence in which plants were studied that should have generated this phenomenon.

Changes in leaf water potential from 06.00 h to 18.00 h are shown in Fig. 4.3.4 (A, B & C). Leaf water potential decreased in all three clones from morning to midday, and then recovered slightly in the afternoon, although this was less marked in TAG14. Leaf water potential was marginally higher in high watered than low watered plants of GU210 and TAG14, but no difference between watering treatments was apparent in GC550. However, pooled values of midday xylem water potentials (Table 4.3.3) revealed that neither watering treatment ($F=0.41$, $P=0.524$) nor clone ($F=0.25$, $P=0.781$) had any significant effect.

Table 4.3.4 Mean midday leaf water potential measured for three *Eucalyptus* spp. clones grown for 14 months in 25 l pots, and subjected to high or low watering treatment. Means are given \pm SEM ($n=6$). Letters represent (under each parameter) the means separation by Scheffe's multiple range tests, showing comparison within the high (upper case lettering) and low (lower case lettering) watering treatments separately ($P<0.05$).

Clones	Treatments	Ψ_{Midday} (MPa)
GC550	High	-2.17 ± 0.44^A
GC550	Low	-2.04 ± 0.59^a
GU210	High	-2.10 ± 0.57^A
GU210	Low	-2.41 ± 0.65^a
TAG14	High	-2.17 ± 0.67^A
TAG14	Low	-2.80 ± 0.55^a

Diurnal patterns of net CO₂ assimilation rate (A) are shown in Fig. 4.3.4 (D, E & F). There were no consistent patterns of differences between watering treatments, although there was a tendency in GC550 for the high watered plants to exhibit higher photosynthetic rates, but the variation was high. In all clones and both treatments net CO₂ assimilation rates reached a maximum around 08.00 h and declined until midday, with sometimes a slight recovery in the afternoon. Analysis of data for 08.00 h, where A reached a maximum ($F=0.01$, $P=0.915$; $F=0.14$, $P=0.872$), and at 12.00 h ($F=0.02$, $P=0.891$; $F=0.16$, $P=0.852$), showed no significant differences for both treatment and clones.

Stomatal conductances showed similar trends to net CO₂ assimilation, reaching a maximum about 08.00 h, with no significant differences among clones or between

watering treatments ($F=0.78$, $P=0.468$; $F=0.25$, $P=0.619$). This trend also appeared at midday for clones and treatments ($F=0.47$, $P=0.630$; $F=0.02$, $P=0.904$) (Fig. 4.3.5. A, B & C). Such early closure of stomata, which was probably a result of low leaf water potential, did not lead to an increase in leaf water potential, which continued to decline until midday. Transpiration continued to increase beyond the point of maximum stomatal conductance (Fig. 4.3.5), as VPD continued to increase. As with other leaf physiological parameters, there were no significant differences between treatments ($F=0.57$, $P=0.455$; $F=0.72$, $P=0.404$) or clones ($F=0.76$, $P=0.477$; $F=0.36$, $P=0.702$).

Stomatal limitation of photosynthesis was apparent from the relationship between net CO_2 assimilation rate and stomatal conductances in both treatments (Fig. 4.3.6. $P<0.000$). This suggests that stomata limited the CO_2 assimilation rate in both watering treatments.

The relationship between leaf water potential and the transpiration rate and photosynthetic active radiation and the net CO_2 assimilation rates for the treatments and clones were negatively correlated in this experiment due to early closure of stomata and their data were not shown.

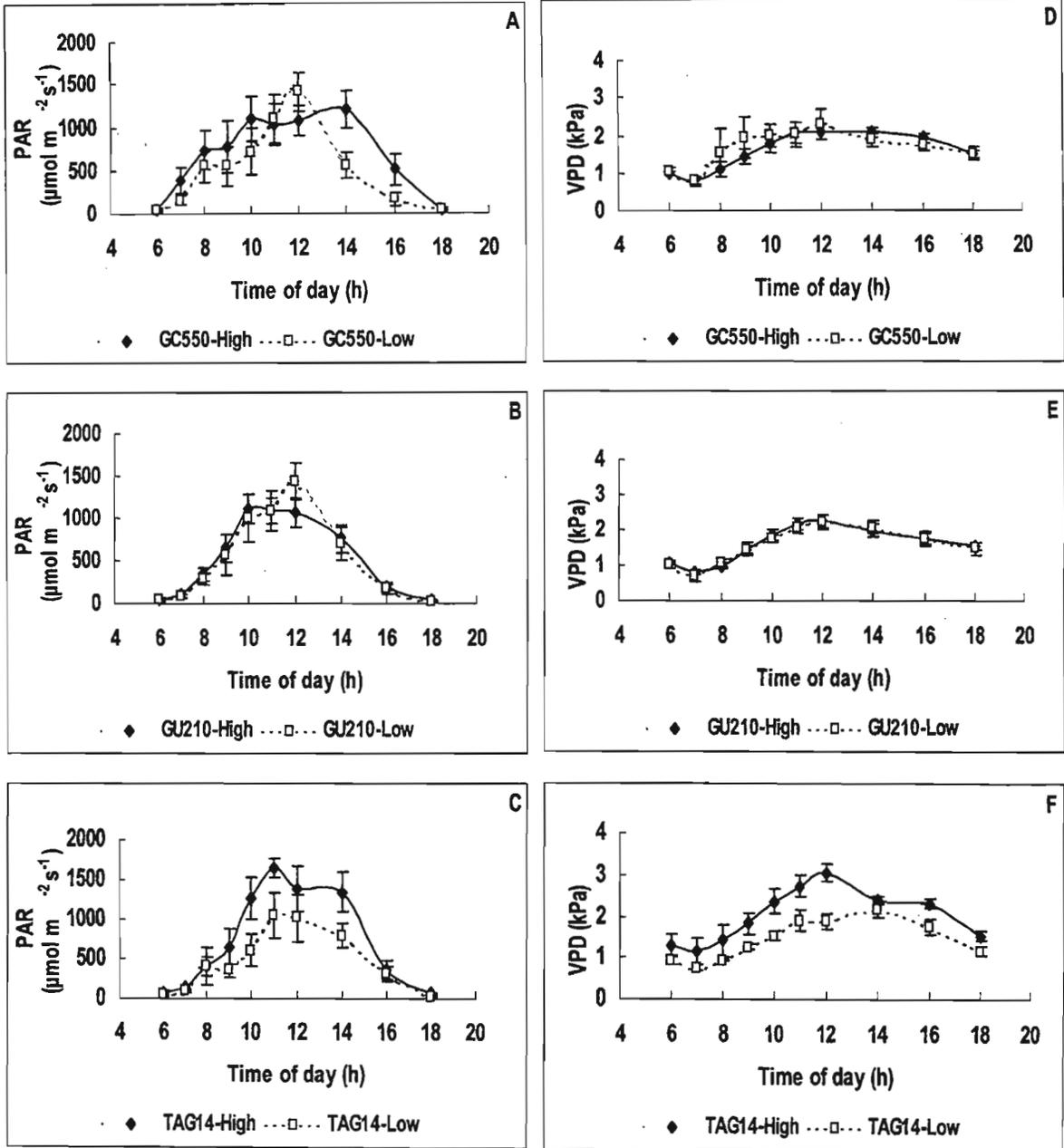


Fig. 4.3.3 Diurnal variations in instantaneous photosynthetically active radiation (PAR) and calculated vapour pressure deficit that were measured concurrently with leaf physiology parameters of three 14 month old *Eucalyptus* spp. clones subjected to high or low watering treatment. Error bars represent the \pm SEM of the mean (n=6).

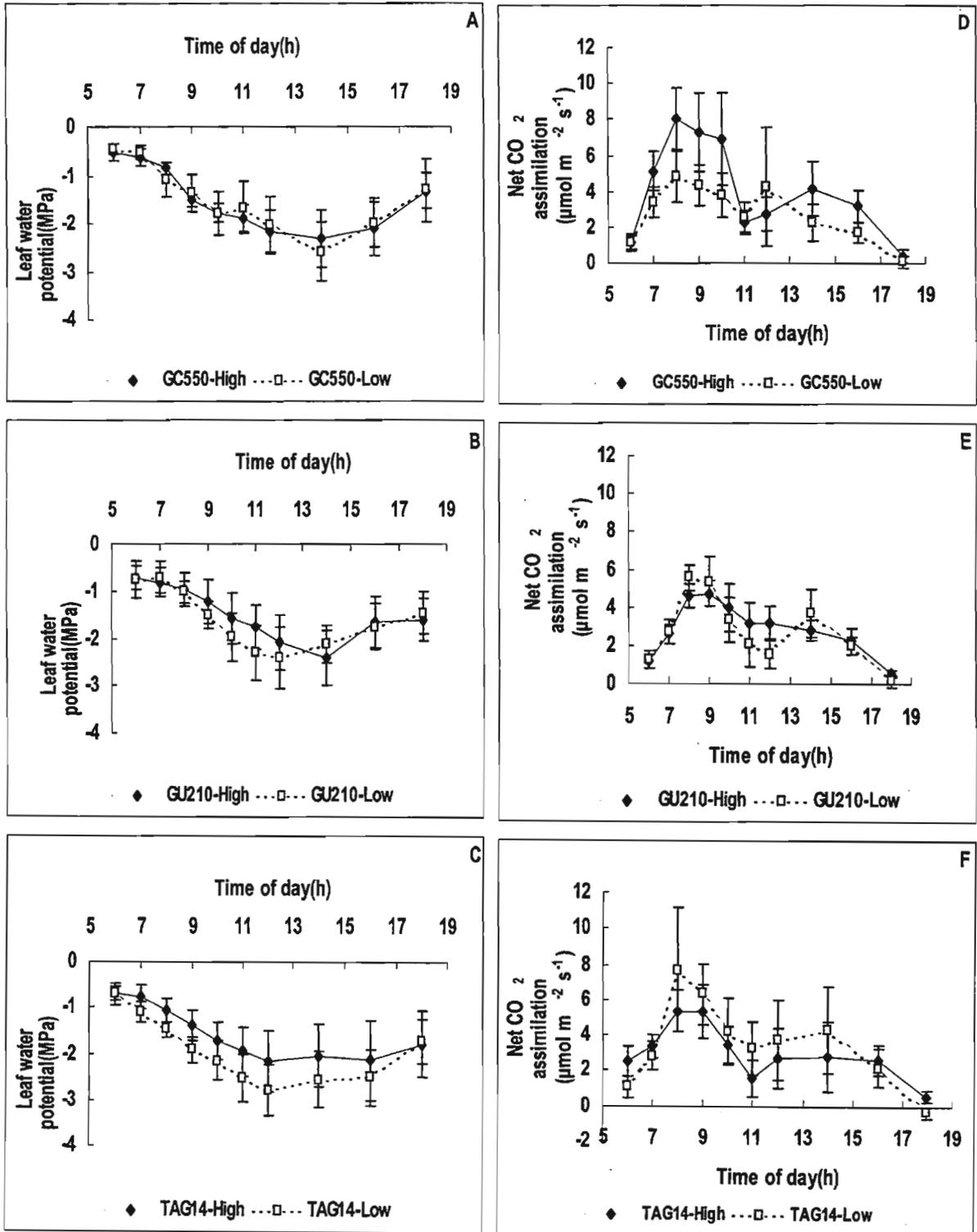


Fig. 4.3.4 Diurnal variations in leaf water potential (A, B & C) and the net CO₂ assimilation rate (D, E & F) of three *Eucalyptus* spp. clones grown for 14 months in 25 l pots, and subjected to high or low watering treatment. Error bars represent the ± SEM of the mean (n=6).

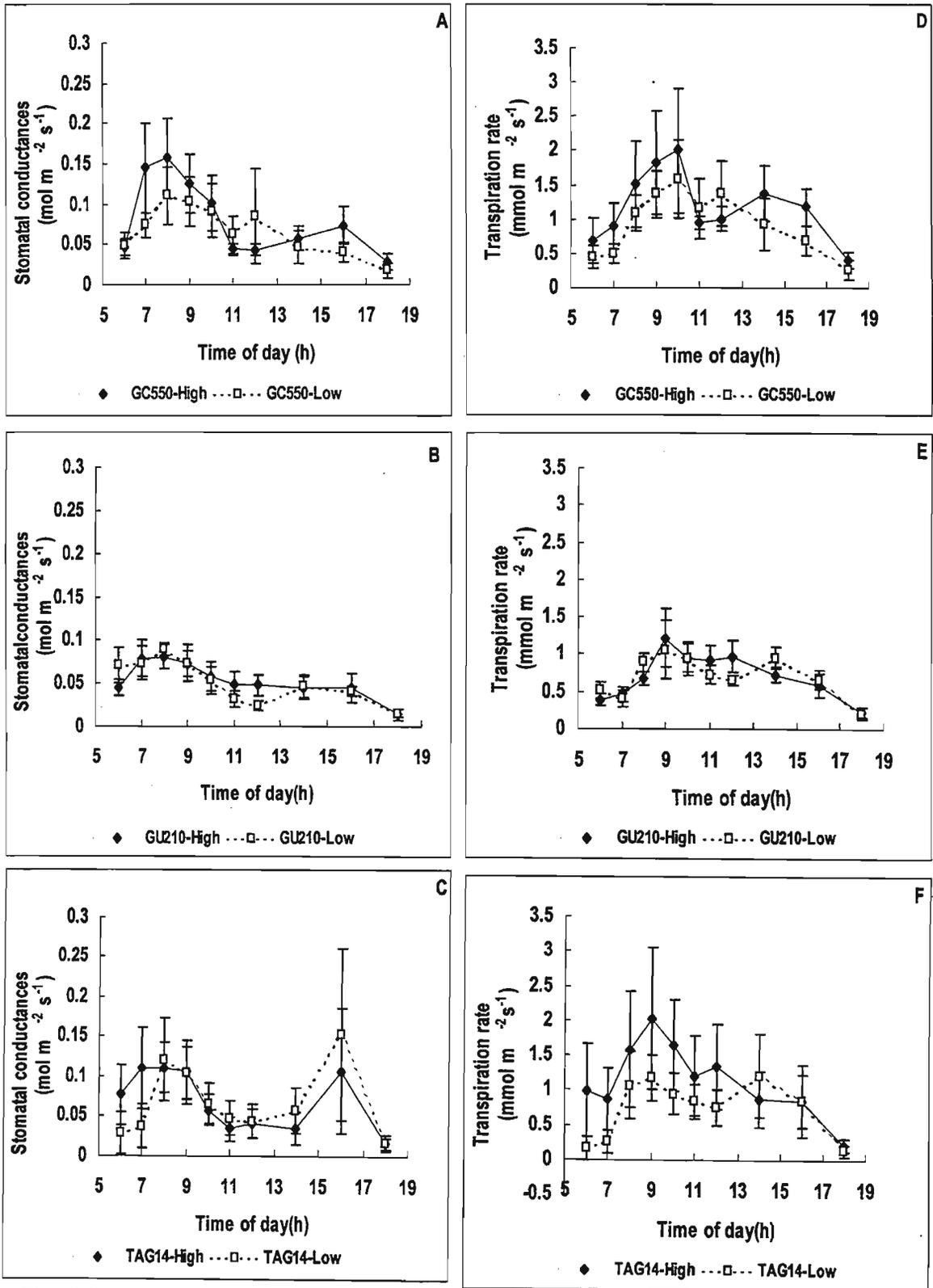


Fig. 4.3.5 Diurnal variations in stomatal conductances (A, B & C) and transpiration rate (D, E & F) of three *Eucalyptus* spp. clones grown for 14 months in 25 l pots, and subjected to high or low watering treatment. Error bars represent the \pm SEM of the mean (n=6).

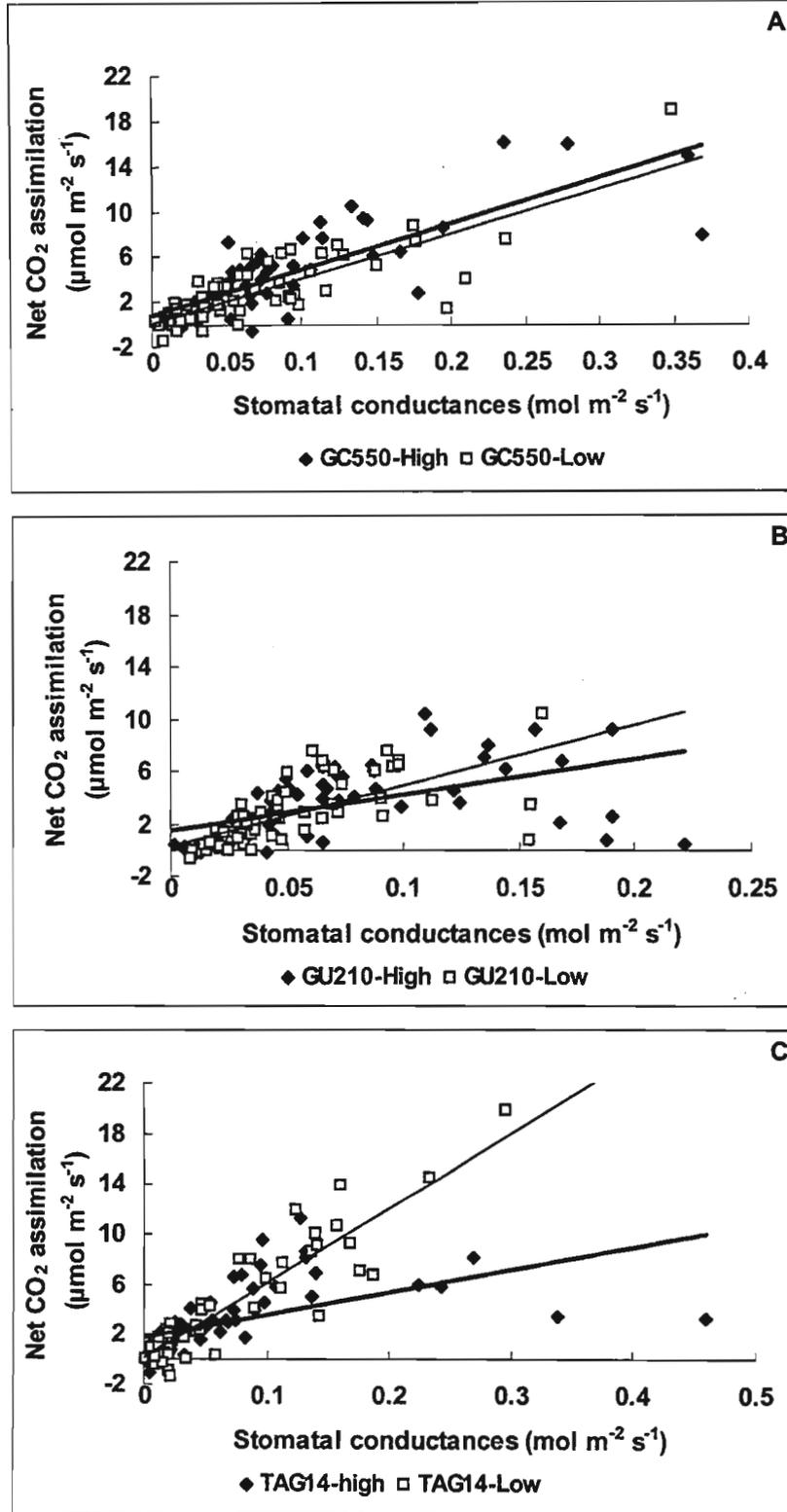


Fig. 4.3.6 The relationship between diurnally measured net CO₂ assimilation rate and stomatal conductances of three *Eucalyptus* spp. clones grown for 14 months in 25 l pots, and subjected to high or low watering treatment. The fitted linear regression lines are for high (bold) and low (thin) watered, respectively.

4.3.4 Xylem cavitation

Cumulative acoustic emissions

The accumulation of UAEs (cUAE) recorded from the main stems of the shoots is shown in Fig. 4.3.7. In all three clones and both watering treatments there was a rise in UAEs from early morning, levelling off around midday or shortly thereafter. The accumulation of events corresponded with the decrease in leaf water potential. High watered plants tended to show more total events cUAE than those subjected to low water; there was a significant difference in total cUAE at 09.00 h ($F=5.02$, $P=0.033$) and calculated EPH at 08.00 h and 09.00 h ($P<0.014$), where high watered clones produced higher number of cUAE events than low watered. This difference could be a result of differences in stem thickness. The high watered plants had greater over-bark diameter at the site of mounting of the detector ($F=40.77$, $P=0.011$). Thus in the high watered plants the detector was in contact with a greater area of stem, and so detected more events. However, there were non-significant differences in the total cUAE (for 12 hours) between watering treatments ($F=1.94$, $P=0.172$).

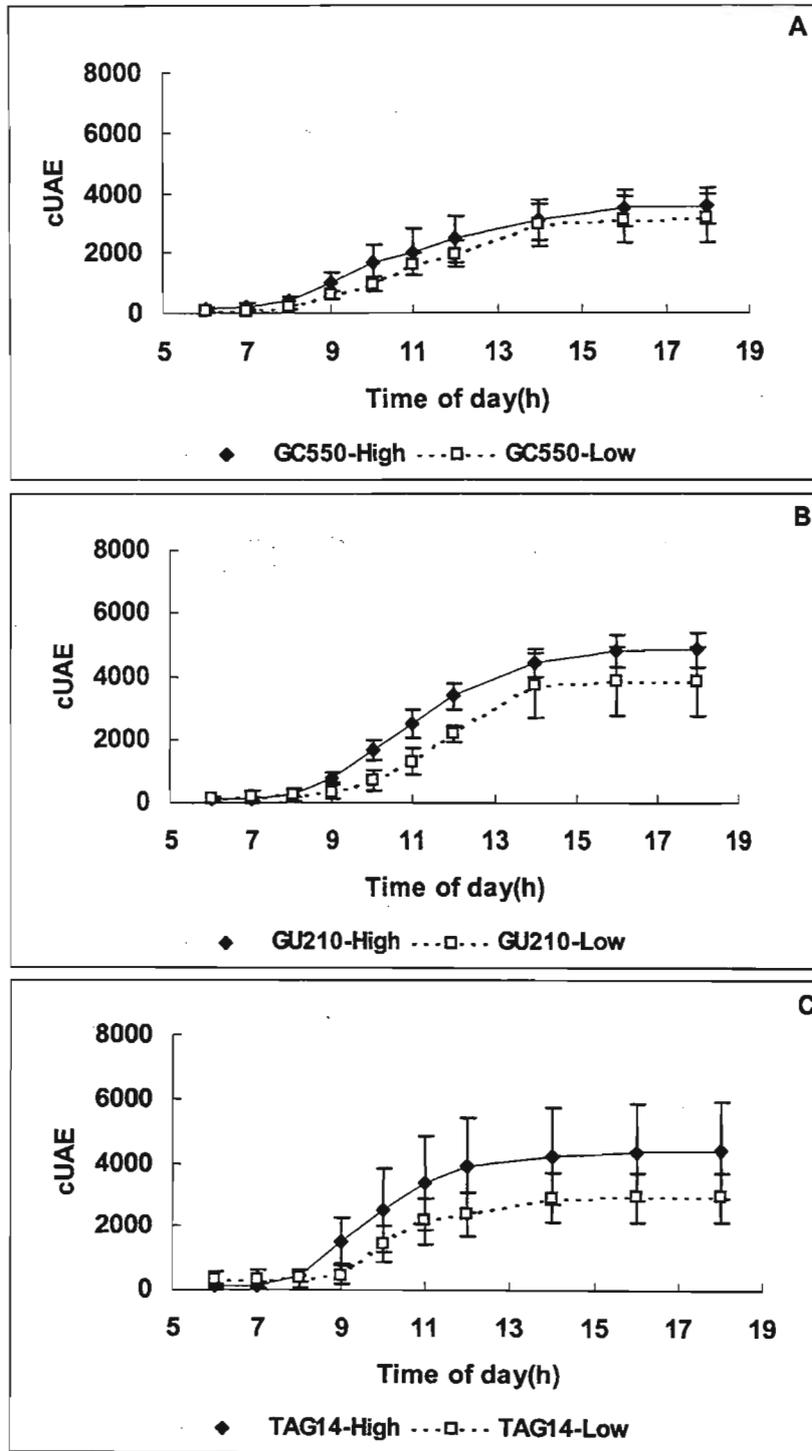


Fig. 4.3.7 Accumulation of ultrasonic acoustic emissions (cUAE), indicating xylem cavitation events, in the main stem of three *Eucalyptus* spp. clones grown for 14 months in 25 l pots, and subjected to high or low watering treatment. Error bars represent the \pm SEM of the mean (n=6).

Events per hour (EPH)

The rate of accumulation of acoustic events, together with the corresponding stomatal conductances are shown in Fig. 4.3.8. Maximum events per hour (EPH_{max}) tended to be slightly higher in high watered plants than in those receiving low water, except for GC550. EPH_{max} occurred later in the morning than maximum stomatal conductance, indicating that the partial stomatal closure that occurred was inadequate to prevent further cavitation events (transpiration continued to increase in response to increasing VPD, Fig. 4.3.5). EPH_{max} occurred slightly earlier in high watered plants (09.00 h to 10.00 h) than in the low watered plants (10.00 h to 11.00 h). Leaf water potentials at EPH_{max} are shown in Table 4.3.5. Leaf water potential corresponding to EPH_{max} were lower in low watered plants in GU210 and TAG14, but a two-way ANOVA showed that neither watering treatment ($F=2.01$, $P=0.165$) nor clone ($F=0.19$, $P=0.823$) had any significant effect on ψ_L at EPH_{max} .

Table 4.3.5 Mean leaf water potential measured at the time of EPH_{max} , with the stem over-bark diameter where UAE sensor was mounted, of three *Eucalyptus* spp. clones grown for 14 months in 25 l pots, and subjected to high or low watering treatment. Means \pm SEM (n=6). Letters represent (under each parameter) the means separation by Scheffe's multiple range tests, showing comparison within the high (upper case lettering) and low (lower case lettering) watering treatments separately ($P<0.05$).

Clones	Treatments	ψ_L, EPH_{max} (MPa)	Stem OBD (mm)
GC550	High	-1.77 ± 0.18^A	14.70 ± 0.61^{AB}
GC550	Low	-1.67 ± 0.53^a	11.83 ± 0.44^a
GU210	High	-1.59 ± 0.55^A	16.03 ± 0.56^A
GU210	Low	-2.30 ± 0.58^a	12.19 ± 0.65^a
TAG14	High	-1.40 ± 0.33^A	13.13 ± 1.30^B
TAG14	Low	-2.15 ± 0.41^a	11.57 ± 0.49^a

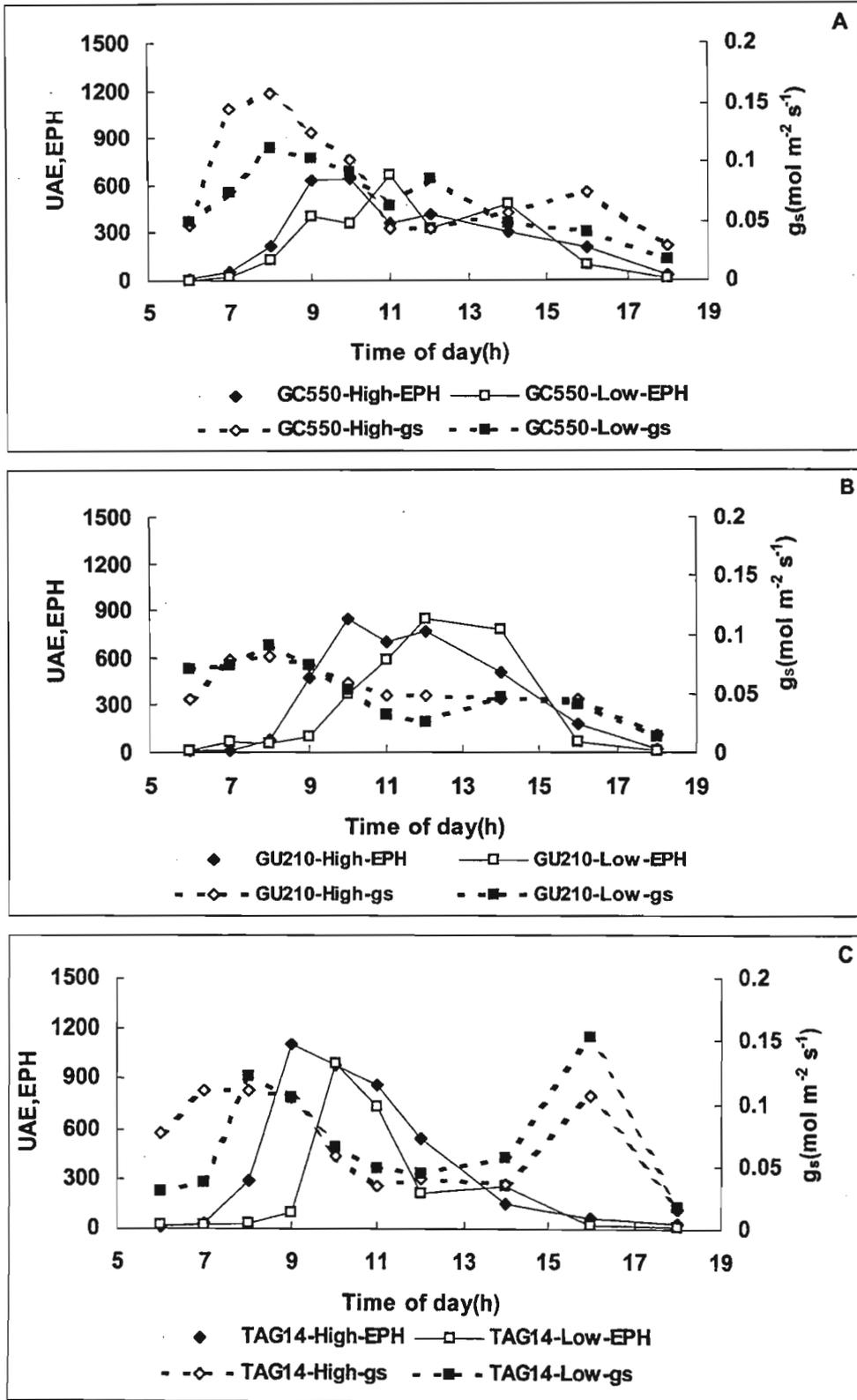


Fig. 4.3.8 Concurrently measured rate of ultrasonic acoustic emissions (events per hour; UAE, EPH) and stomatal conductances of three *Eucalyptus* spp. clones grown for 14 months in 25 l pots, and subjected to high or low watering treatment. Each data point represent the mean (n=6).

UAE as a percentage of maximum cumulated cavitation events

UAEs, expressed as percentages of the maximum CE, were plotted against leaf water potential (Fig. 4.3.9). The critical leaf water potentials triggering xylem cavitations were estimated as the means of the water potentials of all points lying between 5% and 10% of total cUAE ($\psi_{CAV,cUAE,\%}$) (Salleo *et al.*, 2000; Nardini *et al.*, 2001). There was too much scatter in the points to undertake the analytical procedure of two intersecting straight lines used in the data from the field studies (Chapter 3, Fig. 3.2.7). The critical leaf water potentials triggering xylem cavitation are shown in Table 4.3.6. Two-way ANOVA showed that both watering treatment ($F=11.00$, $P=0.004$) and clone ($F=6.21$, $P=0.009$) had significant effects. In GU210 and TAG14 the water potential triggering cavitation events was lower in low watered than high watered plants, whereas in GC550 the opposite was true. The two-way ANOVA showed a significant watering treatment x clone interaction ($F=8.61$, $P=0.002$) on the measured initiation leaf water potentials triggering xylem cavitations. Also, the critical water potential was higher in GC550 than the other clones. These patterns were similar to those of water potential corresponding to EPH_{max} (Table 4.3.5), even though the treatment differences in EPH_{max} were not significantly different. The data suggest that low watered plants of GU210 and TAG14 were less vulnerable to xylem cavitations than their high watered counterparts, although this is not the case for GC550.

Table 4.3.6 Mean threshold leaf water potential triggering xylem cavitation assessed from acoustic emission data ($\psi_{CAV,cUAE,\%}$) (see text for details) of three *Eucalyptus* spp. clones grown for 14 months in 25 l pots, and subjected to high or low watering treatment. Means are given \pm SEM. Letters represent (under each parameter) the means separation by Scheffe's multiple range tests, showing comparison within the high (upper case lettering) and low (lower case lettering) watering treatments separately ($P<0.05$).

Clones	Treatments	$\psi_{CAV,cUAE,\%}$ (MPa)
GC550	High	-0.96 ± 0.21^A
GC550	Low	-0.73 ± 0.19^a
GU210	High	-1.02 ± 0.16^A
GU210	Low	-1.44 ± 0.16^{ab}
TAG14	High	-0.81 ± 0.22^A
TAG14	Low	-2.31 ± 0.43^b

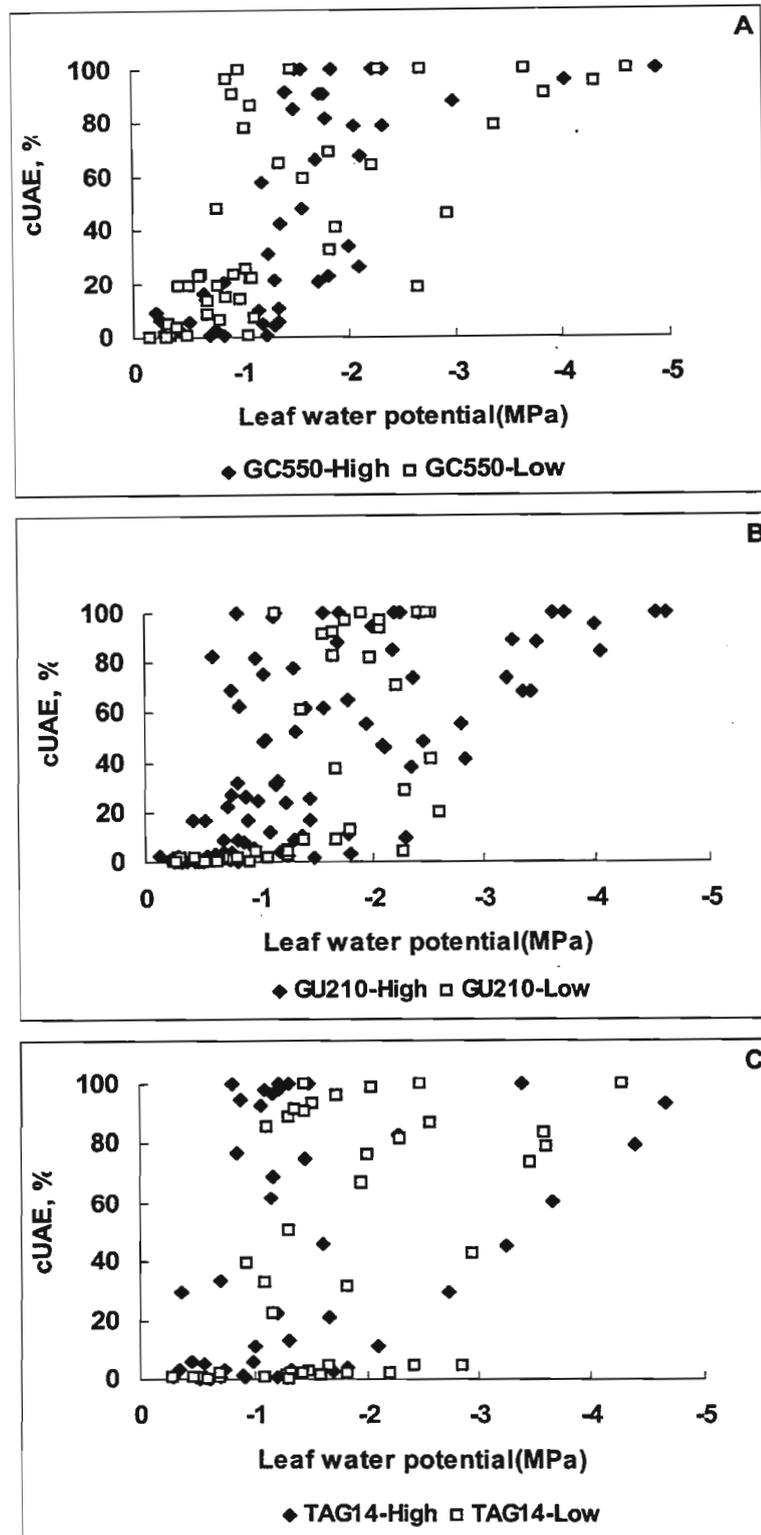


Fig. 4.3.9 Cumulated UAE (cUAE) from the main stem, expressed as percentage of the maximum, plotted against leaf water potential for three *Eucalyptus* spp. clones grown for 14 months in 25 l pots, and subjected to high or low watering treatment.

4.3.5 Positive root pressure

Positive root pressures were recorded both predawn and at midday (Fig. 4.3.10). Overnight root pressure ranges from 0.31 kPa to 3.69 kPa, whereas midday root pressures were higher at 13 kPa to 26 kPa, regardless the treatments. Predawn values showed marginally significant clonal effects ($F=3.11$, $P=0.059$), but watering treatment had no effect ($F=0.27$, $P=0.604$). Neither clone ($F=0.54$, $P=0.591$) nor treatment ($F=1.84$, $P=0.186$) had any effect on midday values.

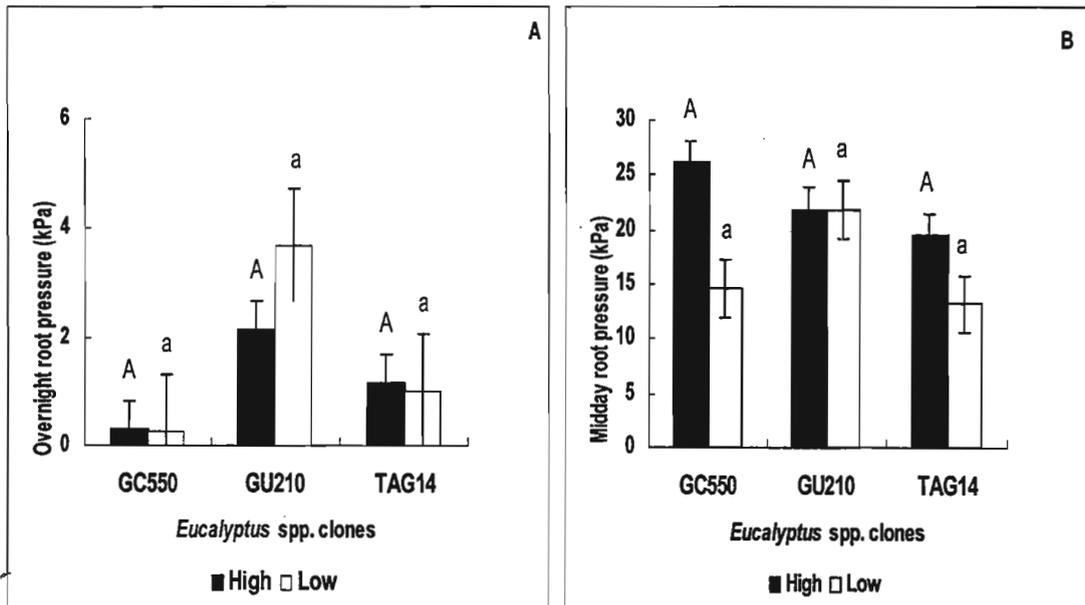


Fig. 4.3.10 Positive xylem root pressure developed overnight and the following midday in three *Eucalyptus* spp. clones grown for 14 months in 25 l pots, and subjected to high or low watering treatment. Error bars represent the \pm SEM (n=6). Letters represent the means separation by Scheffe's multiple range tests, showing comparison within the high (upper case lettering) and low (lower case lettering) watering treatments separately ($P < 0.05$).

4.3.6 Root , shoot and whole plant hydraulic conductances

Absolute root and shoot hydraulic conductances

Absolute hydraulic conductances of the roots and shoots measured by the HPFM are shown in Table 4.3.7. In all three clones root conductances (K_r) were higher for high watered plants than those receiving low water ($F=5.70$, $P=0.024$), but the effect was not significant for shoot conductance (K_s) ($F=2.50$, $P=0.124$). There were no significant differences among the clones in either K_r ($F=0.57$, $P=0.569$) or K_s ($F=1.20$, $P=0.315$).

Table 4.3.7 Absolute hydraulic conductances (raw data from the HPFM) measured of three *Eucalyptus* spp. clones grown for 14 months in 25 l pots, and subjected to high or low watering treatment. Means are given \pm SEM (n=6). Letters represent (under each parameter) the means separation by Scheffe's multiple range tests, showing comparison within the high (upper case lettering) and low (lower case lettering) watering treatments separately ($P<0.05$).

Absolute Hydraulic Conductances ($\text{kg s}^{-1}\text{MPa}^{-1} \times 10^{-5}$)			
Clones	Treatments	K_r	K_s
GC550	High	9.44 \pm 5.27 ^A	6.86 \pm 1.54 ^A
GC550	Low	1.56 \pm 0.26 ^{ab}	5.30 \pm 0.63 ^a
GU210	High	6.54 \pm 3.70 ^A	7.33 \pm 1.22 ^A
GU210	Low	2.68 \pm 0.86 ^a	7.15 \pm 2.08 ^a
TAG14	High	4.65 \pm 2.39 ^A	6.85 \pm 1.05 ^A
TAG14	Low	0.85 \pm 0.22 ^b	4.05 \pm 0.41 ^a

Normalized root and shoot hydraulic conductances

Root hydraulic conductances expressed per unit leaf area ($K_{r/LA}$) are shown in Fig. 4.3.11. A. Values for high watered plants ranged from 21.31×10^{-5} for GC550 to $7.51 \times 10^{-5} \text{ kg s}^{-1} \text{ MPa}^{-1} \text{ m}^{-2}$ for TAG14, and for the low watered plants between 8.22×10^{-5} for GU210 and $3.09 \times 10^{-5} \text{ kg s}^{-1} \text{ MPa}^{-1} \text{ m}^{-2}$ for TAG14; these clonal differences were not significant ($F=1.630$, $P=0.213$). High watered plants had

marginally significant ($F=3.221$, $P=0.083$) higher $K_{r/LA}$ values than low watered plants.

Shoot hydraulic conductances expressed per unit leaf area ($K_{S/LA}$) are shown in Fig. 4.3.11. B. Values from high watered plants ranged from 18.01×10^{-5} for GC550 to $9.79 \times 10^{-5} \text{ kg s}^{-1} \text{ MPa}^{-1} \text{ m}^{-2}$ for GU210. Surprisingly, low watered plants had higher values of $K_{S/LA}$ than high watered plants, ranging from 21.07×10^{-5} for GU210 to $14.87 \times 10^{-5} \text{ kg s}^{-1} \text{ MPa}^{-1} \text{ m}^{-2}$ for TAG14. Two-way ANOVA revealed that $K_{S/LA}$ values were not significantly different among the clones ($F=2.49$, $P=0.100$) but were significantly different between watering treatments ($F=7.17$, $P=0.012$). The higher $K_{S/LA}$ in the low watered plants could be because the low watered plants produced lower leaf area than the high watered plants, and in the shoots at least half of the total hydraulic resistances resided in the leaves (see later).

Root hydraulic conductances expressed per unit root dry mass ($K_{r/trdw}$) are shown in Fig. 4.3.11. C. Values for high watered plants ranged between 4.90×10^{-7} for GC550 and $3.29 \times 10^{-7} \text{ kg s}^{-1} \text{ MPa}^{-1} \text{ g}^{-1}$ for TAG14, with low watered plants having lower root conductances, values ranging 3.04×10^{-7} for GU210 and $1.06 \times 10^{-7} \text{ kg s}^{-1} \text{ MPa}^{-1} \text{ g}^{-1}$ for TAG14. However, these differences were not significant, either among clones ($F=0.44$, $P=0.650$) or between watering treatments ($F=2.28$, $P=0.106$).

Shoot conductances expressed per unit shoot dry mass ($K_{S/tsdw}$) are shown in Fig. 4.3.11. D. High watered plant shoot conductances ranged between 5.02×10^{-7} for TAG14 and $4.47 \times 10^{-7} \text{ kg s}^{-1} \text{ MPa}^{-1} \text{ g}^{-1}$ for GU210. Values for low watered plants were higher, ranging from 8.70×10^{-7} for GU210 to $4.98 \times 10^{-7} \text{ kg s}^{-1} \text{ MPa}^{-1} \text{ g}^{-1}$ for TAG14. Clonal differences were not significant ($F=1.39$, $P=0.265$), but the effect of watering treatment was ($F=5.93$, $P=0.021$).

Whole plant hydraulic conductances expressed per unit leaf area ($K_{P/LA}$) (Fig. 4.3.12) ranged from 8.39×10^{-5} for GC550 to $3.66 \times 10^{-5} \text{ kg s}^{-1} \text{ MPa}^{-1} \text{ m}^{-2}$ for TAG14 for high watered plants, with the low watered plants giving lower values, between 5.23×10^{-5} for GU210 and $2.47 \times 10^{-5} \text{ kg s}^{-1} \text{ MPa}^{-1} \text{ m}^{-2}$ for TAG14. In the case of $K_{P/LA}$, only

clones had significant effect ($F=3.47$, $P=0.045$) and there was no watering treatment effect ($F=1.63$, $P=0.212$). GC550 and GU210 had similar $K_{P/LA}$ values where as GC550 $K_{P/LA}$ was significantly higher than that of TAG14. Further, the clonal effect was apparent under the low watering treatment, where TAG14 had a considerably reduced whole plant conductivity compared with that of GU210 clone.

In this study, high watered plants had higher absolute hydraulic conductances, although the differences were not significant. However, the low water treatment significantly reduced the total leaf area as well as total root dry mass for all three clones. Consequently, scaling of absolute hydraulic conductances by either plant component resulted in significant differences, with low watered plants having higher normalized conductances (except for root conductance normalized by root mass). As such, the efficiency of the hydraulic pathway supplying water from the soil to the leaves was maintained under low watering conditions by reducing the leaf area produced.

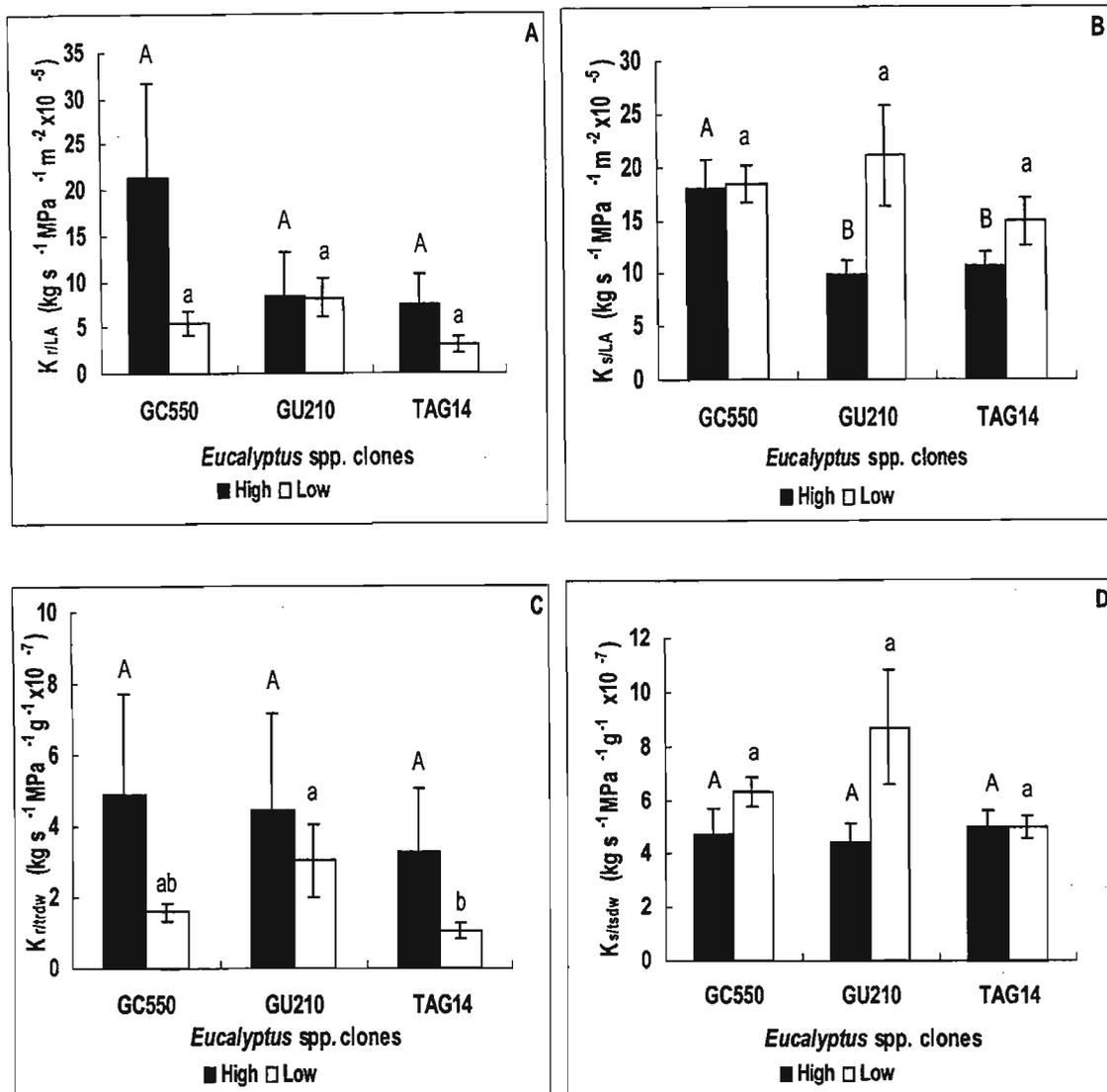


Fig. 4.3.11 Root and shoot hydraulic conductances measured in three *Eucalyptus* spp. clones grown for 14 months in 25 l pots, and subjected to high or low watering treatment. K_r and K_s expressed on a per unit leaf area basis (A, B), K_r expressed per unit total root dry mass (C) and K_s expressed per unit total shoot dry mass (D). Error bars represent the \pm SEM (n=6). Letters represent the means separation by Scheffe's multiple range tests, showing comparison within the high (upper case lettering) and low (lower case lettering) watering treatments separately (P<0.05).

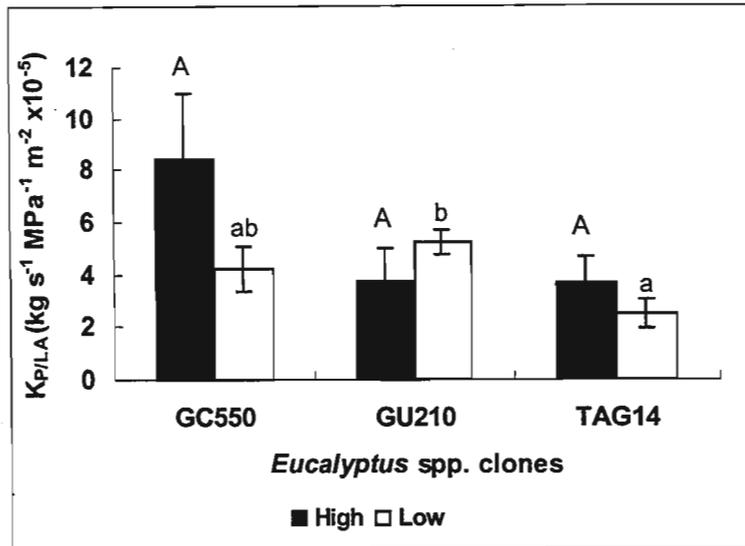


Fig. 4.3.12 Whole plant hydraulic conductances expressed per unit leaf area of three *Eucalyptus* spp. clones grown for 14 months in 25 l pots, and subjected to high or low watering treatment Error bars represent the \pm SEM (n=6). Letters represent the means separation by Scheffe's multiple range tests, showing comparison within the high (upper case lettering) and low (lower case lettering) watering treatments separately (P<0.05).

4.3.7 Relationships between vulnerability to cavitation, growth parameters and hydraulic architecture

Relationship between shoot hydraulic conductances and the xylem vulnerability

The relationship between stem xylem vulnerability, estimated from both mean of ψ_{CAV} , cUAE, % (ψ_L at 5 to 10 cUAE, %) and mean ψ_L at EPH_{max} , and mean shoot hydraulic conductance per unit leaf area are shown in Figs. 4.3.13. A and B, respectively. There was no relationship between xylem vulnerability estimated from either method ($r^2 = 0.02$, $P=0.797$, and $r^2=0.47$, $P=0.130$, respectively) and shoot conductance when data were pooled for both watering treatments. Similarly, no relationship was found when data from each watering treatment were analyzed separately.

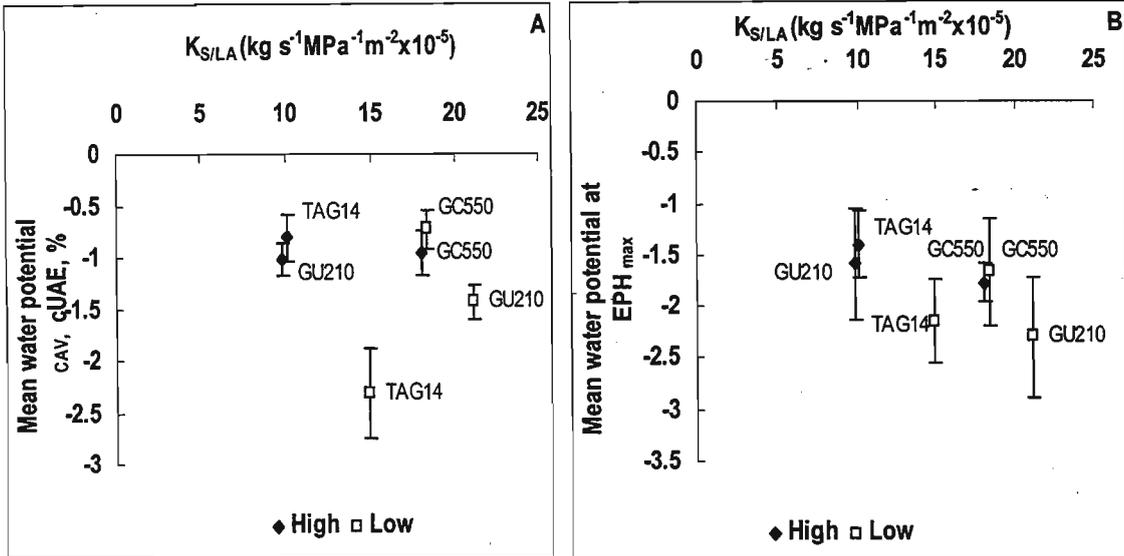


Fig. 4.3.13 Relationships between mean ψ_{CAV} , cUAE, % (A) or mean leaf water potential at EPH_{max} (B) and mean shoot hydraulic conductances of three *Eucalyptus* spp. clones grown for 14 months in 25 l pots, and subjected to high or low watering treatment. Error bars represent the \pm SEM.

Relationship between whole plant hydraulic conductances and productivity

There was no significant relationship between $K_{P/LA}$ and total whole plant dry mass (Fig. 4.3.14) for either high watered plants ($r^2=0.005$, $P=0.772$) or low watered plants ($r^2=0.11$, $P=0.194$) analyzed separately. A similar trend was apparent, when data from the watering treatments were combined ($r^2=0.024$, $P=0.376$). One of the data points (high watered GC550) was an outlier, but removing this point did not change the relationship.

4.3.8 The components of whole plant resistances

The contributions of roots (R_{r*LA}) and shoots (R_{s*LA}), and the components of the shoot to whole plant resistances, normalized to leaf area, are given in Table 4.3.8. Leaf resistance (R_{L*LA}) was the major component of the shoot resistance, contributing at least half of the total for both watering treatments. There were no clonal differences in R_L ($F=2.17$, $P=0.132$), but the high watered plants had leaf resistances significantly

greater than the lower watered plants ($F=8.69$, $P=0.006$), as they produced more leaves than its counterpart and that might have increased the R_L .

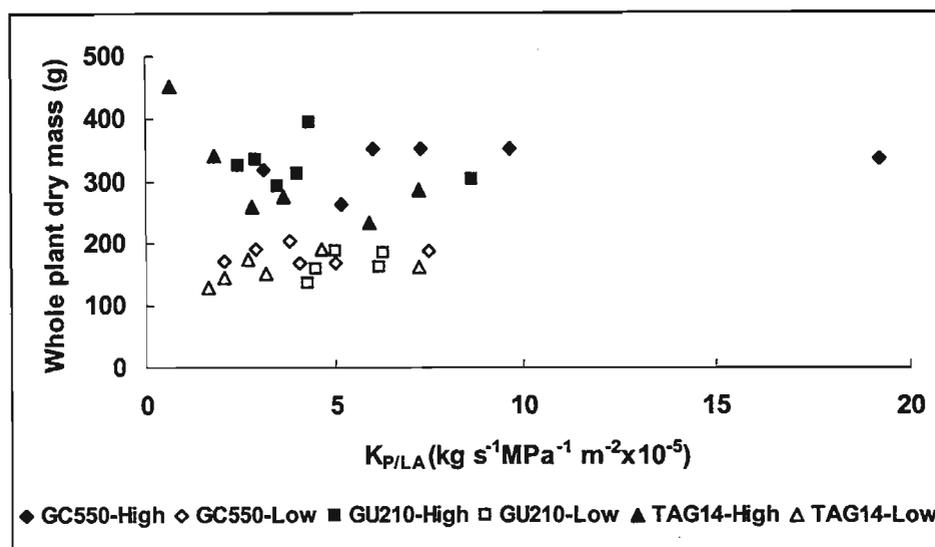


Fig. 4.3.14 The relationship between total plant biomass and whole plant hydraulic conductance ($K_{P/LA}$) obtained from individual plants of three *Eucalyptus* spp. clones grown for 14 months in 25 l pots, and subjected to high or low watering treatment.

Petiole resistances (R_{P*LA}) were lower than any other component of the shoot but were higher for the high watered plants compared with those receiving low water, the difference being marginally significant ($F=4.05$, $P=0.053$). Clonal differences were also marginally significant ($F=2.95$, $P=0.068$). There were significant clonal differences in the resistance of leaf-bearing branches (R_{LBB}) ($F=3.48$, $P=0.044$), but no watering treatment effect ($F=1.23$, $P=0.276$). As R_P and R_{LBB} made only a small contribution to total plant resistance, these clonal differences probably had no physiological significance. The hydraulic resistance non-leaf bearing (R_{NLBB}) and the main stem (R_{MS}) showed no clonal ($F=0.18$, $P=0.837$ and $F=1.26$, $P=0.300$, respectively) differences or effect of watering treatment ($F=1.78$, $P=0.192$ and $F=1.39$, $P=0.248$, respectively).

Table 4.3.8 Contributions of individual plant components to the whole plant resistance in three *Eucalyptus* spp. clones grown for 14 months in 25 l pots, and subjected to high or low watering treatment. Means are given \pm SEM (n = 6). Within column, letters represent the means separation by Scheffe's multiple range tests, showing comparison within the high (upper case lettering) and low (lower case lettering) watering treatments separately (P<0.05).

Hydraulic resistances expressed per unit leaf area ($\text{kg}^{-1} \text{ s MPa m}^2 \times 10^3$)								
Clones	Treatments	R_L	R_P	R_{LBB}	R_{NLBB}	R_{MS}	R_S	R_r
GC550	High	3.88 \pm 0.87 ^A	0.36 \pm 0.07 ^A	0.64 \pm 0.10 ^A	0.76 \pm 0.15 ^A	0.53 \pm 0.06 ^A	6.16 \pm 1.02 ^A	10.10 \pm 3.40 ^A
GC550	Low	3.17 \pm 0.39 ^a	0.32 \pm 0.06 ^a	0.65 \pm 0.11 ^a	0.85 \pm 0.13 ^a	0.65 \pm 0.17 ^a	5.65 \pm 0.49 ^a	22.40 \pm 5.20 ^a
GU210	High	6.71 \pm 0.66 ^A	0.67 \pm 0.21 ^A	1.44 \pm 0.35 ^A	1.15 \pm 0.38 ^A	1.13 \pm 0.38 ^A	11.21 \pm 1.70 ^A	59.50 \pm 46.60 ^A
GU210	Low	3.23 \pm 0.80 ^a	0.38 \pm 0.11 ^a	0.70 \pm 0.12 ^a	0.67 \pm 0.15 ^a	0.64 \pm 0.13 ^a	5.63 \pm 1.08 ^a	14.10 \pm 2.32 ^a
TAG14	High	6.23 \pm 1.58 ^A	0.70 \pm 0.13 ^A	1.54 \pm 0.59 ^A	1.04 \pm 0.17 ^A	0.73 \pm 0.15 ^A	10.13 \pm 1.54 ^A	40.20 \pm 23.40 ^A
TAG14	Low	4.14 \pm 0.96 ^a	0.50 \pm 0.04 ^a	1.41 \pm 0.47 ^a	0.76 \pm 0.23 ^a	0.44 \pm 0.10 ^a	7.26 \pm 0.88 ^a	49.50 \pm 18.90 ^a

Root resistance (R_{r*LA}) was higher than shoot resistance (R_{S*LA}) in both watering treatments. There were no significant clonal or watering treatment effects on R_r (F=0.966, P=0.393; F=0.208, P=0.652), although R_r was marginally higher for low watered plants. In contrast, high watered GU210 showed higher R_r than its low watered counterpart, although the difference was not significant. The hydraulic resistance of whole plant (R_{Plant}) was non-significantly different between watering treatments (F=0.376, P=0.545) (data not shown).

The contribution of R_L to total plant resistance was highest in high watered GC550 (27%) and least in low watered TAG14 (9%). For all clones and both watering treatments roots contributed more to total resistance than did shoots, ranging from 55% for high watered GC550 to 83% for low watered TAG14. The contribution of R_r to R_{Plant} was less in high watered than low watered plants.

Table 4.3.9 Percentage contributions of individual plant components to the whole plant resistances in three *Eucalyptus* spp. clones grown for 14 months in 25 l pots, and subjected to high or low watering treatment. Means are given \pm SEM (n = 6).

Hydraulic resistances (%) expressed per unit leaf area								
Clones	Treatments	R_L	R_P	R_{LBB}	R_{NLBB}	R_{MS}	R_S	R_r
GC550	High	26.75 \pm 6.33	3.26 \pm 1.58	4.82 \pm 1.34	5.20 \pm 0.95	4.65 \pm 1.80	44.70 \pm 8.84	55.30 \pm 8.84
GC550	Low	12.12 \pm 1.08	1.25 \pm 0.15	2.49 \pm 0.36	3.77 \pm 1.13	2.63 \pm 0.65	22.30 \pm 2.63	77.70 \pm 2.64
GU210	High	24.11 \pm 7.64	1.94 \pm 0.55	4.18 \pm 1.16	3.00 \pm 0.77	2.50 \pm 0.40	35.70 \pm 9.64	64.30 \pm 9.64
GU210	Low	16.40 \pm 6.46	2.08 \pm 0.87	3.59 \pm 0.91	3.80 \pm 0.83	3.51 \pm 0.71	29.40 \pm 9.01	70.60 \pm 9.01
TAG14	High	21.46 \pm 6.45	2.46 \pm 0.69	5.55 \pm 2.08	3.25 \pm 0.66	3.07 \pm 1.36	35.00 \pm 9.51	65.00 \pm 9.51
TAG14	Low	9.31 \pm 2.48	1.24 \pm 0.31	3.38 \pm 1.06	1.86 \pm 0.68	1.11 \pm 0.35	16.90 \pm 3.74	83.10 \pm 3.74

Chapter: 4.4 Discussion

4.4.1 Effect of watering treatment on growth of three *Eucalyptus* spp. clones

As was to be expected, there were large differences between watering treatments in this study, but there were no differences amongst clones (Table 4.3.1). This is in accordance with results reported by February *et al.* (1995) who observed differences in total dry mass with watering treatment in *E. grandis* and *E. grandis* x *camaldulensis* clones, but no clonal effect. Similarly, the biomass of all above-ground components and the mean annual increment of stems of field grown *E. globulus* were higher at a mesic site than a xeric site (Macfarlane and Adams, 1998). The effect of watering treatment on biomass production is widespread, and has been reported for, *inter alia*, *Quercus robur* (Fort *et al.*, 1997), and seedlings of lodgepole pine and Douglas-fir (Smit and Van Den Driessche, 1992). In contrast to this (only watering treatment), Le Roux *et al.* (1996) found no significant differences in dry mass accumulation among six clones of *E. grandis* grown for 16 months under two different watering regimes.

In all three clones in this study, the leaf area of high watered plants was higher than that of low watered plants (Table 4.3.2). A reduction in the leaf area produced is considered an early response to drought (Macfarlane and Adams, 1998), as has been shown in a wide range of studies. Drought treatment reduced the total leaf area (with inter-clonal differences) in three *E. globulus* clones (Pita and Pardos, 2001) and the leaf area of *E. grandis* was higher than *E. camaldulensis* in a plantation site (Morris *et al.*, 1998). Average leaf area in *Quercus robur* gradually decreased under decreasing watering regimes, fully droughted plants producing lower leaf area than other treatments (Fort *et al.*, 1997). However, there was no affect of drought or water logging on the biomass of six clonal lines of *E. camaldulenesis*, although there were differences among the clones (Farrell *et al.*, 1996).

Differences in growth in both height and stem over-bark diameter occurred between clones and treatments (Fig. 4.3.1). The tallest clone (GC550) had the lowest diameter, whereas shortest clone (GU210) had the highest stem diameter. Consistent with our data set, effects of drought as well as clonal differences on total dry weight, leaf area and plant height were reported in some pot-grown *E. globulus* clones (Pita and Pardos, 2001). Reduction in mean height in *E. microtheca* (Li *et al.*, 2000a) and both the height and stem diameter in *E. grandis* and *E. grandis* x *camaldulensis* clones (February *et al.*, 1995) under water stress condition has been reported. However, there was no change in growth in height or diameter in *Quercus robur* seedlings when subjected to different watering regimes (Fort *et al.*, 1997).

Thick leaves experience more positive leaf water potentials than thin leaves, suggesting that thick leaves and low specific leaf area (SLA) have physiological characteristics of greater drought tolerance than thin leaves with high SLA (Cavelier and Goldstein, 1989). There were clonal differences in SLA, with GC550 having the thickest leaves (Table 4.3.2). There were no changes in SLA with watering treatment, in accordance with the observation of Macfarlane and Adams (1998) that the SLA of two stands of 6year-old *E. globulus* from mesic and xeric sites did not differ. Specific leaf area seems better related to the evaporative climate than to water availability *per se* (Specht and Specht, 1989). However, in other reports, low water treatment significantly reduced the SLA in *E. microtheca* (Li *et al.*, 2000a) and in *E. globulus*, without any clonal differences (Pita and Pardos, 2001). Kloeppel *et al.* (1998) found differences in SLA between *Larix* spp. (Larches) and deciduous species that are positively correlated with photosynthetic capacity.

The root to shoot ratio for all three clones subjected to both watering treatments was slightly higher than the normally expected 1:1 proportion. This slightly higher carbon investment in the roots possibly increases the hydraulic capacity of the roots. Root to shoot ratio is normally 1:1 (Fort *et al.*, 1997), or even less than this proportion (Li *et al.*, 2000a) in tree seedlings that are grown in well watered conditions. Root to shoot ratios were shown to increase with increasing water stress in *Quercus robur* (Fort *et al.*, 1997) and *E. microtheca* (Li *et al.*, 2000a). However, root to shoot ratios in this

study remained constant, or even decreased, with low watering. This suggests the difference in water availability between the high and low watering treatments was not large enough in these potted saplings to alter carbon allocation adequately to segregate the treatment effect.

4.4.2 Leaf physiology

Are the low levels of gas exchange and lack of differences between treatments a consequence of inadequate water in both treatments?

The instantaneous values of leaf water potential and leaf gas exchange did not differ between the treatments, and values were considerably lower than expected, particularly in the high water treatment. These results are in contrast to other reports showing a clear effect of different watering treatment on leaf transpiration (February *et al.*, 1995; Ray and Sinclair, 1998; Li, 2000; Li *et al.*, 2000a; Pita and Pardos, 2001).

The soil water potential assessed from the predawn leaf water potential during this study (around -0.5 MPa, Fig. 4.3.2) also did not differ among treatments and was somewhat lower than values of -0.18 to -0.28 MPa reported for field grown specimens (Vander Willigen and Pammenter, 1998). Morris *et al.* (1998) have suggested that the weak relationship between rainfall and water use reported in some *Eucalyptus* sp. probably reflects a strong dependence of the trees on the groundwater. Similarly, Heuperman (1995)(*loc. cit.* Morris *et al.*, 1998) demonstrated that roots of *Eucalyptus* species penetrate to the water table around 5m depth to access water. This suggests that in the field *Eucalyptus* spp. rely on the deep soil water concurrently with rainfall, and particularly so during dry periods. This was obviously not the case for the potted saplings in this study, which were totally depend on the applied water, which did not appear to be adequate even in the high watered treatment.

Furthermore, A_{\max} and $g_{s \max}$ occurred as early as 08.00 h (Fig. 4.3.4. D, E, F and 4.3.5. A, B & C). This is totally different from the recorded diurnal leaf gas exchange pattern of field grown *Eucalyptus* spp., where it normally occurs between 10.00 h to

12.00 h (Mielke *et al.*, 1999). As the $g_{s \max}$ occurred early in the morning, the leaf water potential declined only slightly from 09.00 h to late midday. Similar to these observations, Stoneman *et al.* (1994) found highest net CO₂ assimilation rate of drought-stressed seedlings of *Eucalyptus marginata* Donn ex Sm (jarrah) to occur early in the morning, and to be negligible during the rest of the day. For water stressed potted *Copaifera langsdorffii* plants, Prado *et al.* (1994) also observed stomatal closure at to occur as early as 09.00 h. The values of stomatal conductance in the present study were also low; maximum values were between 0.16 to 0.08 mol m⁻² s⁻¹ at leaf water potentials ranging from -0.86 MPa to -1.47 MPa (Fig. 4.3.4. A, B & C). Similarly, drier site populations of *Populus trichocarpa* grown in pots exhibited stomatal conductances of <1 mmol m⁻² s⁻¹ at leaf water potential less than -1.5 MPa (Sparks and Black, 1999). Also, in water stressed *Copaifera langsdorffii* stomatal conductance was 0.12 mol m⁻² s⁻¹ at leaf water potential of -2.0 MPa (Prado *et al.*, 1994).

Stomatal closure has been suggested to be a measure preventing excessive embolisms by maintaining water potential above that triggering xylem cavitation (Saliendra *et al.*, 1995; Nardini and Salleo, 2000; Cochard, 2002). In the case of the three *Eucalyptus* spp. clones in this study, the measure was not entirely successful, as, despite early stomatal closure, leaf water potential continued to decline, and dropped below the critical value of triggering cavitation events ($\psi_{CAV,cUAE,\%}$), as assessed from cUAEs (although the leaf water potential corresponding to EPH_{\max} was similar to measured midday ψ_L , Table 4.3.6 ψ_L ; Fig. 4.3.9). There are reports of leaf water potentials falling below the value triggering xylem cavitations using an UAE method in other species of *Populus balsamifera* (Hacke and Sauter, 1995) and *Pinus sylvestris* L. (Jackson *et al.*, 1995a, b).

In this study maximum CO₂ assimilation rates ranged from 4.5 to 8 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 4.3.4. D, E, F). Battaglia *et al.* (1996) measured much higher rates of 12 to 14 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in *E. globulus* and 16 to 22 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in *E. nitens*. Similarly, Stoneman *et al.* (1994) showed rates around 17 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in well watered *E. marginata* (jarrah)

seedlings, which decreased to about $6 \mu\text{mol m}^{-2} \text{s}^{-1}$ with increased soil water stress. Thus the rates measured for the *Eucalyptus* spp. clones in this study, even those subjected to high watering, can be included in the range of water stressed plants.

The leaf physiological data surprisingly showed no differences between watering treatments, indicating that even plants subjected to the high watering treatment showed water limitation. Despite this, biomass production clearly segregated the treatments, suggesting a lack of association between leaf physiology and biomass accumulation. In this regard, Pereira *et al.* (1992) observed the photosynthetic capacity per unit leaf area of seedlings of *E. globulus* supplied with near optimal fertilizer and irrigation was similar to that of non-irrigated, unfertilized controls, but despite this the irrigated and fertilized plants showed greater biomass accumulation. They concluded that the increase in biomass production of the irrigated and fertilized plants was largely a result of increases in leaf area and radiation interception. A similar interpretation could be placed on the present study: the increased production under high watering treatment was associated with a higher leaf area per plant (Table 4.3.2), not increased gas exchange per unit leaf area. This also is in accord with the observation that the productivity and leaf area of *E. globulus* are closely related (Pereira *et al.*, 1992; Macfarlane and Adams, 1998) and that the water balance of a site of controls both leaf area and productivity in the long term (Macfarlane and Adams, 1998).

It was during the analysis of these data, showing a surprisingly lack of effect of watering treatment on leaf physiological characteristics, that it was realized that an error had been made in calculating the water to be supplied to each plant. The high watering treatment was based on the mean annual rainfall of a site where the clones are grown commercially. This annual rainfall was multiplied by the surface area of the pot, to give the volume of water to be added over a year, allocated on a season basis. What was overlooked was the espacement of planting. In the field each plant has available to it a ground area of 2 m x 3 m, and thus would have available to it considerably more water than that applied to the surface area of a pot 0.34 m x 0.26 m in diameter. It is highly likely that the plants in this experiment were subjected to such

a severe water stress that differences in water availability between the two watering treatments were small compared with the total water deficit faced by even the 'high' watered plants. Consequently, the data presented in this chapter could not be used in comparison with other experiments to assess the influence of age on plant response.

4.4.3 Root, shoot and whole plant hydraulic conductances

Hydraulic conductances measured using the HPFM, (values normalised to leaf area) of the *Eucalyptus* spp. clones measured in this study (Fig. 4.3.11) were within the range of values reported for a number of species (e.g. Tsuda and Tyree, 1997; Tyree *et al.*, 1998; Becker *et al.* 1999; Nardini and Tyree, 1999; Nardini and Pitt, 1999; Sobrado, 2000). Root hydraulic conductances were within the range measured for *Acer saccharinum* (Tsuda and Tyree, 1997), *Olea oleaster* (Lo Gullo *et al.*, 1998), a number of tropical angiosperms and conifers (Becker *et al.*, 1999), and some *Quercus* species (Nardini and Tyree, 1999). Although not significantly different from the other clones, high watered GC550 had high values of $K_{r/LA}$, but values for the other clones were within the ranges of light demanding pioneers and shade tolerant species (Tyree *et al.*, 1998). Values for *E. regnans* (Brodribb and Hill, 2000) were higher than for the clones in this study, except high watered GC550.

The shoot hydraulic conductances ($K_{s/LA}$) measured for *Eucalyptus* spp. clones in this study (Fig. 4.3.11. B) were higher than those of light demanding pioneers and shade tolerant species (Tyree *et al.*, 1998), three mangrove species (Sobrado, 2000), *Acer saccharinum* (Tsuda and Tyree, 1997), and slightly more than many *Quercus* species (Nardini and Tyree, 1999), but slightly lower than the values reported for *E. regnans* (Brodribb and Hill, 2000).

Root hydraulic conductances

The root hydraulic conductances expressed per unit root dry mass ($K_{r/trdw}$) were not significantly decreased by the low watering treatment. However, hydraulic conductances expressed per unit leaf area ($K_{r/LA}$) were marginally significantly ($P=0.083$) reduced by the low watering treatment (Fig. 4.3.11. A). It was only GU210 that did not show a marked effect of watering, probably accounting for a P value greater than 0.05. North and Nobel (1991 & 1992); Huang and Nobel (1993); Lo Gullo *et al.* (1998) and Nardini *et al.* (1999) have all observed drought-induced decreases in root hydraulic conductances.

The root hydraulic conductances measured in this study were obtained using an HPFM and so were maximum values, excluding any embolisms. The major resistances to water flow can be located in the soil, at the soil-root interface, in radial movement of water to the stele (Faiz and Weatherly, 1977; Running, 1980; Faiz and Weatherly, 1982; Kramer and Boyer, 1995) or in the axial flow of water in the root (Kramer and Boyer, 1995). However the HPFM measures conductances by forcing water backwards through the roots, and so do not include root-soil interface or soil resistances. Blizzard (1980) demonstrated that the resistance of the plant is greater than that of the soil. Root hydraulic resistances are comprised of both axial and radial components, but the radial resistances are considerably higher than axial resistances and so it is radial flow that will limit water uptake (Rowse and Goodman, 1981; Frensch and Steudle, 1989; Steudle and Brinckmann, 1989; Lo Gullo *et al.*, 1998; Steudle and Peterson, 1998). Water stress changes the architecture of the non-vascular pathway and decreases the root hydraulic conductance (Lo Gullo *et al.*, 1998). The changes in radial resistances of roots is mainly by the development of the endodermis (Kramer, 1983; Lo Gullo *et al.*, 1998) the formation of exodermis (Perumalla and Peterson, 1986; Lo Gullo *et al.*, 1998), and changes in the hydraulic conductivity of root cells (Radin and Mathews, 1989). According to Huang and Nobel (1993), prolonged drought periods increase the number of suberized peridermal layers and cortical cells of lateral roots in cacti species. Water stress reduced root hydraulic conductances reported in *Olea oleaster* seedlings as a result of a two layered

exodermis with thicker suberized walls, three to four layered endodermis with all cells having suberized tangential walls and diffusing shrinkage of cortex cells (Lo Gullo *et al.*, 1998).

In this study, although watering had an effect on root conductances, there were no differences among clones. Differences have been reported between light demanding pioneer species and shade tolerant species (Tyree *et al.*, 1998), between *Quercus suber* L. and *Quercus cerris* L. during summer periods (Nardini *et al.*, 1999) and between drought adapted and mesophilous *Quercus* species (Nardini and Tyree, 1999). It would appear that the three *Eucalyptus* spp. clones used in this study were sufficiently genotypically related and ecologically similar, that root conductances did not differ.

Shoot hydraulic conductances

Shoot hydraulic conductances expressed per unit leaf area ($K_{S/LA}$) for all three *Eucalyptus* spp. clones were higher for low than for high watered plants, although there were no clonal differences (Fig. 4.3.11. B). This is in contrast to other reports in the literature. Vander Willigen and Pammenter (1998) showed higher leaf specific conductances of branches of 7 to 8-year-old trees of four closely related *Eucalyptus* spp. clones growing in a mesic site compared with a xeric site. February *et al.* (1995) found wider vessels in stem xylem of *E. grandis* and *E. grandis* x *camaldulensis* grown under wet than under dry conditions, and changes in stem vessel characteristics of *Vitis vinifera* L. under water stress decreased leaf specific hydraulic conductivity (Lovisolo and Schubert, 1998). Similarly, maximum leaf specific stem hydraulic conductivities of *Populus trichocarpa* were higher in populations growing at a wet site than a dry site (Sparks and Black, 1999). However, Shumway *et al.* (1991) showed that water stressed seedlings of *Fraxinus pennsylvanica* Marsh. had higher main stem leaf specific conductivities, in agreement with the shoot hydraulic conductance data for the *Eucalyptus* spp. clones.

This difference in the reported effects of water availability may have been because the data reported here for three *Eucalyptus* spp. clones were obtained on whole shoots, whereas other authors used side branches or portions of the main stem, and did not include minor branches and leaves, and so their data may not be representative of whole shoot hydraulic conductances. Leaf bearing branches represent a substantial hydraulic constriction in trees (Zimmermann, 1983; Tyree *et al.*, 1991) and leaves constituted a greater proportion of the hydraulic resistances of the shoots than any other component (Tyree *et al.*, 1993b; Yang and Tyree, 1994; Cochard *et al.*, 1997; Nardini and Pitt, 1999; Nardini and Salleo, 2000). Consequently, the lower leaf area developed as a result of low water treatment in the *Eucalyptus* spp. clones in this study (Table 4.3.2) might have reduced the hydraulic resistances of the shoots relative to the high watered plants which carried a greater leaf area. In accordance with this, both the absolute and the hydraulic resistance of leaves (Table 4.3.8), and their proportional contribution to total plant resistance (Table 4.3.9) was significantly higher for high watered compared with low watered plants.

According to Nardini and Pitt (1999) higher hydraulic efficiency of stems and roots, normalised to leaf area, under water stress is a drought avoidance strategy. The elevated shoot hydraulic conductances (normalised to leaf area) of the low watered *Eucalyptus* spp. clones in this study were a consequence of reduced leaf area, which would improve water supply per unit leaf area, and so could be considered a drought avoidance strategy.

Whole plant hydraulic conductances

The roots constituted a considerable proportion of the total plant hydraulic resistances, and this was higher in low watered plants (71 to 83%) than high watered plants (55 to 65%) (Table 4.3.9). The contribution of roots to total resistance was considerably higher than the approximately 1:1 proportion found in light demanding pioneers and shade tolerant forest species (Tyree *et al.*, 1998), some of the angiosperms and conifers investigated by Becker *et al.* (1999) and some *Quercus* species (Nardini and Tyree, 1999). However, in accordance with the data for the three *Eucalyptus* spp.

clones in this study, *Acer saccharinum* (66%; Tsuda and Tyree, 1997), *Quercus* species (55 to 65%, Nardini and Pitt, 1999; Nardini and Tyree, 1999) and in *Toona australis*, (77%, Brodribb and Hill, 2000) all showed higher root than shoot hydraulic resistances.

Whole plant hydraulic conductances expressed per unit leaf area ($K_{P/LA}$) did not differ between the treatments, but a clonal effect was apparent under low watering treatment (Fig. 4.3.12). This suggests that $K_{P/LA}$ was well coordinated with soil water availability. High $K_{P/LA}$ has been suggested to favour fast growth (Tyree and Ewers, 1996; Tyree *et al.*, 1998; Nardini and Tyree, 1999), although in this study on three *Eucalyptus* spp. clones, differences in growth associated with watering treatment were not associated with differences in $K_{P/LA}$. This is probably because adjustment of $K_{P/LA}$ was brought about predominantly by adjustment of leaf area. There was a clonal effect under the low watering treatment, where TAG14 had a lower whole plant conductivity compared with that of GU210. However, there was no difference in biomass production associated with this difference.

There are no reports in the literature on the long-term effect of watering treatments on $K_{P/LA}$ as measured by the HPFM. However, Wakamiya- Noborio *et al.* (1999) calculated hydraulic conductivity of loblolly pine from the relationship between transpiration rate and the water potential gradient between the root surface and the leaf. They found the hydraulic conductivity of seedlings was higher under irrigated than drought treatments. Also, soil to leaf hydraulic resistances in *Pinus sylvestris* L. increased during the drought period compared with a control watering treatment (Irvine *et al.*, 1998). These reports are at variance to the data from the present study.

4.4.4 Hydraulic segmentation

The concept of hydraulic segmentation suggests that leaf specific hydraulic conductance decreases toward the shoot apex (Zimmermann, 1983; Tyree and Ewers, 1991). Such decline of leaf specific hydraulic conductance permits a more or less equal competition for water in the crowns of trees lacking apical dominance (Tyree

and Ewers, 1996). Plant hydraulic constrictions like petiole–leaf, twig–petiole and stem–twig junctions will lead to a component-to-next-component decrease in water potential, but will confine xylem cavitations to the distal components with low water potential (Tyree and Ewers, 1991). In the shoots of the three *Eucalyptus* spp. clones in this study, hydraulic resistances (normalized to leaf area) increased sequentially towards the apex, from the main stem to the leaves (Table 4.3.8), indicating the hydraulic segmentation observed in several other woody plant species. R_L was significantly higher for high watered plants, as a consequent of higher leaf areas. Xylem cavitations were not measured on components other than main stems, so the effectiveness of hydraulic segmentation to confining cavitations to distal components could not be assessed

4.4.5 Xylem vulnerability to cavitation

UAE detected stem xylem cavitation

Vulnerability to xylem cavitation of the main stem, detected from the critical leaf water potential triggering xylem cavitation ($\psi_{CAV,cUAE,\%}$) differed significantly between treatment and among clones, with a positive interaction between the factors. This is in contrast to a study by Vander Willigen and Pammenter (1998), who showed that the xylem vulnerability (quantified by an hydraulic method) of branches of *Eucalyptus* spp. clones were influenced by genotype but not by water availability (mesic or xeric sites). The $\psi_{L,EPH_{max}}$ was slightly lower than $\psi_{CAV,cUAE,\%}$, and vulnerability assessed this way showed no significant differences between treatments and clones. However, $\psi_{L,EPH_{max}}$ showed similar patterns to $\psi_{CAV,cUAE,\%}$.

Differences in stem xylem vulnerability to cavitation have been reported among a number of species from a wide range of habitats (Tyree and Dixon, 1986; Sperry and Tyree, 1990), and differences have been found between three coffee cultivars (Tausend *et al.*, 2000), and between evergreen sub-tropical tree species growing together (Vander Willigen *et al.*, 2000), all of which is accordance with the genotypic differences shown in this study. The ranking of the *Eucalyptus* spp. clones in this

study (with GC550 being the most vulnerable, and TAG14 the least) differed from that found by Vander Willigen and Pammenter (1998) working with genetically similar clones. In their study a pure *E. grandis* clone, closely related to TAG14, was more vulnerable than a *E. grandis x camaldulensis* clone closely related to GC550. These differences may be a consequence of the age differences of the plants (vulnerability has been shown to vary with tree age (Cochard, 1992; Sperry and Saliendra, 1994; Sperry and Ikeda, 1997)), or may reflect differences in the components of the pathway studied (branches in the case of Vander Willigen and Pammenter (1998) and main stem in this study). Furthermore, the UAE technique does not provide information about the impact of xylem cavitation on hydraulic conductances, making comparisons of data obtained with acoustic and hydraulic techniques difficult (Salleo *et al.*, 2000 and Chapter 2 of this thesis).

Stomatal closure to avoid xylem cavitation was demonstrated in droughted *Quercus* sp. by Cochard *et al.* (1996) and in four maize genotypes (Cochard, 2002). In the present study photosynthesis appeared to be limited by stomatal conductance in all clones and watering treatment (Fig 4.3.6), with the strongest relationship being observed for TAG14. Such strong stomatal limitation of photosynthesis, possibly to prevent the development of excess xylem tension to avoid runaway embolism cycle during period of severe water stress, is associated with the cost of decreased productivity. A model describing runaway embolism cycles (Tyree and Sperry, 1988) has shown that xylem cavitation can be controlled by either stomatal closure or a reduction in leaf area. When data for A and g_s were pooled across watering treatments, GC550 showed stronger relationship between these variables ($r^2=0.65$) than other two clones ($r^2 < 0.48$). Furthermore, the total leaf area was significantly lower than that of the other two clones. These data are in accord with the higher vulnerability of GC550, although the lower conductance and leaf area did not prevent cavitations occurring.

The question of whether vulnerability to cavitation is determined by genotype or whether growth conditions have an effect has been raised by Vander Willigen *et al.* (2000). There are reports that stem xylem vulnerability to cavitation, measured by

hydraulic and UAE methods, was the same in a wet and dry site (Alder *et al.* 1996; Jackson *et al.* 1995b; Vander Willigen and Pammenter, 1998). On the other hand, in this present study two of the clones were less susceptible to xylem cavitation under low than high watering conditions, and there are other reports of differences in xylem vulnerability between mesic and xeric sites consistent with this. Franks *et al.* (1995) reported that seedlings of *E. camaldulensis* collected from a xeric environment showed lower xylem vulnerability than those collected from a mesic site. Similarly, a Douglas fir population from a mesic environment was more vulnerable to cavitation in both stems and roots than two other dry site populations (Kavanagh *et al.*, 1999). Furthermore, Sparks and Black (1999) found interpopulation variation in resistance to drought induced xylem cavitation in *Populus trichocarpa* Torr. & A., with populations from moist environments showing less resistances to drought-induced xylem cavitation than those from dry environments. Also, Alder *et al.* (1996) found that root xylem vulnerability of *Acer grandidentatum* was higher in the riparian (wet) than slope (dry) site. The differences between clones and the effect of watering treatment on the three *Eucalyptus* spp. clones in this study suggest that xylem vulnerability can be driven by the effect of soil water as well as genotype.

The concept of safety versus efficiency of xylem has been discussed by Tyree and Ewers (1996) and by Tyree *et al.* (1994a). The relationship between whole shoot hydraulic conductances and the xylem vulnerability was not clear in this study. The higher xylem vulnerability for high watered plants of two of the *Eucalyptus* spp. clones was associated with lower, rather than higher, whole shoot conductances. This may be because it was the vulnerability of the stem xylem that was assessed using the UAE method, whereas the conductance of whole shoots, including leaves, was measured with the HPFM. Vulnerability has been shown to differ among plant components (Sperry and Saliendra, 1994; Alder *et al.*, 1996; Hacke and Sauter, 1996), and, Salleo *et al.* (2000) showed that leaves are more vulnerable than shoots.

4.4.6 Xylem recovery

Overnight recovery in leaf water potential of the plants of both treatments was apparent (Fig. 4.3.4. A, B & C / Tables 4.3.3 and 4.3.4), indicating refilling of the xylem subsequent to substantial cavitation events. The mechanisms of embolism repair are currently unclear (Holbrook and Zwieniecki, 1999; Tyree *et al.*, 1999), although presumably they comply with the cohesion-tension theory. Root pressure has been suggested to be a process that could lead to embolism repair in short plants (Devlin, 1966; Salisbury and Ross, 1991), Root pressures developed in all clones under both treatments in this study, with values being higher at midday than early morning. The development of positive xylem pressure and the associated recovery from embolisms has been documented by Sperry *et al.* (1987); Sperry *et al.* (1988b); Sperry (1993); Lopez-Portillo *et al.* (2000) and Cochard *et al.* (2001). Values of overnight root pressure range from 3 kPa in *Fagus grandifolia* (Sperry, 1993) to 10 - 100 kPa in vines (Sperry *et al.*, 1987), with a record of 225 kPa in a hemiepiphyte (Lopez-Portillo *et al.*, 2000), but these values are somewhat higher than the overnight pressures measured for the *Eucalyptus* spp. clones in this study. Sperry (1993) reported the presence of middays root pressure of 42 kPa to 86 kPa in *Betula occidentalis*, consistent with the results from this study.

A positive pressure of 10 kPa is required to push a water column to a height of 1m, regardless any xylem tissue friction. The overnight root pressures measured in the *Eucalyptus* spp. clones were less than 4 kPa (Fig. 4.3.10. A), and presumably were unable to push the water to the apex of the plants which were between 0.70 and 1.00 m in height. However, midday root pressures ranged from 13 to 26 kPa and so would have been adequate to refill embolized xylem throughout the entire plant. Root pressure-driven refilling would be expected to be most effective when transpiration rates are low, and so it is difficult to visualize positive root pressures developing and dissolving embolisms in an intact plant at midday. However, embolism repair has been shown to occur concurrently with high transpiration rates (Canny *et al.*, 1997; McCully *et al.*, 1998; McCully, 1999; Zwieniecki *et al.*, 2000), although the mechanisms are not understood. Whether positive midday root pressures were

involved in the refilling of embolized xylem in the *Eucalyptus* spp. clones in this study is not known, but refilling, as evidenced by recovery of leaf water potentials, certainly occurred.

Chapter: 5.1 Introduction

This experiment was undertaken to assess whether there were any age-related changes in the response of potted saplings to watering regime. However, the data from the studies on 14 month old saplings in 25 l pots (Chapter 4) indicated that in that experiment even the high watered plants were severely water stressed, to that extent that treatment effects were not apparent. Thus, in this experiment, the watering regime was changed from May 2001 to November 2001. This ensured that the high watered plants were not stressed during the subsequent 7 months, and so this experiment would show any treatment effects on plant performance. The experimental design and detailed methodology were identical to that for the 14 month-old saplings, except that the plants were grown in 85 l, rather than 25 l pots and the watering regime had been altered.

Chapter: 5.2 Results

5.2.1 Effect of watering treatment on total biomass production and allocation

As with the plants harvested at 14 months old, watering treatments had a significant effect on total plant biomass at 21 months ($F=158.82$, $P<0.001$), with biomass being reduced by 46 to 50% by the low watering treatment (Table 5.2.1), but there were no differences among clones ($F=0.45$, $P=0.643$). Significant reductions in biomass occurred in all plant components, but there were no differences among clones within a watering treatment. The reduction in root growth (50 to 57%) was greater than that in leaves and stems, such that the low watering treatment significantly reduced root:shoot ratios ($F=6.67$, $P=0.004$), but there were no clonal differences ($F=2.15$, $P=0.134$).

Along with leaf biomass, total leaf area was also reduced by low water ($F=60.38$, $P<0.001$) (Table 5.2.2). There were significant clonal differences in leaf area ($F=6.28$, $P<0.001$), with TAG14 producing a greater leaf area than GC550 under high water, but this difference was not apparent under reduced watering. Specific leaf area of

GC550 was lower than that of the other two clones ($F=39.61$, $P<0.001$), but this parameter was not influenced by watering treatment ($F=2.49$, $P=0.125$). Leaf weight ratio was significantly increased by low water ($F=11.03$, $P<0.002$), as a result of the reduction in leaf weight being less than the reduction in root weight. In terms of interaction between treatment and clones, there was no significance.

Table 5.2.1 Growth parameters measured after harvest on three *Eucalyptus* spp. clones grown for 21 months in 85 l pots subjected to high or low watering treatment. Means are given \pm SEM ($n= 6$). Letters represent (under each parameter) the means separation by Scheffe's multiple range tests, showing comparison within the high (upper case lettering) and low (lower case lettering) watering treatments separately ($P<0.05$). Percentage reduction in growth parameters caused by low watering are also shown.

Clones	Treatments	Leaf DW,(g)	Stem DW,(g)	Root DW,(g)	Plant DW,(g)	R:S ratio
GC550	High	259.50 \pm 23.21 ^A	476.00 \pm 47.20 ^A	1207.40 \pm 122.80 ^A	1943.10 \pm 159.00 ^A	1.69 \pm 0.19 ^A
GC550	Low	153.00 \pm 4.30 ^a	291.00 \pm 11.70 ^a	525.50 \pm 25.90 ^a	968.90 \pm 25.20 ^a	1.19 \pm 0.08 ^a
% difference between treatments		41.20	38.92	56.48	50.13	
GU210	High	225.88 \pm 12.10 ^A	468.50 \pm 34.30 ^A	1178.50 \pm 31.70 ^A	1872.90 \pm 63.80 ^A	1.72 \pm 0.10 ^A
GU210	Low	143.50 \pm 3.70 ^a	282.70 \pm 16.64 ^a	529.50 \pm 54.70 ^a	955.70 \pm 40.90 ^a	1.27 \pm 0.17 ^a
% difference between treatments		36.46	39.66	55.07	48.97	
TAG14	High	256.70 \pm 10.80 ^A	502.80 \pm 30.10 ^A	1022.17 \pm 146.00 ^A	1781.60 \pm 146.70 ^A	1.36 \pm 0.70 ^A
TAG14	Low	142.40 \pm 9.30 ^a	309.20 \pm 20.70 ^a	512.60 \pm 32.60 ^a	964.20 \pm 47.80 ^a	1.15 \pm 0.20 ^a
% difference between treatments		44.51	38.51	49.85	45.88	

Table 5.2.2 Growth parameters measured after harvest on three *Eucalyptus* spp. clones grown for 21 months in 85 l pots subjected to high or low watering treatment. Means are given \pm SEM (n= 6). Letters represent (under each parameter) the means separation by Scheffe's multiple range tests, showing comparison within the high (upper case lettering) and low (lower case lettering) watering treatments separately (P<0.05). Percentage reduction in growth parameters caused by low watering are also shown.

Clones	Treatments	LA, (m ²)	SLA, (m ² kg ⁻¹)	LWR	LAR, (m ² kg ⁻¹)
GC550	High	2.53 \pm 0.25 ^A	9.78 \pm 0.36 ^A	0.13 \pm 0.007 ^{AB}	1.30 \pm 0.08 ^A
GC550	Low	1.60 \pm 0.06 ^a	10.53 \pm 0.23 ^a	0.16 \pm 0.005 ^a	1.64 \pm 0.06 ^a
% difference between treatments		37.21			
GU210	High	3.00 \pm 0.24 ^{AB}	13.31 \pm 0.49 ^B	0.12 \pm 0.004 ^A	1.60 \pm 0.10 ^{AB}
GU210	Low	1.97 \pm 0.08 ^a	13.84 \pm 0.57 ^b	0.15 \pm 0.009 ^a	2.07 \pm 0.06 ^b
% difference between treatments		34.40			
TAG14	High	3.40 \pm 0.25 ^B	13.27 \pm 0.60 ^B	0.15 \pm 0.01 ^B	1.95 \pm 0.19 ^B
TAG14	Low	1.95 \pm 0.19 ^a	13.68 \pm 0.51 ^b	0.15 \pm 0.007 ^a	2.02 \pm 0.15 ^b
% difference between treatments		42.64			

Growth in height and stem over-bark diameter

Growth in height and over-bark diameter over the 21 month period is shown in Fig. 5.2.1. Both height (F=12.37, P=0.001) and the stem over-bark diameter (F=94.99, P<0.001) at the end of the experiment were significantly affected by watering treatment (Table 5.2.3). There was also a clonal effect on height (F=15.36, P<0.001), with GC550 being taller than the other clones, but stem over-bark diameter was not influenced by clone (F=2.57, P=0.093).

Effect of pot size on growth

The effect of pot size on growth can also be seen in Table 5.2.3, where heights and over-bark diameters of plants at 14 months of age in 25 l or 85 l are compared. Significantly greater growth in height (F=131.89, P<0.001) and stem over-bark diameter (F=152.35, P<0.001) occurred in plants grown in 85 l pots compared with that of those in 25 l pots over 14 months. This was probably a consequence of root

restriction in the smaller pots: at harvest, roots in 25 l pots (14 months old) were compactly pot bound with roots being rigid and large in diameter; in 85 l pots (21 months old) roots were mostly concentrated at the bottom and top region of the pots, with an increased proportion of thinner and fragile roots. However, although small pots size did reduce growth, the ranking of the clones and the relative effects of the watering treatment were the same in the two pot sizes.

Table 5.2.3 Effects of two watering regimes on the plant height (h) and main stem over-bark diameter measured 100 mm above soil (OBD) at the time of harvest of three *Eucalyptus* spp. clones grown for 14 months in 25 l pots, or 21 months in 85 l pots, and subjected to high or low watering. In addition, the values for plants in 85 l pots at 14 months are also shown. Means are given \pm SEM (n= 6). Letters represent (under each parameter) the means separation by Scheffe's multiple range tests, showing comparison within the high (upper case lettering) and low (lower case lettering) watering treatments separately (P<0.05). Percentage reduction in growth parameters caused by low watering are also shown.

		At 14 months old in 25 l	At 14 months old in 85 l	At 21 months old in 85 l			
Clones	Treatments	h (m)	OBD (mm)	h (m)	OBD (mm)	h (m)	OBD (mm)
GC550	High	0.99 \pm 0.03 ^A	16.70 \pm 0.80 ^A	1.37 \pm 0.05 ^A	23.30 \pm 0.42 ^A	2.10 \pm 0.10 ^A	33.10 \pm 1.10 ^A
GC550	Low	0.85 \pm 0.05 ^a	13.70 \pm 0.23 ^a	1.27 \pm 0.05 ^a	20.00 \pm 0.50 ^a	1.80 \pm 0.10 ^a	26.70 \pm 0.50 ^a
% difference between treatments		14.75	17.85	7.35	13.93	14.52	19.46
GU210	High	0.80 \pm 0.04 ^B	18.50 \pm 0.30 ^A	1.04 \pm 0.03 ^B	24.30 \pm 0.40 ^A	1.60 \pm 0.07 ^B	32.60 \pm 0.60 ^A
GU210	Low	0.70 \pm 0.00 ^b	14.40 \pm 0.25 ^{ab}	0.90 \pm 0.03 ^b	20.40 \pm 0.60 ^a	1.32 \pm 0.09 ^b	26.60 \pm 0.80 ^a
% difference between treatments		13.38	22.14	15.91	15.06	16.63	18.40
TAG14	High	0.90 \pm 0.03 ^B	16.50 \pm 0.75 ^A	1.10 \pm 0.08 ^B	23.00 \pm 1.04 ^A	1.70 \pm 0.10 ^B	31.00 \pm 1.00 ^A
TAG14	Low	0.80 \pm 0.01 ^{ab}	15.00 \pm 0.35 ^b	1.02 \pm 0.03 ^{ab}	19.80 \pm 0.41 ^a	1.50 \pm 0.10 ^{ab}	26.00 \pm 0.60 ^a
% difference between treatments		9.36	10.41	10.91	12.68	9.91	16.13

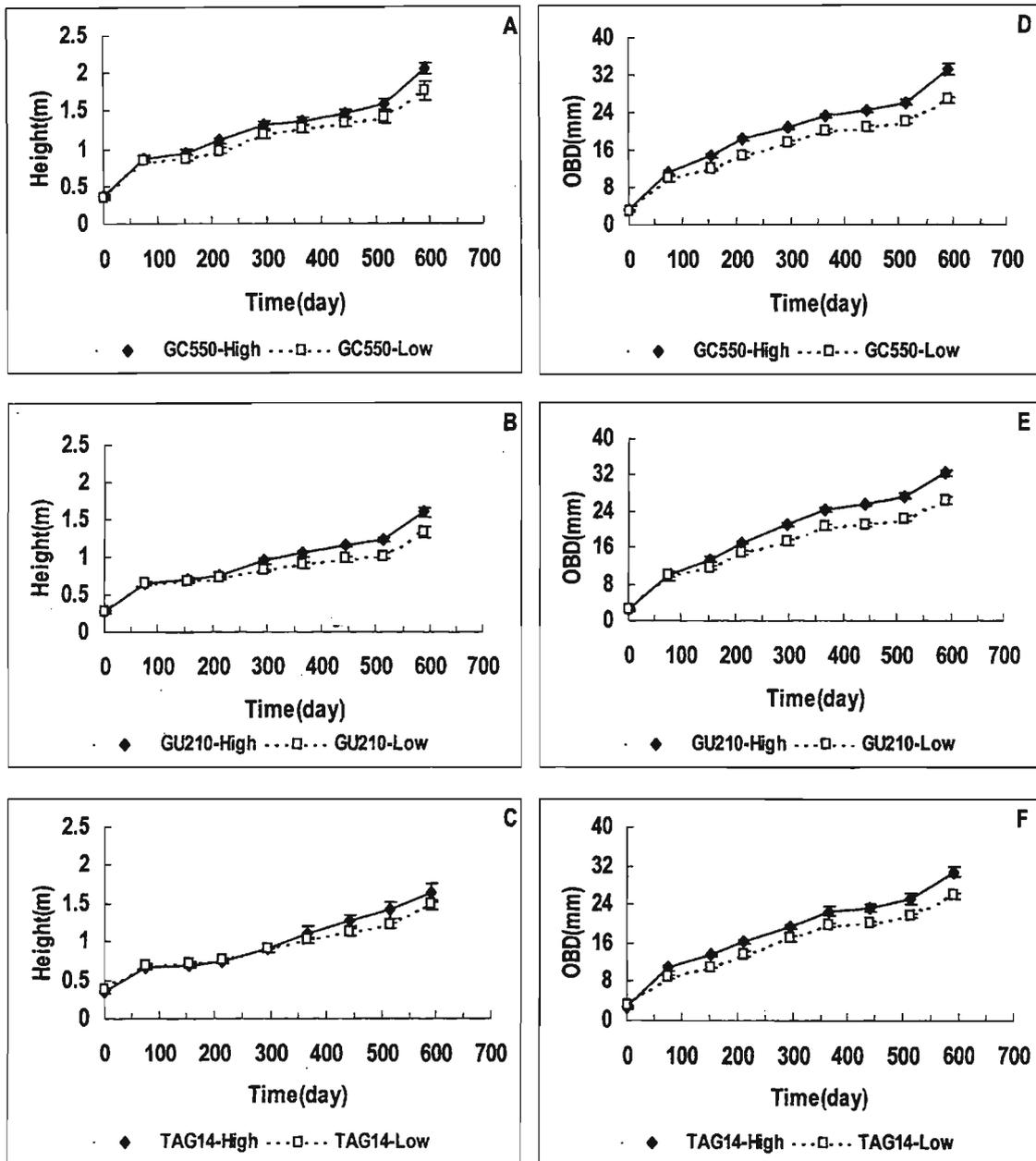


Fig. 5.2.1 Growth in terms of height (A, B & C) and stem over-bark diameter (D, E & F), as a function of time from the implementation of watering treatments for three *Eucalyptus* spp. clones grown in 85 l pots for 21 months. Error bars represent the \pm SEM of the mean (n=6).

5.2.2 Predawn leaf water potential

Predawn leaf water potential (which can be used as an assessment of soil water potential) was measured from 17th of June 2001 to 22nd of November 2001 on a monthly basis for both high and low watering treatments (Fig. 5.2.2). Mean monthly predawn leaf water potential of high watered plants was consistently higher than that of those receiving low water, although the effect was not significant in June, and August.

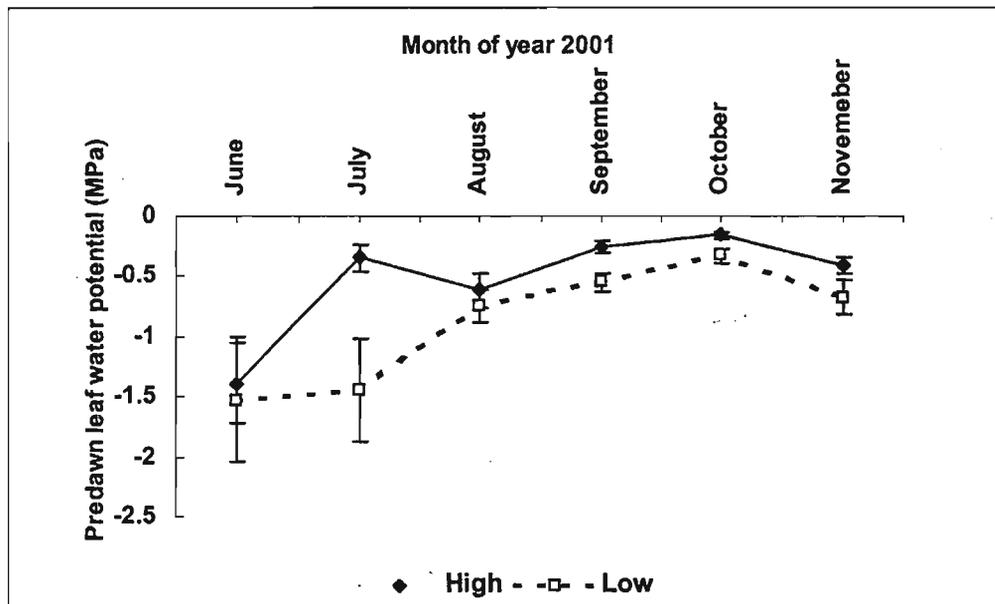


Fig. 5.2.2 Mean monthly predawn leaf water potential from June to November 2001 for the three *Eucalyptus* spp. clones grown in 85 l pots and subjected to high or low watering. Error bars represent the SEM of the mean for each month (n=10).

When the data collected over the experimental period were pooled, the mean predawn water potential for plants receiving high water was -0.49 ± 0.07 MPa (SEM) compared with -0.81 ± 0.11 MPa (SEM) for low watered plants. However, a two-way ANOVA revealed only marginal significant difference between watering treatments ($F=3.37$, $P=0.069$) effect, but there was a clear clonal effect, with GU210 showing lower predawn water potentials than the other two species (Table 5.2.4).

Table 5.2.4 Mean predawn and midday leaf water potential measured for three *Eucalyptus* spp. clones grown in 85 l pots for 21 months and subjected to high or low watering. Means are given \pm SEM. Within column, upper case lettering (high water) and lower case lettering (low water) indicate significantly different values at $P < 0.05$ separately for treatment. (ANOVA followed by Scheffe's multiple range test).

Clones	Treatments	Ψ_{PDL} (MPa)	Ψ_{Midday} (MPa)
GC550	High	-0.42 ± 0.08^{AB}	-1.70 ± 0.12^A
GC550	Low	-0.49 ± 0.05^a	-1.98 ± 0.17^a
GU210	High	-0.79 ± 0.17^A	-2.43 ± 0.15^B
GU210	Low	-1.17 ± 0.20^b	-2.58 ± 0.37^a
TAG14	High	-0.23 ± 0.07^B	-1.96 ± 0.44^{AB}
TAG14	Low	-0.48 ± 0.11^a	-2.64 ± 0.59^a

5.2.3 Diurnal changes in leaf water potential

Changes in diurnal leaf water potential from 06.00 h to 16.00 h are shown in Fig. 5.2.4. In all clones leaf water potential began to decrease gradually from morning to midday and then, except for GU210, showed slight recovery in the afternoon. Leaf water potential of high watered plants was consistently higher than those receiving low water throughout the day. Pooled middays xylem water potential data revealed that both watering treatment ($F=4.62$, $P=0.040$) and the clone ($F=5.15$, $P=0.012$) had significant effects (Table 5.2.4), with GU210 showing the lowest midday leaf water potentials.

5.2.4 Diurnal pattern of leaf gas exchange

Mean vapour pressure deficits during the days, when gas exchange measurements were made are shown in Fig. 5.2.3. Essentially the same pattern occurred during sampling of all clones at both watering levels. Net CO_2 assimilation rates (A) from 06.00 h to 16.00 h are shown in Fig. 5.2.4 (D, E & F). Rates were consistently higher in all three clones subjected to higher watering treatment compared with those of low watered plants. They initially increased as the morning progressed, but began to

decline before midday, with the decline occurring earlier in plants receiving low water. Midday rates were significantly higher for high watered plants ($F=12.71$, $P<0.001$), but there were no clonal differences ($F=0.05$, $P=0.950$).

Stomatal conductances showed similar patterns to net CO_2 assimilation rates pattern, with initial increases followed by stomatal closure (Fig. 5.2.5.). This occurred earlier in the day in the plants receiving low water (07.00 h to 08.00 h) than in those subjected to high watering (08.00 h to 10.00 h). Values for high watered plants were consistently higher than the low watered plants throughout the day.

The relationship between net CO_2 assimilation and stomatal conductance measured during the course of the day was linear in all cases (Fig. 5.2.6.), suggesting stomatal control of CO_2 assimilation. However, the relationship for high watered TAG14 was weaker ($r^2 = 0.37$) than for low watered plants of this clone ($r^2 = 0.74$), and for the other clones under both treatments (r^2 ranging from 0.61 to 0.73), suggesting that high watered TAG14 may have been less limited by stomata.

Transpiration (Fig. 5.2.5. D, E & F) followed similar patterns to stomatal conductance. Midday values of both g_s and E were significantly higher for high watered plants than low watered plants ($F=13.43$, $P=0.001$, and $F=12.63$, $P=0.001$, respectively). However, there were no significant differences among the clones in either g_s ($F=1.06$, $P=0.358$) or E ($F=0.75$, $P=0.481$). In all instances, transpiration continued to increase after stomatal conductance began to decline, this in response to increasing VPD.

Instantaneous leaf level water use efficiency (A/E) is shown in Fig. 5.2.6. GC550 and TAG14 showed marginally higher water use efficiency under high watering regimes than under low water, but there was no significant effect of either watering treatment ($P>0.05$) or clones ($P>0.05$) when midday data were subjected to a two-way ANOVA.

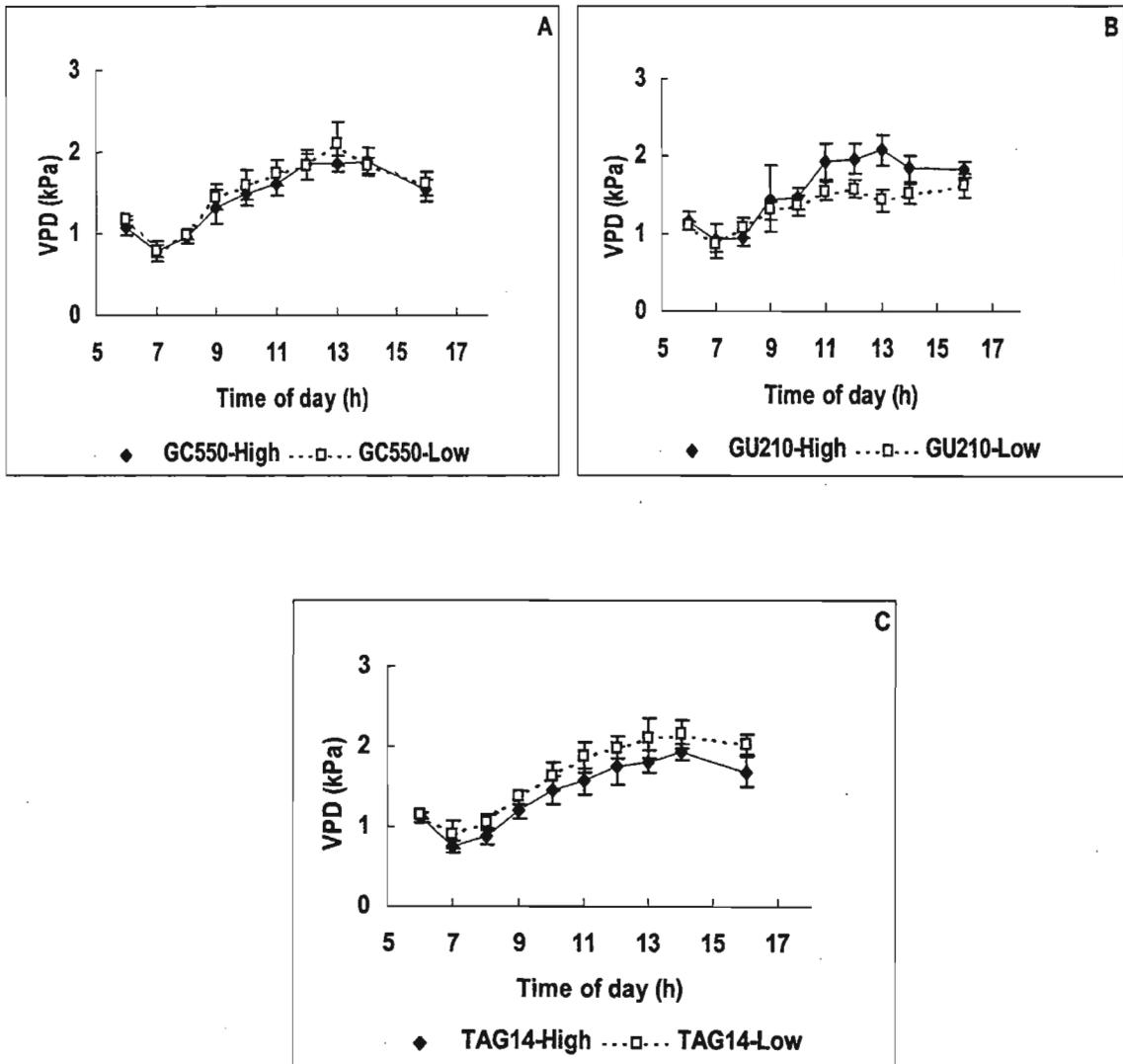


Fig. 5.2.3 Diurnal variations in instantaneous calculated vapour pressure deficits that were measured concurrently with leaf physiology parameters of three *Eucalyptus* spp. clones grown in 85 l pots for 21 months and subjected to high or low watering treatment. Error bars represent the \pm SEM of the mean ($n=6$).

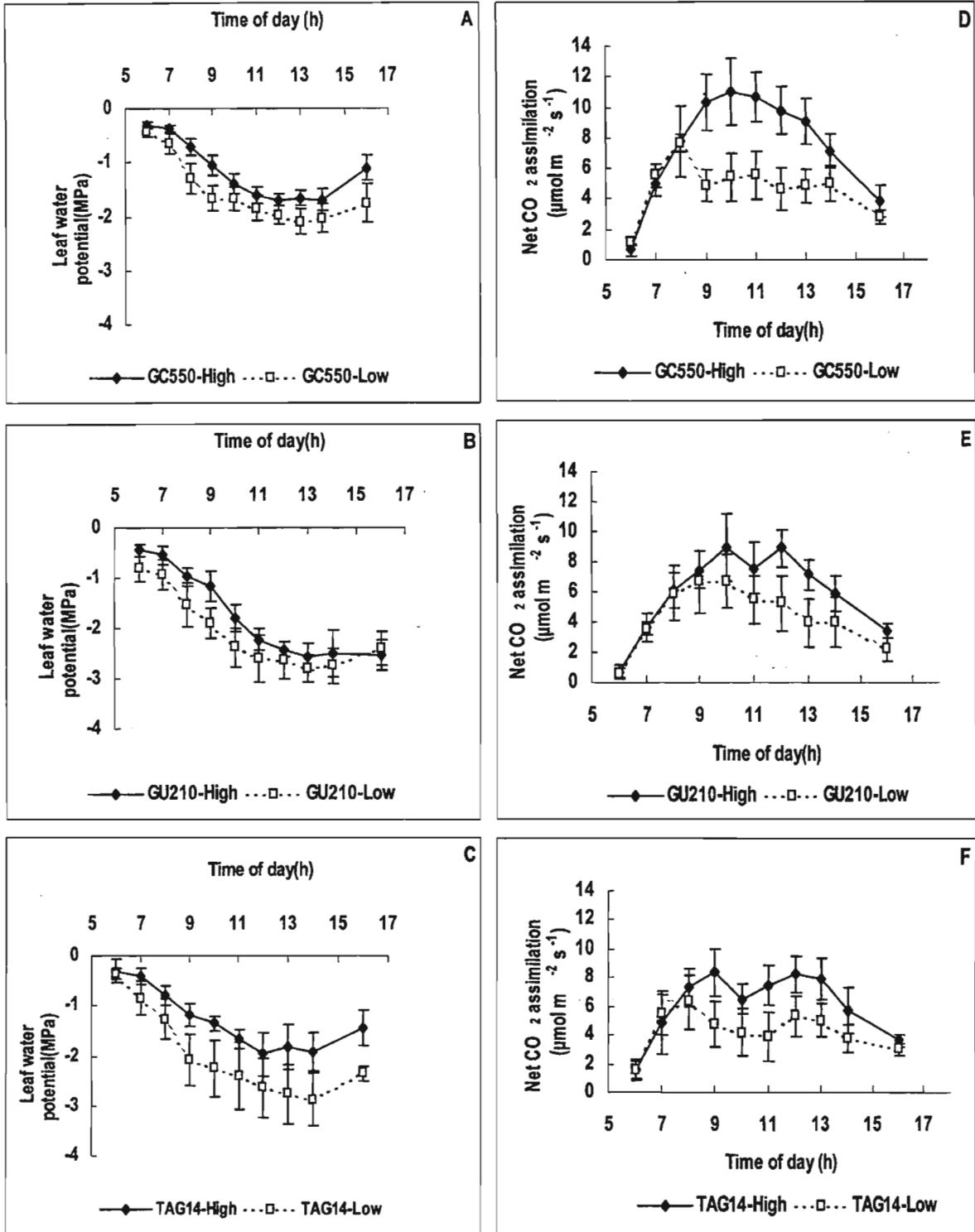


Fig. 5.2.4 Diurnal variations in leaf water potential (A, B & C) and the net CO₂ assimilation rate (D, E & F) of three *Eucalyptus* spp. clones grown for 21 months in 85 l pots, and subjected to high or low watering treatment. Error bars represent the \pm SEM of the mean (n=6).

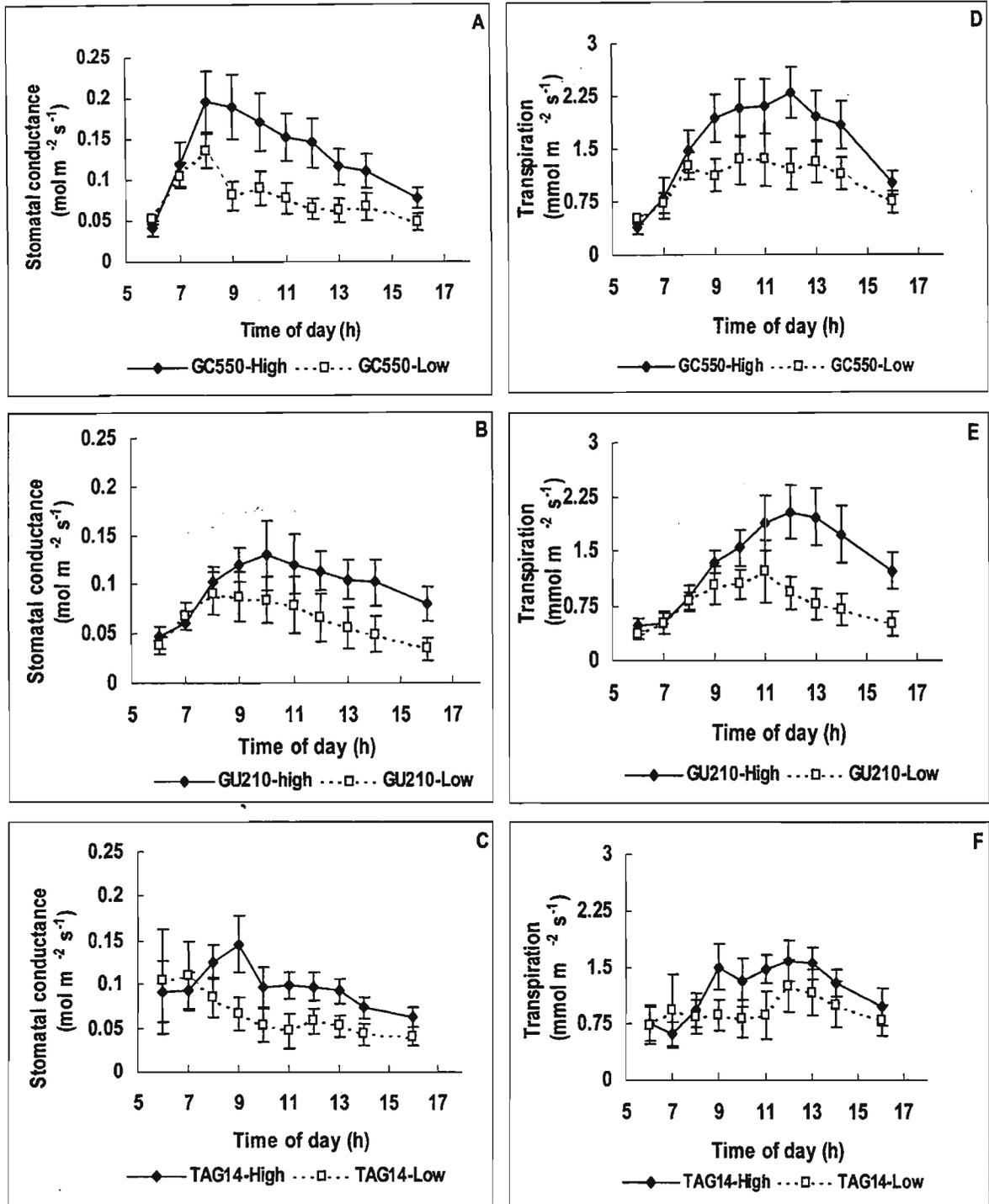


Fig. 5.2.5 Diurnal variations in stomatal conductances (A, B & C) and transpiration rate (D, E & F) of three *Eucalyptus* spp. clones grown for 21 months in 85 l pots, and subjected to high or low watering treatment. Error bars represent the \pm SEM of the mean ($n=6$).

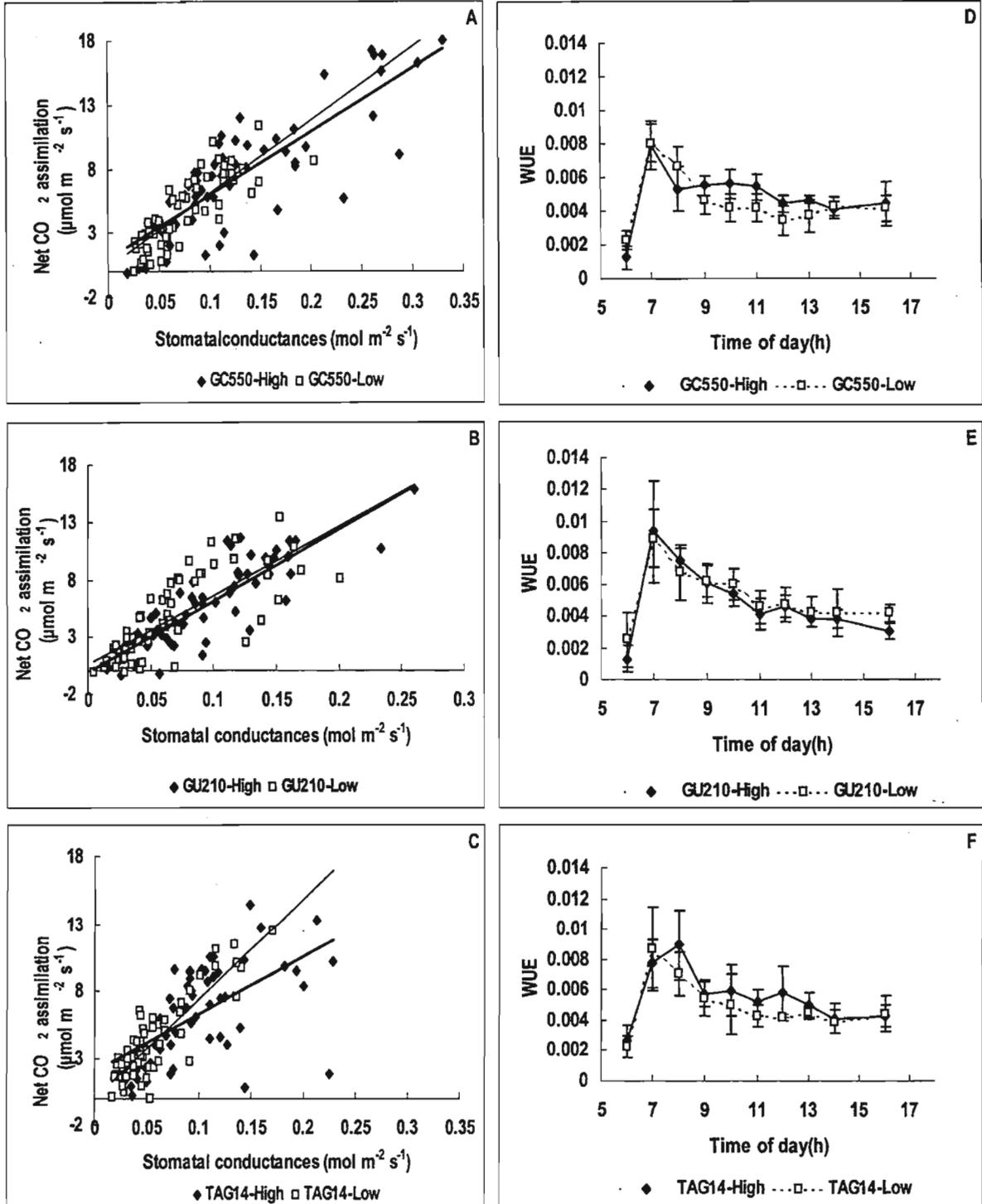


Fig. 5.2.6 The relationship between diurnally measured net CO₂ assimilation rate and stomatal conductances (A, B and C), and instantaneous leaf level water use efficiency (D, E and F) of three *Eucalyptus* spp. clones grown for 21 months in 85 l pots, and subjected to high or low watering treatment. The fitted linear regression lines are for high (bold) and low (thin) watered, respectively. Error bars represent ± SEM (n=6).

5.2.5 Relationship between transpiration and water potential

Linear regressions were fitted to the relationships between transpiration rates and the corresponding leaf water potentials (Fig. 5.2.7; each line is extended to the maximum transpiration rate measured). The regression slopes (bulked data for all plants of each clone and treatment) were subjected to linear regression and the slopes compared. Both treatment ($P < 0.039$) and clone ($P < 0.05$) had significant effects, with low watered plants having steeper slopes than those receiving high water, indicating a lower hydraulic resistance to water transport from soil to leaves in the high watered plants. Under high water conditions GU210 exhibited a steeper slope than the other clones.

Regression equations-

a) GC550- High- $y = -0.50x - 0.16$, $r^2 = 0.76$ ($P = 0.000$)

b) GC550- Low- $y = -2.76x + 0.65$, $r^2 = 0.52$ ($P = 0.000$), (pair wise-a vs. b- $F = 24.35$, $P = 0.000$)

c) GU210- High- $y = -1.03x - 0.13$, $r^2 = 0.80$ ($P = 0.000$)

d) GU210- Low- $y = -5.01x + 0.34$, $r^2 = 0.31$ ($P = 0.039$), (pair wise-c vs. d- $F = 7.39$, $P = 0.009$)

e) TAG14-High- $y = -0.55x - 0.33$, $r^2 = 0.64$ ($P = 0.000$)

f) TAG14-Low- $y = -2.50x - 0.18$, $r^2 = 0.55$ ($P = 0.000$), (pair wise-e vs. f- $F = 23.43$, $P = 0.000$)

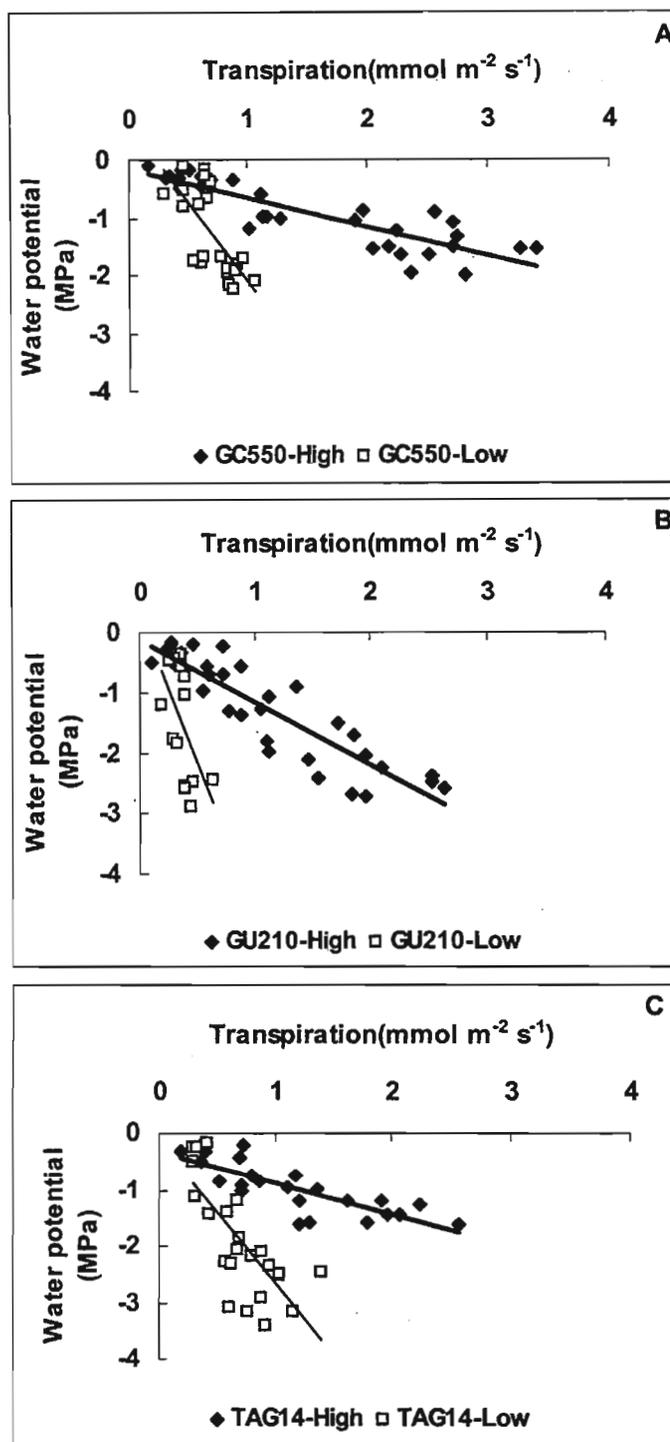


Fig. 5.2.7 Relationship between leaf water potential and transpiration rate for three *Eucalyptus* spp. clones grown in 85 l pots for 21 months and subjected to high (bold line) and low (thin line) watering treatments.

5.2.6 Xylem cavitation

Cumulative ultrasonic acoustic emissions

The diurnal pattern of the accumulation of ultrasonic acoustic emissions (cUAE) in the main stems of the three *Eucalyptus* spp. clones subjected to high and low watering treatments are shown in Fig. 5.2.8. cUAE began to rise from 07.00 h, increased to midday and then levelled off during the afternoon. In low watered plants of the clones GU210 and TAG14 acoustic emissions were initiated earlier and accumulated faster than in plants receiving high water, whereas in GC550 there was little difference between treatments. The accumulation of acoustic emissions (and probably cavitation events) corresponded with the decline in water potential from morning to midday, and the higher values recorded in low watered plants corresponded with the lower water potentials measured in these plants compared with that of high watered plants. The low watered plants total cUAE at midday was higher than the high watered ($F=4.48$, $P=0.043$), but no clonal differences were apparent ($F=0.49$, $P=0.612$). It is possible that differences in cUAEs were a consequence of differences in stem diameters, with more events being recorded from thicker stems. However, for both treatments, the UAE sensor head was smaller than the stem, and also the stem diameter of the high watered plants was greater than that of the low watered plants. Thus the higher cUAE measured for low watered plants was an effect of watering treatment, not the geometry of the sensor relative to stem diameter.

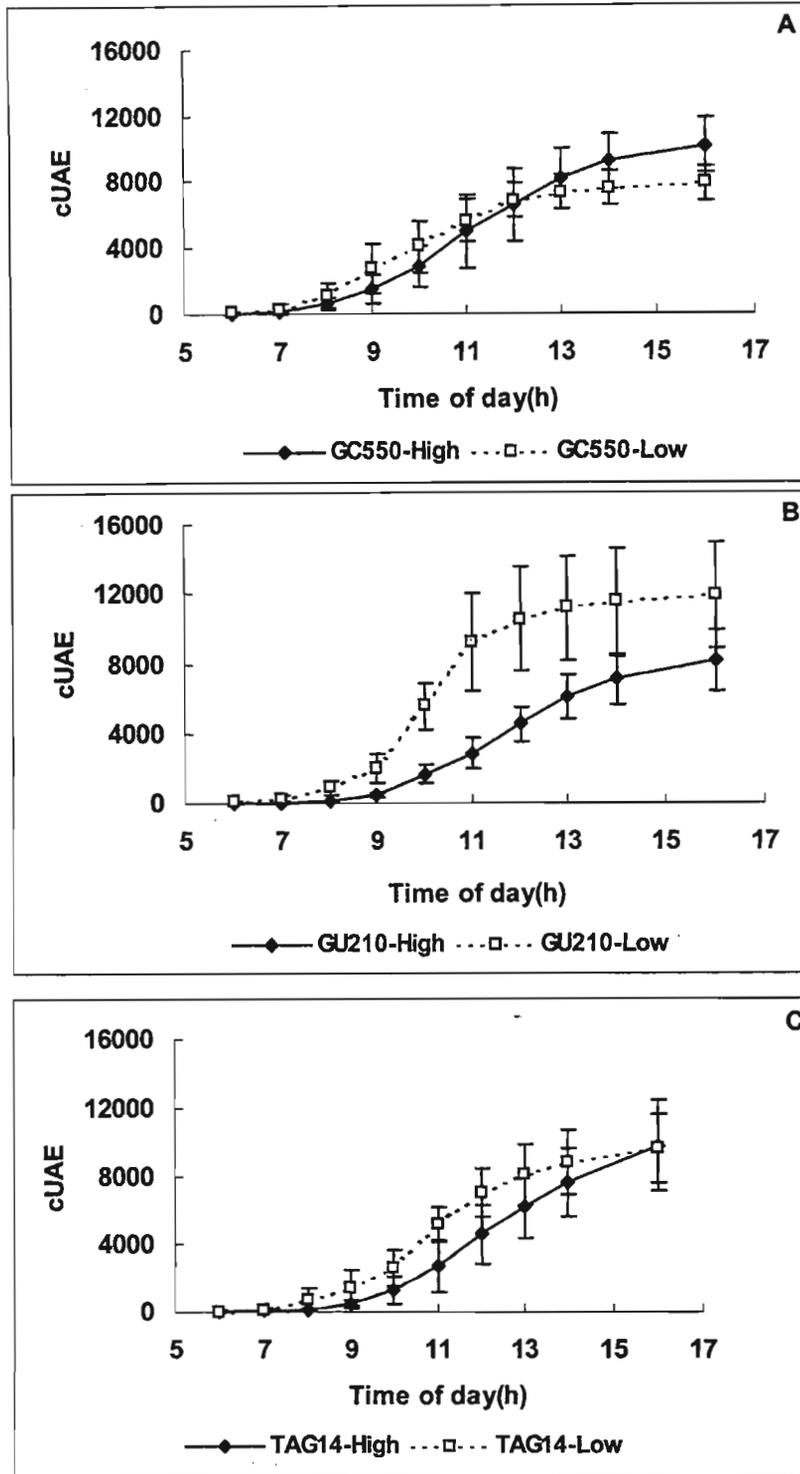


Fig. 5.2.8 Accumulation of ultrasonic acoustic emissions (cUAE), indicating xylem cavitation events, in the main stem of three *Eucalyptus* spp. clones grown for 21 months in 85 l pots, and subjected to high or low watering treatment. Error bars represent \pm SEM of the mean (n=6).

Events per hour (EPH)

The rate of accumulation of acoustic events, together with the corresponding stomatal conductances are shown in Fig. 5.2.9. Maximum events per hour (EPH_{max}) were marginally higher for low watered than high watered plants during early hours, except for GC550, where treatment had no effect. EPH_{max} for high watered plants occurred between 11.00 h and 12.00 h, whereas in low watered plants EPH_{max} occurred earlier, between 09.00 h and 11.00 h. EPH_{max} occurred later in the morning than maximum stomatal conductance, indicating that the partial stomatal closure that occurred was inadequate to prevent further cavitation events. A total EPH_{max} at middays (13.00 and 14.00 h) were significantly higher for high watered plants ($P < 0.011$), though there was no clonal effect ($P > 0.348$). Leaf water potentials corresponding to EPH_{max} are shown in Table 5.2.5. For all clones, leaf water potential at EPH_{max} was lower for low watered than for high watered plants. However, two-way analysis of variance indicated that this treatment effect was not significant ($F = 1.40$, $P = 0.252$), although the clonal effect was significant ($F = 4.90$, $P = 0.015$), with GU210 showing EPH_{max} at lower water potentials than the other clones.

Table 5.2.5 Mean leaf water potential measured at the time of EPH_{max} , and the stem over-bark diameter where UAE sensor was mounted for three *Eucalyptus* spp. clones grown in 85 l pots for 21 months, subjected to high or low watering treatment. Means are given \pm SEM ($n = 6$). Within column, upper case lettering (high water) and lower case lettering (low water) indicate significantly different values at $P < 0.05$ separately for treatment. (ANOVA followed by Scheffe's multiple range test).

Clones	Treatments	ψ_L, EPH_{max} (MPa)	Stem OBD (mm)
GC550	High	-1.60 ± 0.16^A	29.12 ± 1.06^A
GC550	Low	-1.66 ± 0.23^a	23.68 ± 0.54^a
GU210	High	-2.43 ± 0.15^B	31.85 ± 0.86^A
GU210	Low	-2.59 ± 0.47^a	26.10 ± 0.47^b
TAG14	High	-1.96 ± 0.44^{AB}	29.50 ± 1.27^A
TAG14	Low	-2.41 ± 0.67^a	23.97 ± 0.39^a

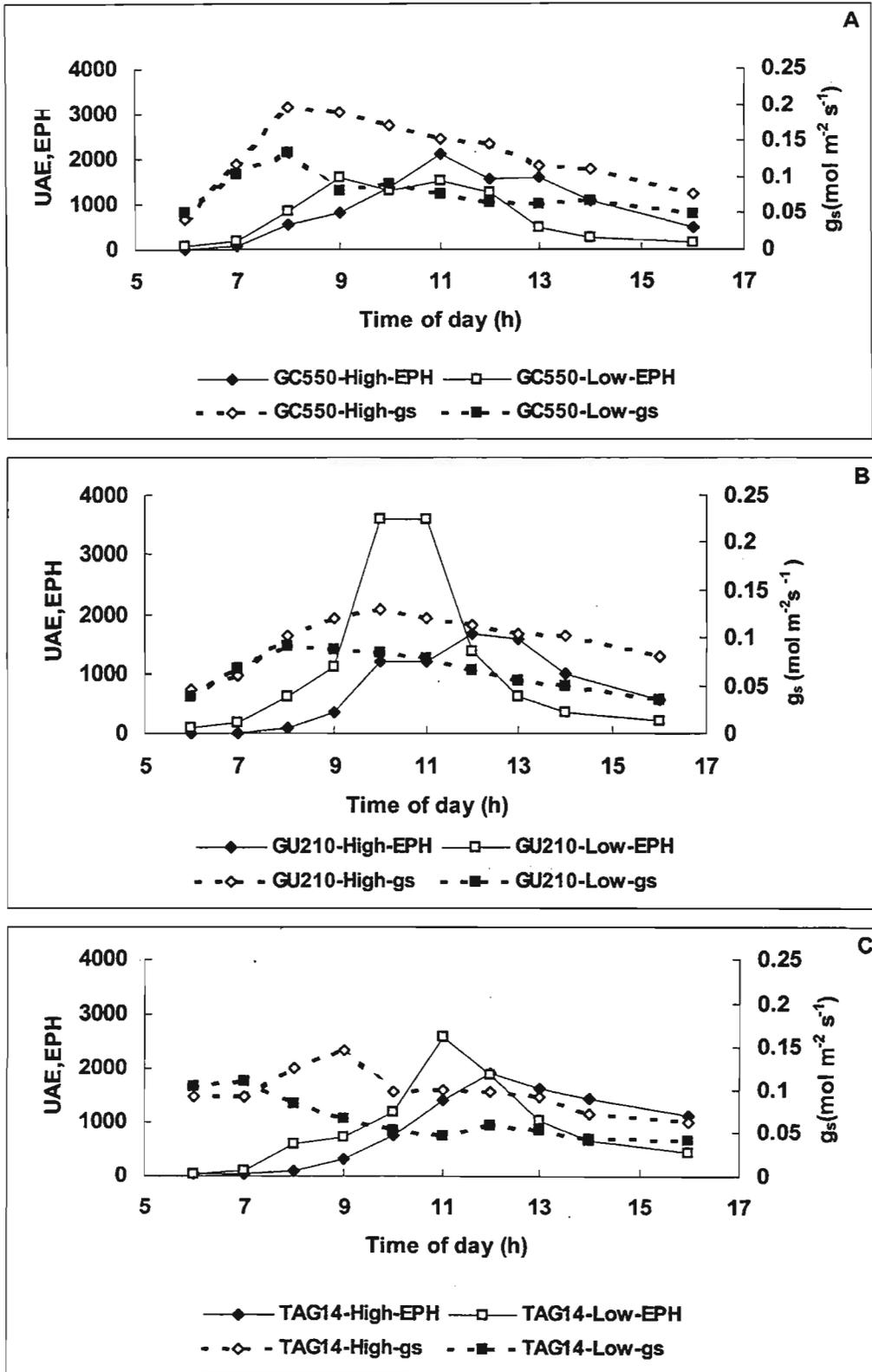


Fig. 5.2.9 Concurrently measured rate of ultrasonic acoustic emissions (events per hour; UAE, EPH) and stomatal conductances of three *Eucalyptus* spp. clones grown for 21 months in 85 l pots, and subjected to high or low watering treatment. Each data point represent the mean (n=6).

cUAE as a percentage of maximum events

Cumulated acoustic emission events expressed as percentages of the maximum are plotted against leaf water potential in Fig. 5.2.10. The critical leaf water potentials triggering xylem cavitation ($\psi_{CAV,cUAE,\%}$) were estimated as the mean water potential of all the data points lying between 5% to 10% of the maximum events (Table 5.2.6). In GU210 and TAG14 water potentials triggering cavitation events were lower in low watered than high watered plants, the reverse being the case for GC550. However, a two-way ANOVA revealed that there were no significant differences of initiation of leaf water potential between treatments ($F=1.02, P=0.331$) as well as among the clones ($F=2.64, P=0.112$). The analytical procedure of two intersecting straight lines (<20% and >40 % cUAE) was used to calculate the less arbitrary indicator of the threshold water potential generating substantial stem xylem cavitation events (see Chapter 3, Fig. 3.2.7), except for TAG14, as the points showed too much scatter (Fig. 5.2.10). The values of the critical thresholds estimated this way were -0.71 MPa and -0.25 MPa for low and high watered GC550, and as -0.92 MPa and -0.96 MPa for high and low watered GU210. Among the two clones, GC550 appeared to more vulnerable than GU210.

Table 5.2.6 Mean critical leaf water potential triggering xylem cavitation ($\psi_{CAV,UAE,\%}$) for three *Eucalyptus* spp. clones grown in 85 l pots for 21 months and subjected to high or low watering treatment. Means are given \pm SEM. Within column, upper case lettering (high water) and lower case lettering (low water) indicate significantly different values at $P<0.05$ separately for treatment. (ANOVA followed by Scheffe's multiple range test).

Clones	Treatments	$\psi_{CAV,cUAE,\%}$ (MPa)
GC550	High	-0.99 \pm 0.00 ^A
GC550	Low	-0.67 \pm 0.19 ^a
GU210	High	-1.02 \pm 0.22 ^A
GU210	Low	-1.70 \pm 0.36 ^a
TAG14	High	-1.40 \pm 0.06 ^A
TAG14	Low	-1.80 \pm 0.60 ^a

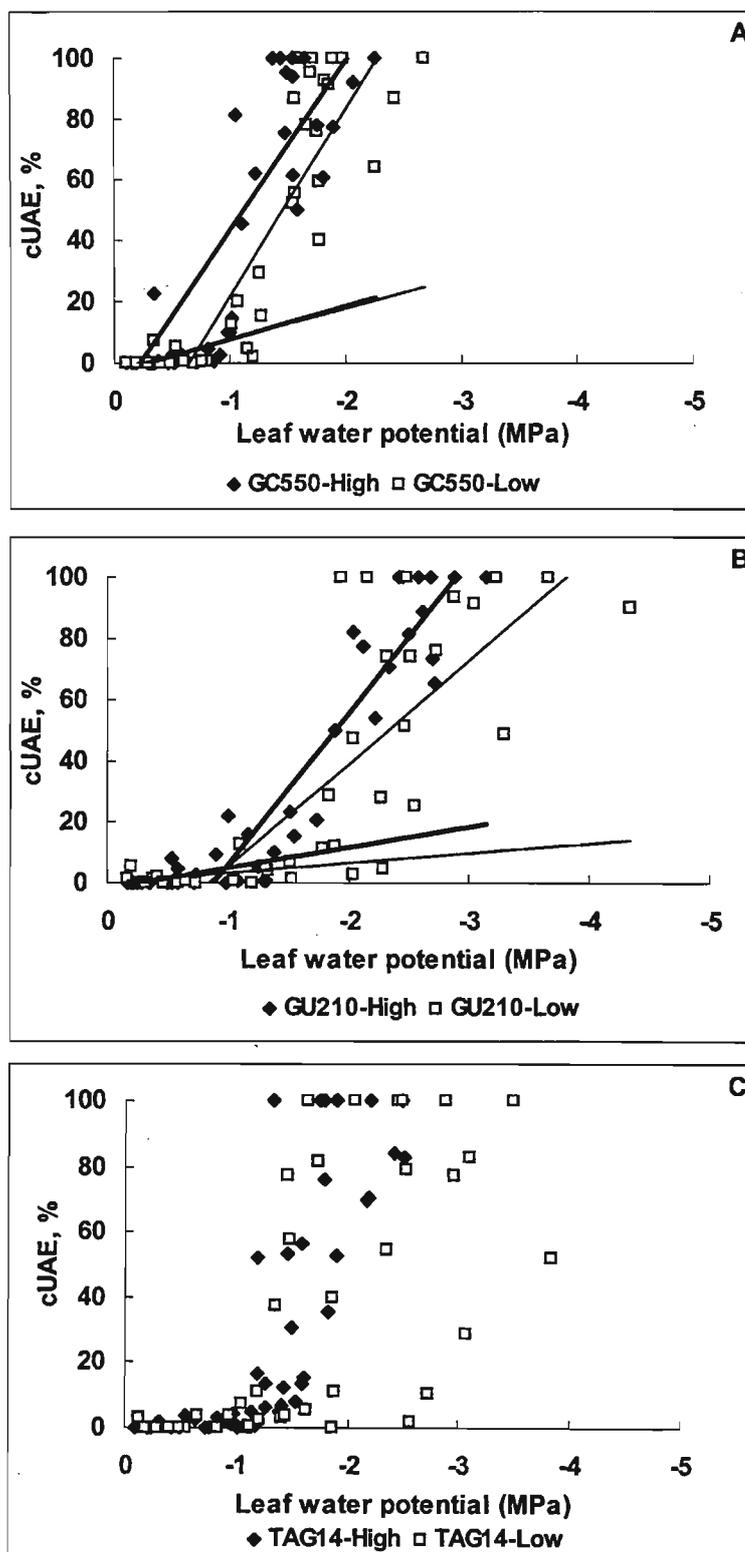


Fig. 5.2.10 Cumulated UAE (cUAE) from the main stem, expressed as percentage of the maximum, plotted against leaf water potential for three *Eucalyptus* spp. clones grown for 21 months in 85 l pots, and subjected to high or low watering treatment. Data represent mean (n=6). See text for the meaning of regression lines.

Largely, the three techniques, except line intersection method, which used only for two clones, for estimating vulnerability to cavitations of the main stem xylem gave similar results. Although two-way ANOVAs indicated no significant effect of watering treatment, in both techniques the critical water potential was lower in low than high watered plants (with the exception of GC550 using cUAEs). This does suggest that plants subjected to the low watering treatment were less vulnerable to cavitation than those receiving high water, and that GC550 was more vulnerable than GU210.

5.2.7 Positive root pressure

Overnight root pressures did not develop in the 21 months old saplings and only very low (1 to 3 kPa) midday root pressures were detected (Fig. 5.2.11). There were no treatment ($F=2.50$, $P=0.124$) or clonal ($F=0.95$, $P=0.397$) effects on the root pressures observed.

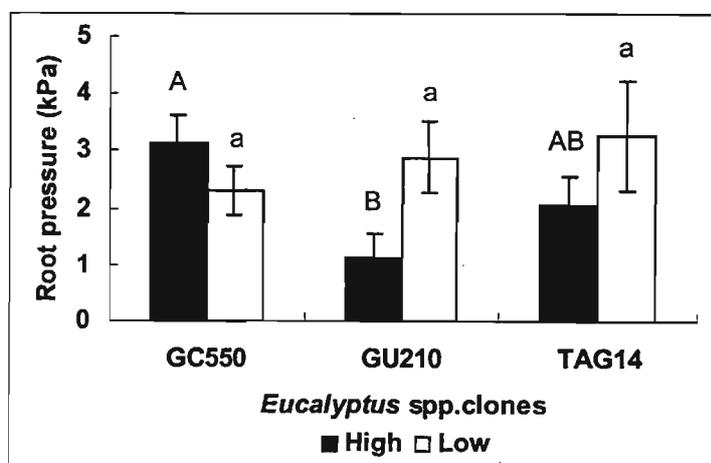


Fig. 5.2.11 Positive xylem root pressure developed by midday in three *Eucalyptus* spp. clones grown for 21 months in 85 l pots, and subjected to high or low watering treatment. Error bars represent the SEM ($n=6$). Letters represent the means separation by Scheffe's multiple range tests, showing comparison within the high (upper case lettering) and low (lower case lettering) watering treatments separately ($P<0.05$).

5.2.8 Root, shoot and whole plant hydraulic conductances

Absolute hydraulic conductances measured by the HPFM are shown in Table 5.2.7. The values of K_r ($F=47.13$, $P=0.000$) and K_s ($F=47.47$, $P=0.000$) were significantly higher in high watered than low watered plants, and there were also significant clonal differences (for K_r ; $F=3.60$, $P=0.041$ and for K_s ; $F=3.70$, $P=0.037$).

Table 5.2.7 Absolute hydraulic conductances (raw data from the HPFM) measured on three *Eucalyptus* spp. clones grown for 21 months in 85 l pots, and subjected to high or low watering treatment. Means are given \pm SEM ($n=6$). Letters represent (under each parameter) the means separation by Scheffe's multiple range tests, showing comparison within the high (upper case lettering) and low (lower case lettering) watering treatments separately ($P<0.05$).

Absolute Hydraulic Conductances ($\text{kg s}^{-1}\text{MPa}^{-1} \times 10^{-5}$)			
Clones	Treatments	K_r	K_s
GC550	High	21.10 \pm 2.55 ^A	56.10 \pm 3.35 ^A
GC550	Low	6.98 \pm 0.27 ^a	35.90 \pm 2.39 ^a
GU210	High	18.50 \pm 2.04 ^A	57.60 \pm 4.65 ^A
GU210	Low	5.52 \pm 0.85 ^a	44.50 \pm 1.27 ^b
TAG14	High	19.80 \pm 1.98 ^A	50.60 \pm 2.99 ^A
TAG14	Low	14.50 \pm 3.36 ^b	36.50 \pm 2.80 ^a

Root hydraulic conductances expressed per unit leaf area ($K_{r/LA}$) are shown in Fig. 5.2.12.A. Two-way ANOVA revealed that $K_{r/LA}$ values did not differ significantly among the clones ($F=2.34$, $P=0.114$), but they did differ significantly between watering treatments ($F=4.38$, $P=0.045$), with higher $K_{r/LA}$ values in high watered GC550 and GU210 plants, with the reverse being true for TAG14.

Shoot hydraulic conductances expressed per unit leaf area ($K_{s/LA}$) are shown in Fig. 5.2.12. B. Values for high watered plants tended to be slightly lower than for the low watered plants, the effect being marginally significant ($F=3.84$, $P=0.059$). Differences among clones were significant ($F=8.94$, $P=0.001$).

Root hydraulic conductances expressed in per unit total root dry mass ($K_{r/trdw}$) are shown in Fig. 5.2.12. C. The patterns shown are similar to those for root conductance

expressed per unit leaf area. Differences between treatments were not significant ($F=0.56$, $P=0.459$), but the clonal effect was ($F=9.42$, $P=0.001$), with low watered TAG14 having higher values than the other clones.

Shoot conductances expressed per unit total shoot dry mass ($K_{S/tsdw}$, $\text{kg s}^{-1}\text{MPa}^{-1} \text{g}^{-1}$) are shown in Fig. 5.2.12. D. Values for the high watered plants were slightly lower than for the low watered plants but the treatment differences were not significant ($F=3.90$, $P=0.058$). There was, however, a significant clonal effect ($F=3.45$, $P=0.045$).

Whole plant hydraulic conductances expressed in per unit leaf area ($K_{P/LA}$) showed similar patterns to root conductances (Fig. 5.2.13). The watering treatment had significant effects ($F=5.82$, $P=0.022$), with low watered plants of GC550 and GU210 having lower whole plant conductances than their high watered counterparts, with the reverse being true for TAG14. The clonal effect was marginally significant ($F=2.91$, $P=0.070$), and there was a significant treatment x clone interaction ($P=0.010$).

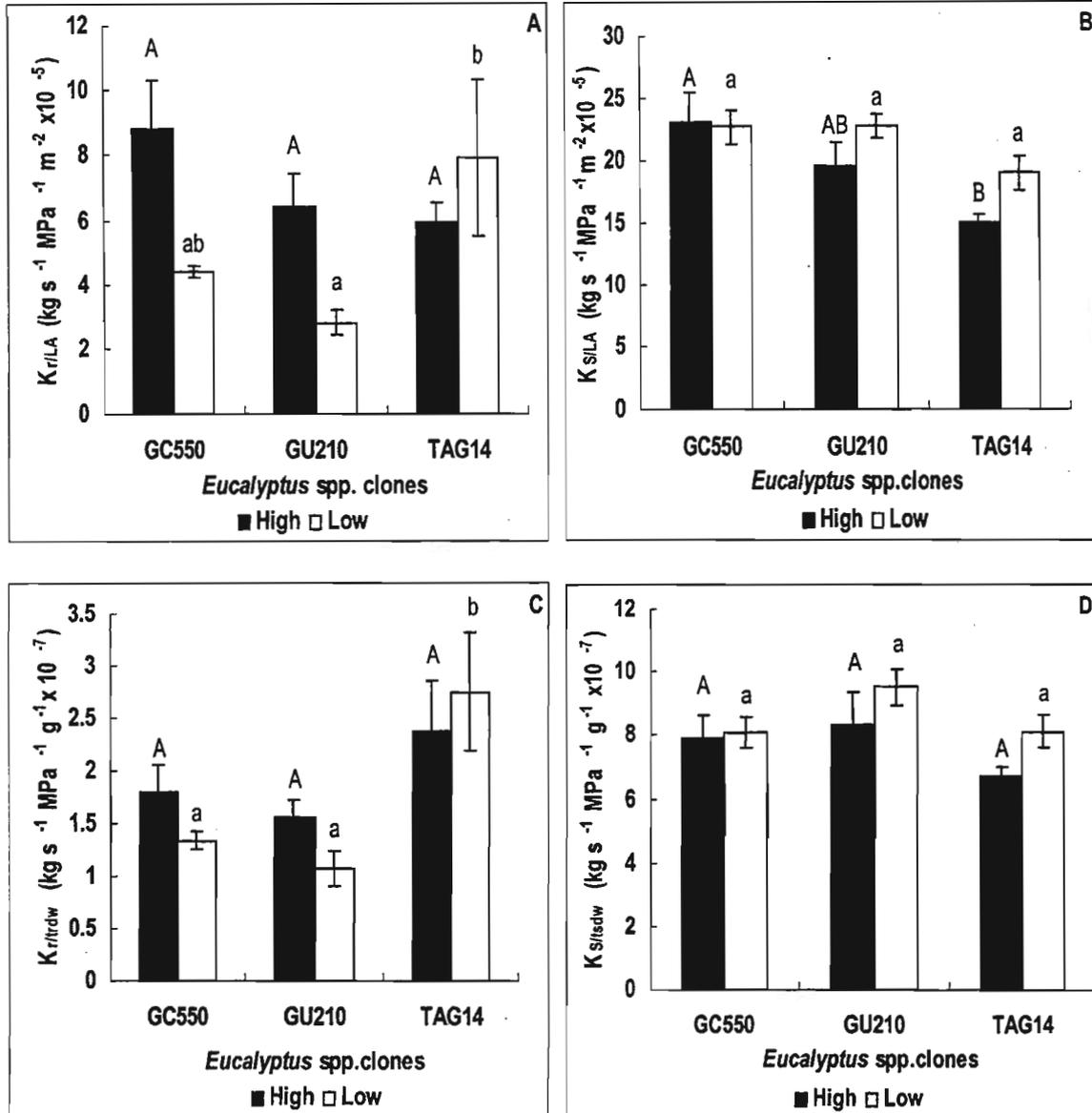


Fig. 5.2.12 Root and shoot hydraulic conductances measured in three *Eucalyptus* spp. clones grown for 21 months in 85 l pots, and subjected to high or low watering treatment. K_r and K_s expressed on a per unit leaf area basis (A, B), K_r expressed per unit total root dry mass (C) and K_s expressed per unit total shoot dry mass (D). Error bars represent the \pm SEM ($n=6$). Letters represent the means separation by Scheffe's multiple range tests, showing comparison within the high (upper case lettering) and low (lower case lettering) watering treatments separately ($P < 0.05$).

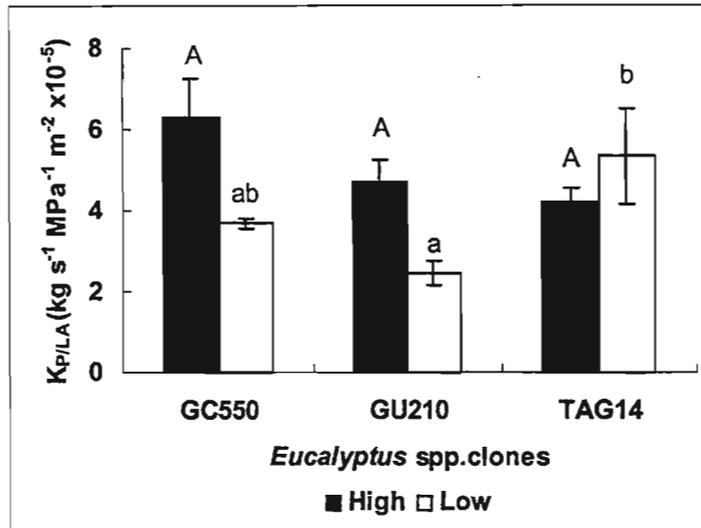


Fig. 5.2.13 Whole plant hydraulic conductances expressed per unit leaf area of three *Eucalyptus* spp. clones grown for 21 months in 85 l pots, and subjected to high or low watering treatment. Error bars represent the \pm SEM (n=6). Letters represent the means separation by Scheffe's multiple range tests, showing comparison within the high (upper case lettering) and low (lower case lettering) watering treatments separately (P<0.05).

5.2.9 Relationships between xylem cavitation, growth parameters and hydraulic architecture

Relationship between shoot hydraulic conductances and the xylem vulnerability

The relationship between stem xylem vulnerability, estimated from both mean of ψ_{CAV} , cUAE, % and mean of ψ_L at EPH_{max} and mean shoot hydraulic conductance per unit leaf area are shown in Fig. 5.2.14. There were no significant relationships between xylem vulnerability estimated from either method ($r^2 = 0.12$, $P = 0.512$, and $r^2 = 0.03$, $P = 0.734$, respectively) when data from both treatments were combined, or when each treatment was analysed separately.

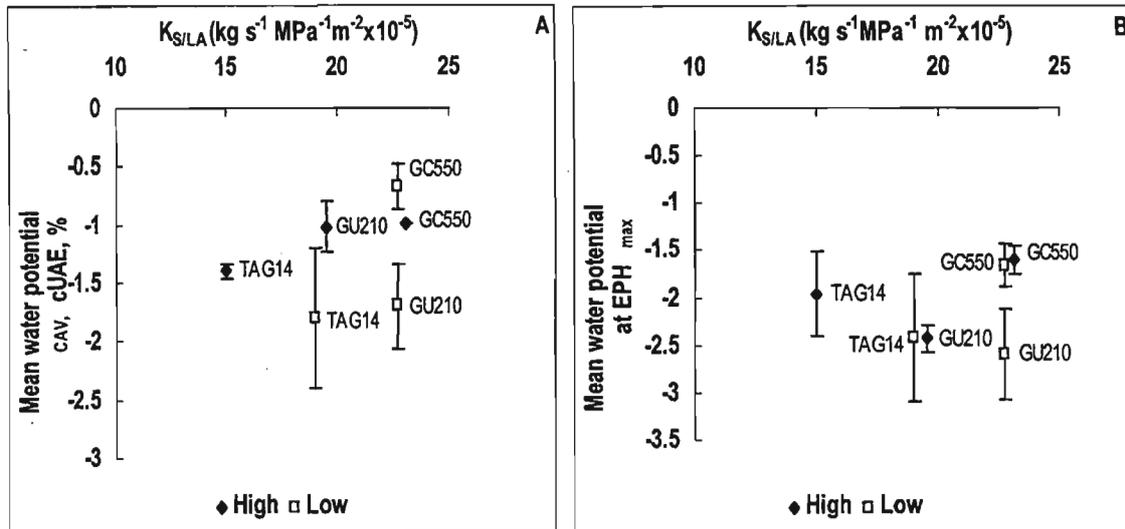


Fig. 5.2.14 Relationships between mean water potential at ψ_{CAV} , cUAE, % (A) or EPH_{max} (B) and mean shoot hydraulic conductances of three *Eucalyptus* spp. clones grown for 21 months in 85 l pots, and subjected to high or low watering treatment. Error bars represent the \pm SEM.

Relationship between hydraulic characteristics and the leaf physiology and growth parameters

The relationship between maximum $K_{P/LA}$ or $R_{Plant*LA}$ and the leaf physiology (all individual data values of minimum leaf water potential, maximum stomatal conductances) are shown in Fig. 5.2.15. This Figure permits assessment of whether there were any relationships between maximum whole plant hydraulic capacity of the clones under two watering treatments, and leaf level physiological characteristics.

A lack of correlation was observed between $R_{Plant*LA}$ and the minimum leaf water potential for high watered (Fig. 5.2.15.A, $r^2 = 0.03$) as well as for low watered clones ($r^2=0.026$). A similar trend appeared when both treatment values were pooled for linear regression analysis ($r^2=0.08$, $P=0.094$).

The relationship between whole plant hydraulic conductances ($K_{P/LA}$) and the maximum stomatal conductances ($g_{s \max}$) showed (Fig. 5.2.15. B) weak relationships separately for high watered ($r^2=0.13$) and low watered plants ($r^2=0.05$). Data pooled for both treatments showed there was a weak relationship as r^2 is 0.14. However, statistical analysis of the regression showed a P value of 0.025, which is significant.

Furthermore, a relationship was established between the $K_{P/LA}$ of *Eucalyptus* spp. clones and the total produced whole plant dry mass for both pooled high and low watering treatments (Fig. 5.2.15. C). A correlation between these two parameters were not totally significant ($r^2=0.10$, $P=0.066$). A reason for the lack of correlation between these two parameters is that two of the data points of TAG14 clone grown under low water treatment and GC550 under high watered showed highest $K_{P/LA}$ with lowest produced biomass. When these data points were removed, the correlation between $K_{P/LA}$ and whole plant dry mass was significant ($r^2=0.36$, $P=0.000$). In another attempt, TAG14 subjected to low water which showed highest hydraulic conductances with lowest dry mass, was removed (total of six data points) with an outlier data point of GC550 of high watered. As a result correlation between $K_{P/LA}$ and the dry mass was further improved for both watering treatments ($r^2=0.49$, $P=0.000$).

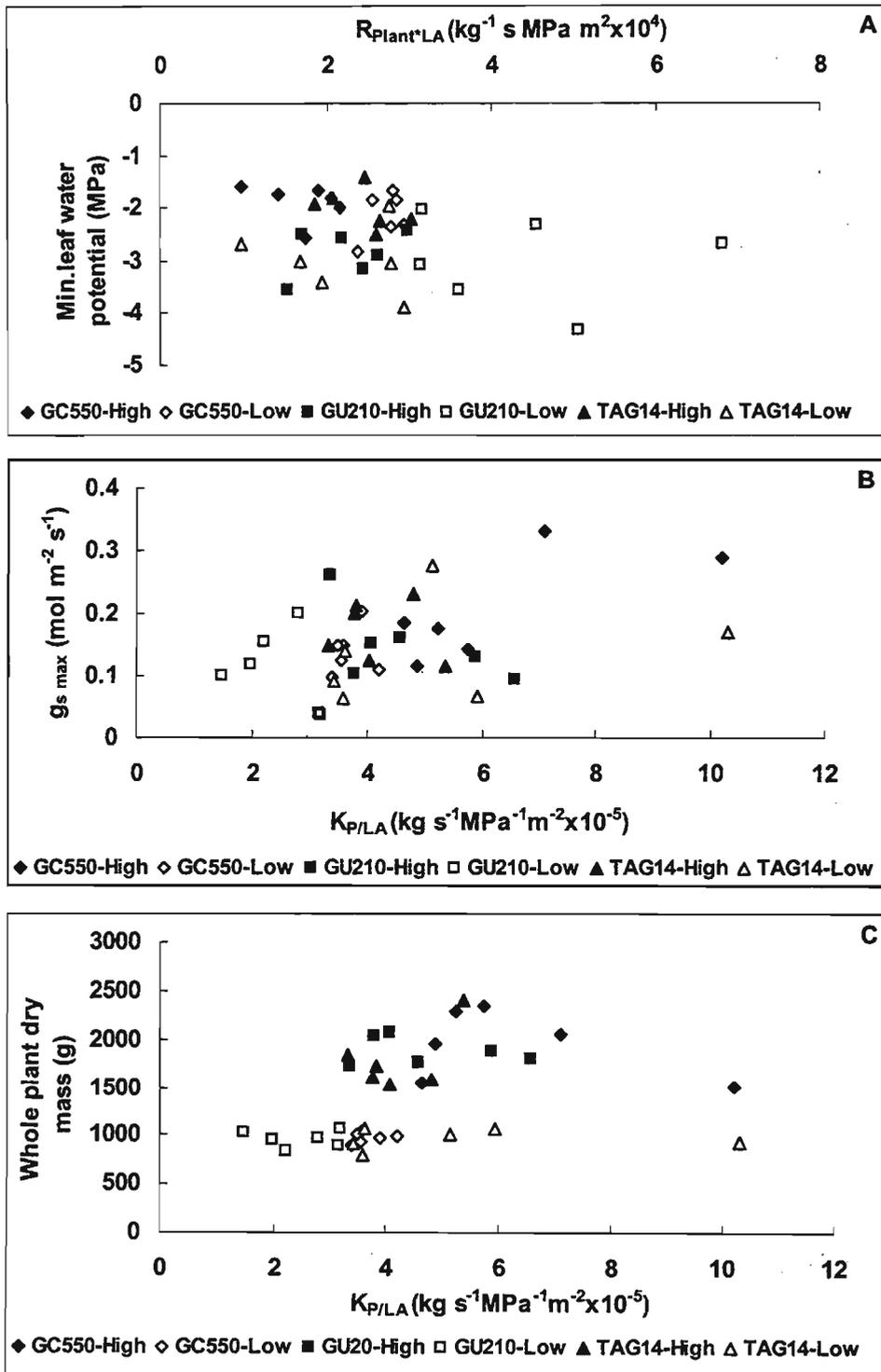


Fig. 5.2.15 The relationship between $R_{Plant*LA}$ and minimum leaf water potential (A), between $K_{P/LA}$ and the maximum stomatal conductance (B) and between $K_{P/LA}$ and the whole plant dry mass (C) obtained from individual plants of three *Eucalyptus* spp. clones grown for 21 months in 85 l pots, and subjected to high or low watering treatment.

5.2.10 The components of whole plant resistances

The contributions of roots (R_{r*LA}) and shoots (R_{s*LA}), and the components of the shoot to whole plant resistances, normalized to leaf area, are given in Table 5.2.8. Watering treatment had a significant effect on whole shoot resistances ($F=8.23$, $P=0.007$), and the clonal effect was also significant ($F=12.95$, $P=0.000$). However, there was no clone by treatment interaction ($F=2.35$, $P=0.113$) (see also Fig. 5.2.12. B). Leaf resistance (R_{L*LA}) was the major component of the shoot resistance, contributing at least half of the total for both watering treatments. High watered plants tended to have higher values of R_L , but the differences were not significant ($F=0.15$, $P=0.705$). Clonal differences, however, were significant ($F=8.48$, $P=0.001$). Resistances of petiole and branches (R_{p+B}) were next major contributors to shoot resistance. The effects of treatment ($F=6.94$, $P=0.013$) and clone ($F=6.94$, $P=0.013$) were both significant with TAG14 having the highest values. The resistance of the main stem (R_{MS}) was the lowest of all the components, and showed a significant increase under high water ($F=6.30$, $P=0.018$), but there was no clonal effect ($F=1.34$, $P=0.277$). In this experiment, the resistance of petioles (R_P) was not measured, as the petioles were too numerous to clip off.

The resistances of the roots (R_r) were considerably higher than those of the shoots. Both watering treatment and clone had a significant effect on R_r ($F=18.21$, $P=0.000$, and $F=9.02$, $P=0.001$, respectively) (see also Fig. 5.2.12. A). Reduced water availability increased root resistances, except in TAG14 where treatment had no effect. GU210 had the highest root resistances under both treatments. Whole plant resistance (R_{Plant}) was significantly affected by both treatment ($F=14.94$, $P=0.001$) and clone ($F=8.02$, $P=0.002$) (see also Fig. 5.2.13), with low watering increasing resistance, except for TAG14, and GU210 having the highest resistance.

Table 5.2.8 Contributions of individual plant components to the whole plant resistance in three *Eucalyptus* spp. clones grown for 21 months in 85 l pots, and subjected to high or low watering treatment. Means are given \pm SEM (n = 6). Within a column, letters represent the means separation by Scheffe's multiple range tests, showing comparison within the high (upper case lettering) and low (lower case lettering) watering treatments separately (P<0.05).

Hydraulic resistances expressed per unit leaf area ($\text{kg}^{-1} \text{ s MPa m}^2 \times 10^3$)							
Clones	Treatments	R_L	R_{P+B}	R_{MS}	R_S	R_r	R_{Plant}
GC550	High	2.18 \pm 0.17 ^A	1.47 \pm 0.12 ^A	0.84 \pm 0.10 ^A	4.49 \pm 0.36 ^A	12.60 \pm 1.74 ^A	17.10 \pm 1.98 ^A
GC550	Low	2.53 \pm 0.33 ^a	1.34 \pm 0.06 ^a	0.62 \pm 0.04 ^a	4.49 \pm 0.33 ^a	22.80 \pm 0.83 ^a	27.30 \pm 0.945 ^a
GU210	High	2.33 \pm 0.27 ^A	1.99 \pm 0.42 ^A	0.98 \pm 0.23 ^A	5.31 \pm 0.48 ^A	17.20 \pm 2.30 ^A	22.50 \pm 2.52 ^{AB}
GU210	Low	2.13 \pm 0.14 ^a	1.39 \pm 0.02 ^a	0.92 \pm 0.04 ^b	4.40 \pm 0.17 ^a	39.50 \pm 6.39 ^b	43.90 \pm 6.33 ^b
TAG14	High	3.29 \pm 0.23 ^B	2.21 \pm 0.10 ^A	1.23 \pm 0.29 ^A	6.73 \pm 0.29 ^B	17.80 \pm 1.81 ^A	24.50 \pm 1.87 ^B
TAG14	Low	2.93 \pm 0.32 ^a	1.83 \pm 0.10 ^b	0.61 \pm 0.03 ^a	5.36 \pm 0.38 ^a	16.50 \pm 3.25 ^a	21.80 \pm 3.48 ^a

The percentage contribution of the different components of the water conducting pathway are given in Table 5.2.9. For all clones and both treatments the major resistance to water flow was located in the roots, ranging from 72% in high watered TAG14 to 89% in low watered GU210. In plants subjected to the low watering treatment roots contributed a higher proportion of the total resistance than in their high watered counterparts, except for TAG14, where treatment had no effect on proportional contribution. The contribution of roots to total resistance tended to be higher in GU210 than the other clones.

Table 5.2.9 Percentage contributions of individual plant components to the whole plant resistances in three *Eucalyptus* spp. clones grown for 21 months in 85 l pots, and subjected to high or low watering treatment. Means are given \pm SEM (n = 6).

Hydraulic resistances (%) expressed per unit leaf area						
Clones	Treatments	R_L	R_{P+B}	R_{MS}	R_S	R_r
GC550	High	13.20 \pm 1.22	8.88 \pm 0.72	5.05 \pm 0.57	27.10 \pm 2.35	72.90 \pm 2.35
GC550	Low	9.26 \pm 1.02	4.92 \pm 0.20	2.29 \pm 0.20	16.50 \pm 1.00	83.50 \pm 1.00
GU210	High	10.50 \pm 1.10	8.91 \pm 1.46	5.06 \pm 1.72	24.50 \pm 3.06	75.50 \pm 3.06
GU210	Low	5.29 \pm 0.84	3.44 \pm 0.48	2.29 \pm 3.48	11.00 \pm 1.64	89.00 \pm 1.64
TAG14	High	13.8 \pm 1.57	9.20 \pm 0.58	5.01 \pm 1.05	28.00 \pm 1.99	72.00 \pm 1.99
TAG14	Low	14.4 \pm 1.76	9.72 \pm 2.19	3.15 \pm 0.59	27.30 \pm 4.25	72.70 \pm 4.25

Chapter: 5.3 Discussion

As much of the literature pertinent to this study has been discussed in Chapter 4 (the 14 month study), to avoid too much repetition, this discussion is somewhat curtailed.

5.3.1 Effect of watering treatment on total biomass production and allocation

As was the case with the 14 month studies (Chapter 4), reduced water availability reduced biomass production by the three *Eucalyptus* spp. clones (Tables 5.2.1 and 5.2.2). Growth reductions are a common response to water limitation, as was discussed in Chapter 4. Perhaps the most surprising result was that there was a greater effect on roots than shoots, such that low watering reduced root: shoot ratios. This is in contrast to many reports in the literature, including studies on *Eucalyptus* species and clones (e.g. *E. microtheca*, Li, 2000; Li *et al.*, 2000a). It has been suggested that drought affects the growth of the shoot more than it does photosynthesis, so that the amount of assimilate available for root growth is thereby increased (Passioura, 1981). The advantages of an increase in the root: shoot ratio of a plant during drought to match water supply to evaporative demand are almost self-evident, and it is difficult to understand why the reverse occurred with the three clones in this study. Possibly, it could be a consequence of the extensive selection for high stem yield during the breeding of these clones. A similar trend in root: shoot ratios was found in the 14 month study, although the effect was not significant.

Most measures of growth were adversely affected by reduced watering, as was the case with the 14 month study, and as with that study, there were no clonal effects on the response. There were clonal differences in leaf area, with TAG14 having a greater area than GC550 under high water conditions, but the clones all responded similarly to watering treatment. Specific leaf area also showed a clonal effect, being lower for GC550 than the other clones, but this characteristic was unaffected by watering treatment. It has been suggested that leaves with low SLA have higher leaf water potentials and greater drought tolerance than leaves of high SLA (Cavalier and Goldstein, 1989). Midday leaf water potentials of GC550 were higher than those of

the other clones (Table 5.2.4), but this was not translated into greater 'drought tolerance' measured in terms of physiological characteristics, such as stomatal conductance and photosynthesis, or biomass accumulation. Although there are reports of water stress decreasing SLA in *Eucalyptus* species and clones (Li *et al.*, 2000a; Pita and Pardos, 2001), this effect was not apparent in the present study, and Li (2000) reported an actual increase in SLA with water stress.

5.3.2 *Leaf physiology*

As reported for many other plants, including some *Eucalyptus* spp. (Stoneman *et al.*, 1994; February *et al.*, 1995; Ray and Sinclair, 1998; Pita and Pardos, 2001; Li *et al.*, 2000a; Li, 2000), the low water treatment decreased leaf water potential, stomatal conductance, transpiration rates, and net CO₂ assimilation rates (Figs 5.2.4 and 5.2.5), leading to reduced growth. Values of g_s and A recorded for these plants were somewhat lower than those recorded in the field study (Chapter 3) and for other commercial *Eucalyptus* spp. clones growing under field conditions (Pereira *et al.*, 1986; Dye and Olbrich, 1993; Mielke *et al.*, 2000; Clearwater and Meinzer, 2001). Additionally, maximum values were achieved earlier in the day (07.00 h to 10.00 h) than reported for field grown material (Chapter 3 and Mielke *et al.*, 1999). Such differences could be a pot effect (Ray and Sinclair, 1998), or due to a water stress, as *Eucalyptus* spp. will access deep water tables (Morris *et al.*, 1998; Mielke *et al.*, 1999; Burgess *et al.*, 2001). The only clonal effect in the present study was on midday leaf water potentials, where GC550 operated at higher values than GU210 under high water treatment (Table 5.2.4), commensurate with the lower SLA of GC550. Similarly, Farrel *et al.* (1996) showed no differences among clone in leaf physiological responses to water stress, although different populations of *E. microtheca* differed in A, E and leaf level WUE under two watering regimes (Li, 2000).

Partial stomatal closure in response to water stress frequently leads to greater decrease in E than A, giving rise to a higher instantaneous leaf level WUE (A/E). Such a response in *Eucalyptus* species and clones has been demonstrated by February *et al.*

(1995), Li (2000) and Li *et al.* (2000a). This increase in WUE with partial stomatal closure is because of the non-linear response of A to g_s . However, in the present study the relation between A and g_s was linear, with no differences in slope among clones or between treatments (Fig. 5.2.6). Consequently, instantaneous leaf level WUE did not vary with treatment (Fig. 5.2.6).

5.3.3 Root and shoot hydraulic conductances

Shoot hydraulic conductances

Shoot hydraulic conductances expressed either per unit leaf area or per unit shoot dry mass were not affected by watering treatment, but interclonal differences were apparent. (Figs 5.2.12.B and D). The lack of watering effect suggests that reduction in leaf area to maintain constant hydraulic capacity was a primary adjustment to low watering conditions. This is in contrast to the 14 month study (Chapter 4) where, surprisingly, low watered plants had higher values of $K_{S/LA}$. Both decreases (Vander Willigen and Pammenter, 1998; Lovisolo and Schuber, 1998; Sparks and Black, 1999) and increases (Schumway *et al.*, 1991) in leaf specific conductivity in response to water stress have been reported. However, many of those measurements were made on excised branches or stems using low pressure water flow methods, and would not have included the resistance of the leaf laminae, which constitute a high proportion of shoot resistance. The interclonal differences observed in the present study were not apparent in some closely related *Eucalyptus* spp. clones (Vander Willigen and Pammenter, 1998). However, consistent with this interclonal effect were the observed differences in shoot hydraulic conductances among three mangrove species (Sobrado *et al.*, 2000), differences between some *Quercus* species (Nardini and Tyree, 1999) and the higher shoot conductances of pioneer species relative to shade tolerant forest species (Tyree *et al.*, 1998).

Root hydraulic conductances

Root hydraulic conductances were considerably lower than shoot conductances, and so would have a more marked effect on the ability of the plant to conduct water from the soil-root interface to the leaves. Root hydraulic conductance expressed per unit root mass is a measure of the efficiency of the roots, the investment of carbon required to achieve a certain conductance. Root conductance normalised to leaf area is a measure of the capacity of the roots to supply water to the leaves.

There were interesting clonal differences in root hydraulic conductance per unit root mass, low watered TAG14 having higher values, indicating more efficient roots (Fig. 5.2.12. C). When the data were expressed per unit leaf area, high watering resulted in higher root conductances in GC550 and GU210, with the reverse occurring in TAG14 (Fig. 5.2.12. A). This pattern of TAG14 showing higher conductances under low watering was apparent in data expressed per unit root mass and the effect was significant. Reductions in root hydraulic conductivity in response to water stress have been reported in two cacti (Huang and Nobel, 1993), some other xerophytic species (North and Nobel, 1992), *Olea oleaster* (Lo gullo *et al.*, 1998), and *Quercus cerris* (Nardini *et al.*, 1999). Additionally, some drought adapted *Quercus* have lower root conductances than mesophilous species (Nardini and Tyree, 1999). However, summer droughts appeared to increase, rather than decrease, root conductances of *Quercus subre*, but not other *Quercus* species (Nardini *et al.*, 1999; Nardini and Pitt, 1999).

Whole plant conductances

As the roots constitute the major hydraulic resistance (between 72% and 89%), whole plant conductances, expressed per unit leaf area, largely reflected those of the roots (Fig. 5.2.13). The proportional contribution of roots to whole plant resistance in the three *Eucalyptus* spp. clones in this study is somewhat higher than normal (Tyree *et al.*, 1998; Becker *et al.*, 1999; Nardini and Tyree, 1999). Treatment effects differed among clones, with low water decreasing $K_{P/LA}$ in GC550 and GU210, but increasing

it in TAG14. Absolute shoot hydraulic conductances were reduced by similar proportions to the reduction in leaf area and root weight, such that when shoot conductances were normalised the differences between treatments was small. However, the treatment effects on absolute root conductances were not similar to the changes in leaf area and root mass, such that when root conductances were normalised, treatment effects were apparent. Root conductances were lower than shoot conductances, so the effect of treatment on root conductances was carried through to whole plant conductances. The relationship between transpiration rate and leaf water potential (Fig. 5.2.7) can be taken as a measure of whole plant conductance (evaporative flux, EF, method). There was agreement between the EF method and HPFM method for GC550 and GU210, but the EF method indicated a decrease in whole plant conductance with low water in TAG14, rather than the increase measured with the HPFM. This could be because the HPFM measures maximum conductance values, and does not account for the soil-root interface resistance. It is possible that *in vivo*, this (together with any xylem embolisms) constituted a large resistance, giving rise to the observed response of water potential to transpiration. In support of this is the observation that whole plant conductance measured by the HPFM is reduced by about half in GC550 and GU210, but by about 80% when assessed from the EF method.

5.3.4 Xylem vulnerability to cavitation

Vulnerability of the main stem xylem to cavitation was assessed by three methods; water potential corresponding to the maximum rate of acoustic emissions ($\psi_{L,EPH_{max}}$, Table 5.2.5), the water potential corresponding to the initiation of extensive cavitations (assessed as the mean water potential of data points lying between 5% and 10% of total accumulated acoustic emissions; $\psi_{CAV,cUAE,\%}$, Table 5.2.6), and as the point of intersection of lines describing the relationship between water potential and $cUAE,\%$ fitted to high and low values of $cUAE,\%$ (this technique could not be used for TAG14 because of the wide scatter of points for the low water treatment; Fig. 5.2.10). All the techniques indicated higher vulnerability of GC550, although only for $\psi_{L,EPH_{max}}$ was the effect significant. Watering treatment had no effect on

vulnerability. The study of 14 month old material showed a similar effect of GC550 being more vulnerable, although that study also indicated that low watering reduced the vulnerability of both GU210 and TAG14. Although there is no doubt that vulnerability to cavitation does vary among genotypes (e.g. Sperry and Tyree, 1990; Tausend *et al.*, 2000; Vander Willigen *et al.*, 2000), there are conflicting data concerning the influence of water stress on the vulnerability of the same genotype. Jackson *et al.* (1995b) and Vander Willigen and Pammenter (1998) have shown no differences in the vulnerability of the same genotypes grown on mesic or xeric sites, whereas Sperry and Saliendra (1994), Alder *et al.* (1996), Franks *et al.* (1995), Kavanagh *et al.* (1999) and Sparks and Black (1999) showed an effect of growing conditions on vulnerability.

Water released by cavitation events is available for transpiration, and so a high vulnerability to cavitation may indicate drought tolerance, permitting survival of long dry season, rather than drought sensitivity, particularly if the capacitance is large. Trees with greater storage capacity maintained maximum rates of transpiration for a substantially longer fraction of the day than trees with smaller water storage capacity (Goldstein *et al.*, 1998). This factor of high vulnerability creating a substantial capacitance may not have been significant in the small potted plants of the present study, but may be of importance in larger field grown trees. GC550 was the most vulnerable of the three clones studied, and is considered by field foresters to be the most drought tolerant.

There has been some interest in the concept of efficiency versus safety. In this study there was no relationship between efficiency, measured as $K_{S/LA}$, and vulnerability, assessed as either $\psi_{CAV,CUAE,\%}$ or $\psi_{L,EPH_{max}}$ (Fig. 5.2.14).

Xylem recovery

Positive root pressure is believed to be an important mechanism of refilling of xylem embolisms in short plants. The development of positive xylem pressure and associated xylem recovery has been documented by Sperry *et al.* (1987), Sperry *et al.* (1988a),

Sperry (1993) and Lopez-Portillo *et al.* (2000). In the present study there was no development of positive overnight root pressures, and the pressures observed at midday were inadequate to refill the xylem (Fig. 5.2.11). Jaquish and Ewers (2001) similarly found a lack of root pressure in two tree species under freezing temperatures. Additionally, positive root pressures are totally absent from conifers (Devlin, 1966).

Xylem cavitation is a transient process and occurs on daily basis (Salleo *et al.*, 2000). Presumably near-atmospheric pressures must develop to permit the embolisms to dissolve. There are several reports of xylem recovery in the absence of any positive root pressure, and embolism repair concurrently with high transpiration has been reported (Canny *et al.*, 1997; McCully *et al.*, 1998; McCully, 1999 and Zwieniecki *et al.*, 2000).

5.3.4 Relationship between hydraulic characteristics and leaf physiology and growth

Assessment of the influence of xylem vulnerability on leaf physiology is complicated by the fact that xylem cavitations were measured on the main stem, whereas xylem vulnerability is known to differ among plant parts (Sperry and Saliendra, 1994; Alder *et al.*, 1996; Hacke and Sauter, 1996; Sperry and Ikeda, 1997; Kavanagh *et al.*, 1999). This is of particular concern in the present study because of the considerable contribution of roots to total hydraulic resistance; cavitations in stems may have little influence on whole plant conductance. Interestingly, there is an almost 1:1 relationship between vulnerability, assessed as $\psi_{L,EPH_{max}}$ (Table 5.2.5), and the mean midday water potential developed (Table 5.2.4), when data from all clones and both treatments are combined. The average of the difference between midday leaf water potential and $\psi_{L,EPH_{max}}$ is 0.1MPa, and the slope of the relationship between the two variables is $0.89(r^2)$ and $P=0.004$. This does suggest that minimum leaf water potentials developed are limited by the vulnerability of the main stem to cavitation. The relationship is not as good if vulnerability is assessed as $\psi_{CAV,cUAE,\%}$ (Table

5.2.6), with midday leaf water potentials on average being 0.95 MPa lower than the water potentials considered to initiate cavitations. However, the linear relationship is not significant (r^2 is 0.46 and $P=0.139$), and so there are indications that minimum leaf water potential is limited by vulnerability. This implies stomatal control to reduce transpiration rates to prevent extensive cavitation occurring. However, in contrast to this, Vander Willigen and Pammenter (1998) showed that midday leaf water potentials dropped below water potentials corresponding to PLC_{50} in branches of some closely related *Eucalyptus* spp. clones

A large body of evidence exists that decreasing hydraulic conductances by various manipulations causes stomata to close (Sperry and Pockman, 1993; Saliendra *et al.*, 1995; Mencuccini and Grace, 1996; Schafer *et al.*, 2000; Hubbard *et al.*, 2001). (As an aside it should be noted that any relationships between leaf physiology and stem conductances could be misleading as whole plant conductance could be limited more by non-vascular conductances of roots and leaves than by the vascular conductances of stems; Zotz *et al.*, 1998). Under normal conditions, soil-to-needle hydraulic conductances and stomatal conductances have shown to decrease with increasing soil water stress in *Pinus sylvestris* L. (Irvine *et al.*, 1998) and two loblolly pine from seed, from two populations, but grown together (Wakamiya-Noborio *et al.*, 1999). In *Quercus petraea*, whole tree specific hydraulic conductance (calculated from slope of a linear regression between leaf water potential and sap flow density) decreased in response to the development of drought, but maintained the leaf water potential above the cavitation threshold (Cochard *et al.*, 1996). Stomatal conductances of *Betula occidentalis* decreased in response to decreased soil-plant hydraulic conductances (Saliendra *et al.*, 1995). Such closure of stomata were adequate to prevent the development of substantial xylem embolism (Cochard *et al.*, 1996; Irvine *et al.*, 1998). In the present study on three *Eucalyptus* spp. clones, the relationships between hydraulic characteristics and leaf physiological characteristics were weak (Fig. 5.2.15). There was no relationship between whole plant hydraulic resistance and the minimum leaf water potential developed, although this lack of relationship perhaps could be expected if stomata are responding to prevent massive cavitation events.

There was, however, a weak but significant relationship between whole plant hydraulic conductance and maximum stomatal conductance, consistent with the concept of some hydraulic limitation. When the entire data set was included, there was no relationship between whole plant conductance and plant growth (biomass). However, if two outlier points are removed, the relationship becomes significant.

A factor possibly contributing to the weak relationships between hydraulic and leaf physiological characteristics is the anomalous increase in hydraulic conductivity of TAG14 under low water treatment (an anomaly not observed when the evaporative flux method was used to estimate whole plant conductance). The leaf physiological characteristics of TAG14 responded to low water in the same manner as the other clones. However, if the low watered TAG14 was removed from the data set no real improvement in the relationships were observed.

Chapter: 6 Manipulation of Root Hydraulic Conductance by Chilling

Chapter: 6.1 Introduction

A fundamental question posed in this thesis is whether low hydraulic conductances can lead to reduced stomatal conductances and hence reduced carbon assimilation and growth. There are reports of relationships between conductance of various components of the hydraulic pathway and growth. Vander Willigen and Pammenter (1998) reported a positive relationship between growth of *Eucalyptus* spp. clones and their hydraulic characteristics, and in other studies of Lovisolo and Schubert (1998) and Schubert *et al.* (1999), reductions in vegetative growth of *Vitis vinifera* L were associated with lower shoot hydraulic conductivity. Although these correlations may be persuasive, direct experimental evidence of a causative relationship between hydraulic conductance and growth is lacking. A number of approaches have been used to demonstrate that changes in hydraulic conductance can effect leaf level physiology. Sperry *et al.* (1993), and Saliendra *et al.* (1995) demonstrated that decreasing conductance by stem notching caused lower leaf level physiology in *Betula occidentalis*. Also, stem hydraulic conductivity has been systematically reduced using an air- injection method in *Betula occidentalis* (Sperry and Pockman, 1993) and in Ponderosa pine seedlings (Hubbard *et al.*, 2001), to assess the leaf physiology. A problem with this approach, particularly with respect to the *Eucalyptus* spp. clones used in this study, is that the major resistances to water flow are located in the roots and manipulation of branch or main stem resistances will have little effect on total soil-to-leaf resistance. Additionally, either the studies have been short-term, and the long-term effects of such manipulations have not been observed (Sperry *et al.*, 1993; Sperry and Pockman, 1993; Saliendra *et al.*, 1995; Hubbard *et al.*, 1999; Hubbard *et al.*, 2001), or re-establishment of conductances after the manipulations occurred (Saliendra *et al.*, 1995; Hubbard *et al.*, 2001). A non-invasive technique to manipulate resistances is to chill the roots, and this has been demonstrated to increase root resistances and stomatal conductances in on angiosperm (*Eucalyptus regnans*) and conifers (Brodribb and Hill, 2000), in *Quercus robur* L. (Cochard *et al.*, 2000), in *Phaseolus vulgaris* L. (Vernieri *et al.*, 2001) and in *Spinacia oleracea* L. (Fennell and

Markhart, 1998). However, these experiments have also been conducted over the short-term only, and data on long-term effects are lacking.

This Chapter describes two studies undertaken on the effects of chilling of the roots (achieved by chilling the soil) of three month old potted saplings of the clones GU210 and TAG14. The first study was short-term; roots were chilled over-night and the physiological effect of chilling was assessed the following morning. Having established that root chilling did affect hydraulic conductance, the experiment was repeated, but chilling was imposed for three months. This was to assess whether there were long-term adaptations to the chilling treatment, and whether chilling induced reductions in hydraulic conductance were associated with reductions in growth. The specific questions asked were: does root chilling reduce root hydraulic conductivity? If so, is this reduction associated with either, or both, reduced leaf water potentials or transpiration rates? Are there reductions in stomatal conductance associated with reduced transpiration? If stomatal conductance is reduced, is this associated with a reduction in carbon dioxide assimilation? And, finally, do reductions in carbon assimilation lead to reductions in biomass accumulation?

Chapter: 6.2 Materials and Methods

6.2.1 Plant growth and soil chilling

For the short-term preliminary experiment the *Eucalyptus* spp. plants of each of the clones GU210 and TAG14 were grown in 15 l black plastic potting bags (height 0.3m, diameter 0.9m) in the open. Pots were watered twice weekly to field capacity. In addition, soil-foliar nutrients and fungicides were used as described in Chapters 4 and 5. At that time of study, the plants were 18 months old with the heights of 1.25m, and stem over-bark diameter at 0.1m above soil surface was 17.5mm. Short-term root chilling was applied to four replicate plants of each clone and physiological measurements were made after overnight chilling and on four unchilled control plants of each clone.

For the long-term studies macro-cuttings of each of the clones were planted in 3 l black plastic potting bags and grown in the open for six weeks. Six replicates of each clone and treatment (chilled and non-chilled) were arranged randomly on a bench in the open, and to acclimate the treatment plants, soil temperature was gradually reduced to 15°C over a period of ten days. Plants were grown under these conditions for a further 60 days, after which physiological characteristics were measured, and the plants harvested to collect growth data. Throughout the experiment plants were watered four times weekly to field capacity.

Soil chilling was achieved by winding copper tubing (OD 9.5 mm, ID 8 mm) around the pots to make an effectively continuous water jacket, and water from a cooling water bath was passed through the tubing. Insulating material (bubble wrap) was wrapped outside the copper tubing. The temperature of the bath was set so as to achieve a soil temperature of 15°C during the day. Temperatures were regularly monitored with thermocouples inserted in the soil, and in the chilling treatment varied between 15°C during the day and 10°C at night (plants were in the open and so soil temperatures fluctuated with diurnal changes in air temperature), and in the controls ranged from a minimum measured value of 17°C on a cool night to a maximum value of 34°C during a warm day, although temperatures were generally in the range of 20 - 25°C. The chilling temperatures chosen were based on preliminary observations that short-term exposure to a temperature of 2°C and longer-term exposure to temperatures below 10°C caused irreversible root damage.

6.2.2 Physiological and growth measurements

In the short-term experiment soil was chilled overnight and leaf gas exchange and water potential (pressure chamber) were measured at two-hourly intervals from 06.30 h to 13.30 h (with the chilling treatment still being applied). After this the stems were excised above ground level and root and shoot hydraulic conductances measured with the HPFM. Total leaf area and root mass were measured to normalize hydraulic data.

During the long-term experiment plant height and stem over-bark stem diameter were measured every ten to fifteen days from the onset of root chilling till the end of experiment. After the treatment had been applied for 60 days leaf water potential and the leaf gaseous exchange parameters were measured from 06.00 h to 12.00 h., at one hour intervals from a single leaf per plant using a thermocouple psychrometer and the infrared gas analyzer, as described for other potted plants. After measuring these physiological parameters, plants were cut above soil level and hydraulic resistances of the root and shoot, and the components of the shoot, were measured using the HPFM. The plant material was then collected and dried for growth determination.

Chapter: 6.3 Results: Short term study

6.3.1 Predawn and diurnal leaf water potentials

Soil water potential (ψ_s), assessed from the predawn leaf water potential (ψ_{PDL}), showed (Table 6.3.1) that the soil chilling treatment significantly decreased ψ_s compared with that of the control plants in both clones ($F=6.86$, $P= 0.022$). However, the changes were both in the opposite direction to, and greater than, that expected purely from a change in temperature (a decrease in temperature should increase water potential; from $\psi = RT \ln a_w$, where R is the gas constant, T absolute temperature and a_w the activity of water; as $a_w < 1$, $\ln a_w$ will be negative). This suggests that predawn leaf water potentials were not a good estimate of soil water potential. The observed effect was possible a consequence of incomplete overnight equilibration between plant and soil water in the chilled systems. There were no significant differences in ψ_{PD} between the clones ($F=0.87$, $P=0.370$).

Table 6.3.1 Mean predawn leaf water potential of two *Eucalyptus* spp. clones subjected to root chilling. Means are given \pm SEM (n=4). Different upper case (control) and lower case (chill) letters indicate significantly different values at $P<0.05$ separately for treatment.

Clones	Treatments	Predawn ψ_L (MPa)
GU210	Control	-0.77 \pm 0.11 ^A
GU210	Chill	-0.97 \pm 0.08 ^a
TAG14	Control	-0.70 \pm 0.90 ^A
TAG14	Chill	-0.91 \pm 0.08 ^a

As the morning progressed ψ_L in GU210 declined gradually, with slight recovery after midday; values for plants with chilled roots were slightly but consistently lower than those of the controls (Fig. 6.3.1). In TAG14 there was little decline in leaf water potential after about 08.00 h, with no consistent differences between treatments.

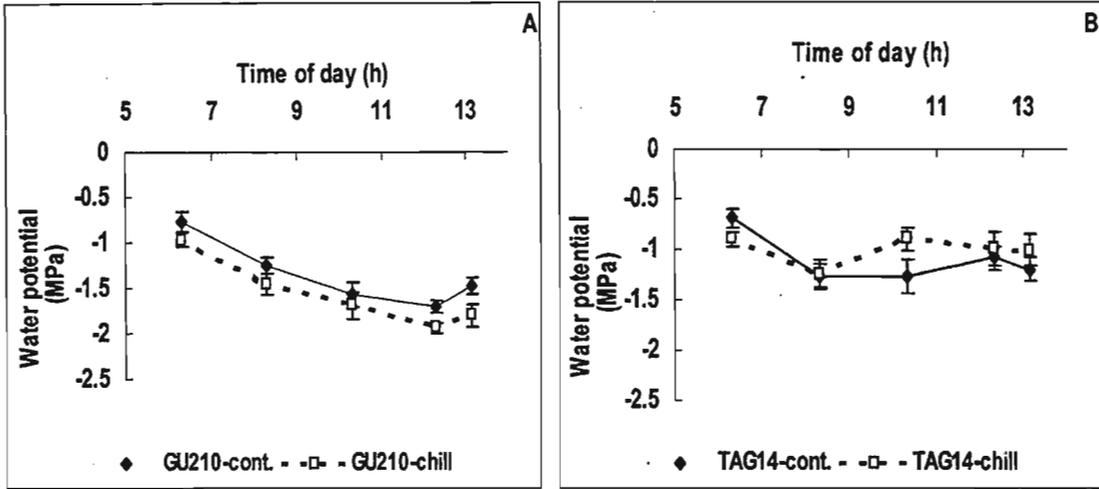


Fig. 6.3.1 Leaf water potential from morning to midday of control plants of GU210 (A) and TAG14 (B) and plants subjected to short-term root chilling. Error bars represent the \pm SEM (n=4).

6.3.2 Diurnal pattern of leaf gas exchange

Root chilling had very little effect on stomatal conductances (g_s) of GU210, and a marginal effect on TAG14 (Fig. 6.3.2. A, B). A similar response was observed for net CO_2 assimilation rates (Fig. 6.3.2. C, D), and for both conductance and assimilation, values in TAG14 were considerably lower than those in GU210. For both parameters measured at midday the clonal differences were significant, but treatment differences were not. The low stomatal conductance and lack of increase during the course of the morning in TAG14 mirrored the high and almost constant leaf water potential of this clone in both treatments.

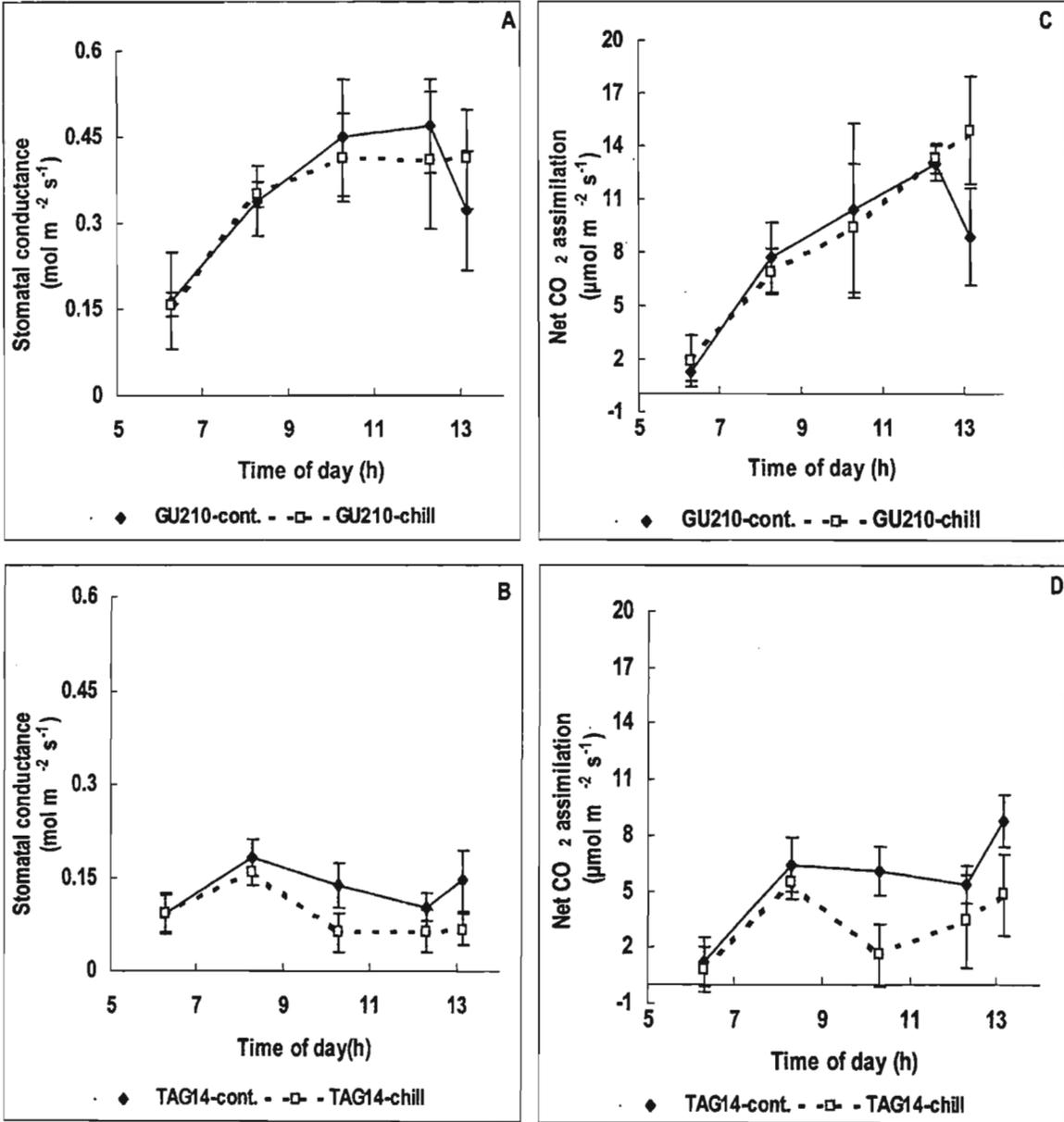


Fig. 6.3.2 Stomatal conductances (A, B) and net CO₂ assimilation (C, D) of plants of clones GU210 (A, C) and TAG14 (B, D) subjected to short-term root chilling with controls. Error bars represent the ± SEM (n=4).

6.3.3 Relationship between transpiration and the water potential

The relationships between transpiration rate and leaf water potential are shown in Fig. 6.3.3. In TAG14 soil chilling had no effect on the slope of the fitted regression lines, indicating no effect of root temperature on plant hydraulic conductance. In GU210 root chilling significantly increased the slope of the line ($P=0.004$, pair-wise comparison), indicating an increase in the resistance of the soil-to-leaf water flow pathway. The slopes of the lines for both TAG14 control and root-chilled TAG14 were similar.

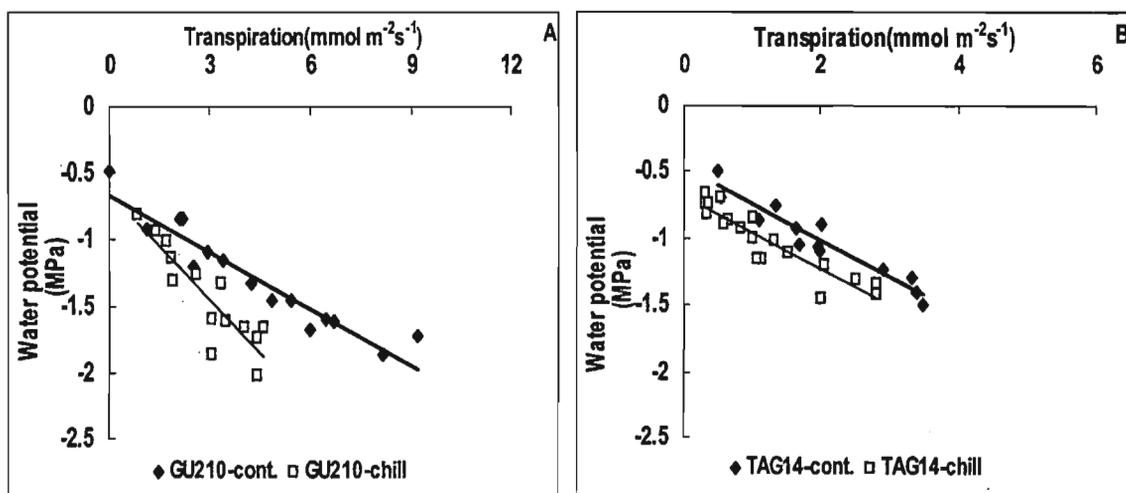


Fig. 6.3.3 The relationship between transpiration rate and the leaf water potential for plants of clones GU210 and TAG14 subjected short-term soil-root-chilling (fine unbroken regression line) with their controls (bold unbroken regression line). Fitted regression lines extended to the maximum transpiration rate achieved by each clone with treatment.

The equations of the fitted regression lines are:

- a) Control clone of GU210 - $y = -0.14x - 0.66$, $r^2 = 0.90$ ($P = 0.000$)
- b) Chilled clone of GU210 - $y = -0.26x - 0.66$, $r^2 = 0.78$ ($P = 0.000$) (pair wise, a vs. b; $F = 10.18$, $P = 0.004$)
- c) Control clone of TAG14 - $y = -0.27x - 0.47$, $r^2 = 0.91$ ($P = 0.000$)
- d) Chilled clone of TAG14 - $y = -0.27x - 0.68$, $r^2 = 0.84$ ($P = 0.000$) (pair wise, c vs. d; $F = 0.00$, $P = 0.965$)

6.3.4 Root, shoot and whole plant hydraulic conductances

The effect of short-term root chilling on root hydraulic conductances expressed per unit leaf area ($K_{r/LA}$) are shown in Fig. 6.3.4. A. The chilling treatment reduced root conductance of TAG14 by 50%, and that of GU210 by 59%, this treatment effect being significant ($F=10.04$, $P=0.008$). There were no differences between clones ($F=0.87$, $P=0.370$). When the data were expressed per unit total root dry mass ($K_{r/trdw}$) the reduction in conductivity was 53% for TAG14 and 56% for GU210, the treatment effect being significant ($F=49.89$, $P=0.000$) (Fig. 6.3.4. B). As was to be expected with short term chilling, there was no effect of short-term root chilling on shoot hydraulic conductances (data not shown). As root resistances make up the majority of whole plant resistance in these clones, the decrease in conductance caused by root chilling translated to decreases in whole plant conductance expressed per unit leaf area ($K_{P/LA}$) (Fig. 6.3.4.C), the treatment effect being significant ($F=11.36$, $P=0.006$).

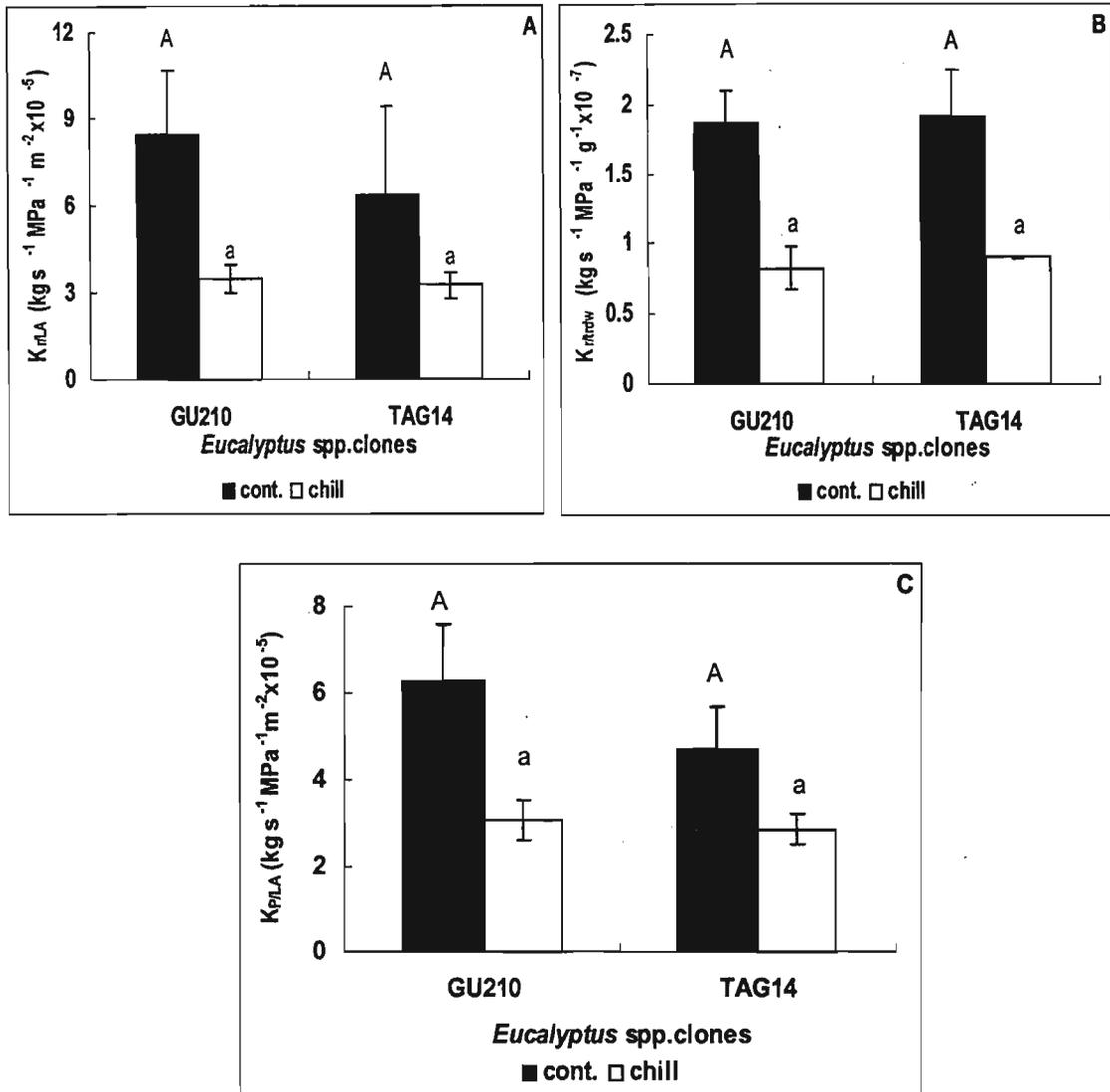


Fig. 6.3.4 Root and whole plant hydraulic conductances measured of *Eucalyptus* spp. clones subjected to short-term root chilling, and control plants. K_r and K_p expressed per unit leaf area basis (A & C) and K_r expressed per unit total root dry mass basis (B). Error bars represent the \pm SEM (n=4). Different capital (control) and small (chill) letters indicate significantly different values at $P < 0.05$ separately for treatment.

6.3.5 Some relationships between whole plant hydraulic characteristics and the leaf physiology

The relationships between $R_{\text{Plant} \cdot \text{LA}}$ [whole plant resistance linearly related to leaf water potential ($\Psi_L = \Psi_{\text{soil}} - E \cdot R_{\text{Plant} \cdot \text{LA}}$)] and the leaf physiology data points were shown in Fig. 6.3.5. The relationship between $R_{\text{Plant} \cdot \text{LA}}$ and the minimum leaf water potential (all individual data points; Fig. 6.3.5. A), showed a very weak but significant relationship ($r^2=0.25$, $P=0.049$). When the two treatments were analysed separately, the relationships were not significant.

Relationship established between $K_{\text{P/LA}}$ and the maximum stomatal conductance (g_{smax}), where data pooled for both treatments showed there was very weak non significant relationship (Fig. 6.3.5. B, $r^2=0.11$, $P=0.214$). Whereas similar weak relationship appeared for controlled clones ($r^2=0.36$, $P=0.119$), compared with that of chilled clones, which chilled showed a lack of relationship ($r^2=0.01$, $P=0.803$). Similarly, the relationships were also not significant if the treatments were analysed separately.

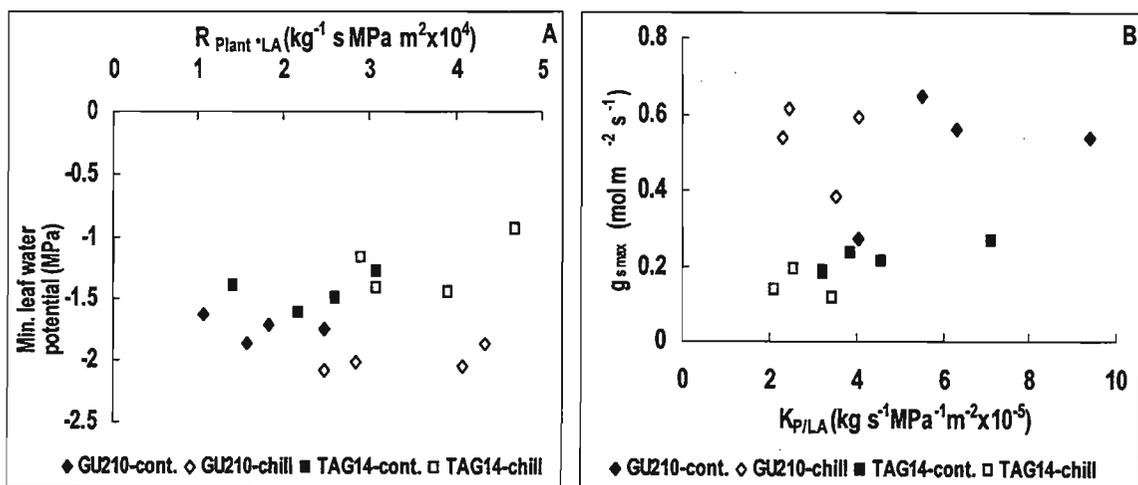


Fig. 6.3.5 Relationship between $R_{\text{Plant} \cdot \text{LA}}$ and minimum leaf water potential (A) and $K_{\text{P/LA}}$ and maximum stomatal conductances (B) for short-term root chilled *Eucalyptus* spp. clones together with control values.

Chapter: 6.4 Results: Long-term study

6.4.1 Effect of root chilling on whole plant biomass allocation

The growth parameters for the control plants and those subjected to the long-term root chilling treatment are presented in Tables 6.4.1 and 6.4.2. The chilling treatment significantly reduced total plant biomass in both clones ($F=4.62$, $P=0.045$), as well as the individual components ($F=6.25$, $P=0.022$ for leaves; $F=5.93$, $P=0.026$ for stems), but with exception of roots ($F=2.13$, $P=0.162$), with the reduction being greater in GU210 than TAG14 (although within individual components clonal differences were not significant). The proportional reduction in biomass in GU210 was similar in all plant parts, at about 35%, whereas in TAG14 leaf dry mass was reduced by a higher proportion (20%) than root dry mass (10%), with total plant mass being reduced by about 13%. There were also significant reductions in leaf area with root chilling ($F=13.93$, $P=0.002$), and as a consequence of slight (non-significant) reductions in specific leaf area, reductions in total leaf area were greater than those in leaf mass. Adjustment to the root chilling treatment by reallocation of biomass was not apparent in the root: shoot ratio or leaf weight ratio, but the ratio of leaf area to root mass did decline with root chilling, more so in TAG14 (22% reduction) than in GU210 (8% reduction). Total leaf area (evaporating surface) divided by total root dry mass (absorbing surface), is a measure of the root to leaf hydraulic efficiency, which was higher for unchilled plants compared with those chilled (Table 6.4.2), although the differences were not significant. (treatments; $F=0.36$, $P=0.555$ and clones ; $F=0.89$, $P=0.357$).

In addition to changes in biomass, there were also visible morphological effects of long-term root chilling, particularly in TAG14. Up to 90% of the leaves were pale and pink in colour (suggesting anthocyanin deposition) and small streaks appeared like little yellow blotches on the upper surfaces. The mature leaves were folded along the

midrib and orientated along the axes of the stems. Roots excavated from the chilling treatment were long and thick, compared with the densely packed thin and fragile roots of the control treatment. The roots were not visibly pot bound in either treatment at the time of excavation.

Height and the stem over-bark diameter (OBD) increased during the experiment period (Fig. 6.4.1), although growth in both height and stem over-bark diameter were only marginally lower for root-chilled plants compared with control plants. This is perhaps not surprising as biomass (which was significantly reduced) is related to volume, not linear dimensions of tissue.

Table 6.4.1 Growth parameters of two *Eucalyptus* spp. clones subjected to long-term root chilling and for the control plants. Means are given \pm SEM (n = 5 to 6). Under each parameter, different capital (control) and small (chill) letters indicate significantly different values at $P < 0.05$ separately for treatment. Differences in growth parameters between treatments shown as percentage.

Clones	Treatments	LA (m ²)	Leaf DW, (g)	Stem DW, (g)	Roots DW, (g)	Plant DW, (g)	SLA (m ² kg ⁻¹)
GU210	Control	0.10 \pm 0.01 ^A	9.46 \pm 1.28 ^A	6.45 \pm 1.10 ^A	19.27 \pm 4.18 ^A	35.18 \pm 6.31 ^A	10.85 \pm 0.64 ^A
GU210	Chill	0.06 \pm 0.01 ^a	6.34 \pm 1.48 ^a	4.18 \pm 0.63 ^a	12.52 \pm 1.71 ^a	23.05 \pm 2.26 ^a	10.32 \pm 1.09 ^a
% difference between treatments		38.07	32.94	35.18	35.01	34.48	4.88
TAG14	Control	0.10 \pm 0.01 ^A	9.10 \pm 0.82 ^A	6.73 \pm 0.52 ^A	18.60 \pm 1.77 ^A	34.43 \pm 2.25 ^A	10.92 \pm 0.69 ^A
TAG14	Chill	0.07 \pm 0.01 ^a	7.27 \pm 0.48 ^a	5.82 \pm 0.58 ^a	16.79 \pm 2.99 ^a	29.87 \pm 3.36 ^a	9.75 \pm 0.35 ^a
% difference between treatments		28.92	20.17	13.54	9.75	13.24	10.71

Table 6.4.2 Growth parameters of two *Eucalyptus* spp. clones subjected to long-term root chilling and for the control plants. Means are given \pm SEM (n = 5 to 6). Under each parameter, different capital (control) and small (chill) letters indicate significantly different values at $P < 0.05$ separately for treatment. Differences in growth parameters between treatments shown as percentage.

Clones	Treatments	LWR	LAR ($m^2 kg^{-1}$)	R:S	LA/RDW ($m^2 kg^{-1}$)	Height (m)	OBD (mm)
GU210	Control	0.28 \pm 0.02 ^A	3.07 \pm 0.35 ^A	1.18 \pm 0.13 ^A	5.96 \pm 0.95 ^A	0.35 \pm 0.03 ^A	5.71 \pm 0.36 ^A
GU210	Chill	0.27 \pm 0.05 ^a	2.64 \pm 0.33 ^a	1.44 \pm 0.51 ^a	5.20 \pm 0.99 ^a	0.32 \pm 0.02 ^a	4.70 \pm 0.46 ^a
% difference between treatments						10.06	17.75
TAG14	Control	0.26 \pm 0.02 ^A	2.87 \pm 0.24 ^A	1.20 \pm 0.13 ^A	5.47 \pm 0.73 ^A	0.41 \pm 0.02 ^A	5.84 \pm 0.24 ^A
TAG14	Chill	0.25 \pm 0.03 ^a	2.46 \pm 0.25 ^a	1.30 \pm 0.22 ^a	4.74 \pm 0.78 ^a	0.39 \pm 0.01 ^b	5.60 \pm 0.18 ^a
% difference between treatments						4.58	4.78

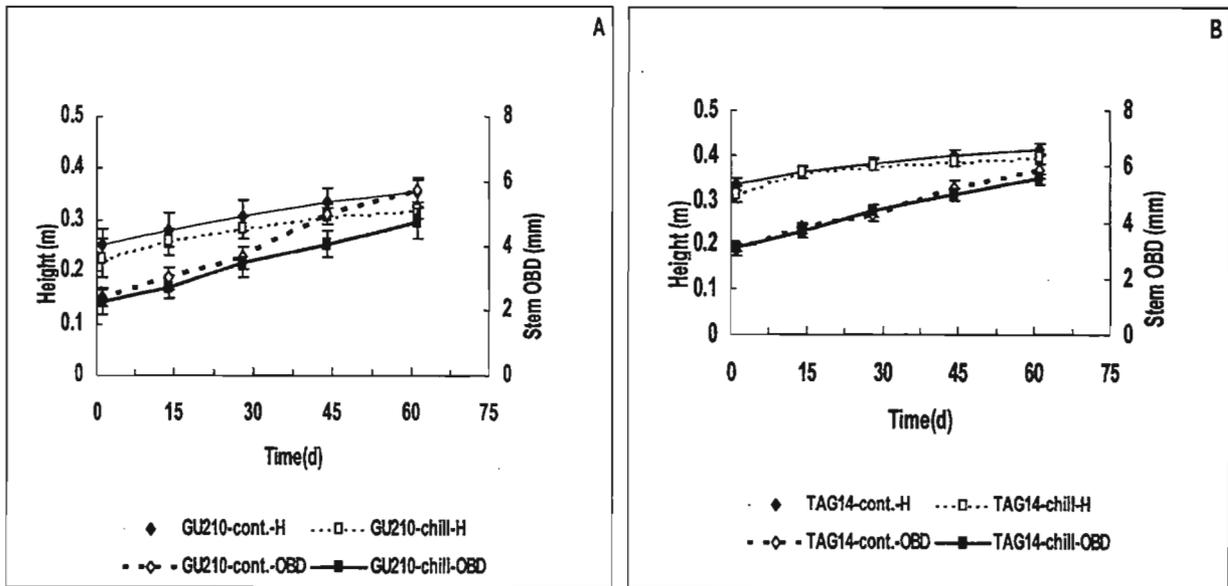


Fig. 6.4.1 Growth in terms of height and stem over-bark diameter (OBD) for GU210 (A) and TAG14 (B) clones subjected to long-term root-chilling with controls. Error bars represent the \pm SEM (n = 5 to 6).



Fig. 6.4.2 Effect of long-term root chilling at 15°C on two-selected *Eucalyptus* spp. clones grown in 3-l pot at above ground shoots (A) with control plant (B).

6.4.2 Predawn and diurnal leaf water potentials

The uppermost pot soil surface wetting was more visible and persistent in soil chilled plants compared with that of the controlled plants grown pot soil surface, which often showed dry surface in the afternoon, despite the watering pattern being the same.

As was the case for the short-term chilling, predawn leaf water potentials were lower in plants subjected to long-term soil and root chilling than those of control plants ($F=4.22$, $P=0.050$) (Table 6.4.3), suggesting incomplete equilibration between plant and soil overnight. There were no clonal differences in predawn leaf water potential ($F=0.96$, $P=0.337$).

Table 6.4.3 Mean predawn leaf water potential of two *Eucalyptus* spp. clones subjected to long-term root chilling. Means are given \pm SEM ($n=4$). Different upper case (control) and lower case (chill) letters indicate significantly different values at $P<0.05$ separately for treatment.

Clones	Treatments	Predawn ψ_L (MPa)
GU210	Control	-0.10 ± 0.024^A
GU210	Chill	-0.15 ± 0.025^a
TAG14	Control	-0.09 ± 0.020^A
TAG14	Chill	-0.12 ± 0.004^a

As the morning progressed leaf water potential (ψ_L) decreased marginally more in the root-chilled plants relative to control plants, up to about 09.00 h (Fig. 6.4.3). However, by 10.00 h the ψ_L of chilled plants either levelled off, or even increased slightly, whereas in control plants it continued to decline until midday. These data suggest that stomatal closure was occurring in the plants subjected to root chilling, leading to a decrease in transpiration and consequently no further decline in ψ_L .

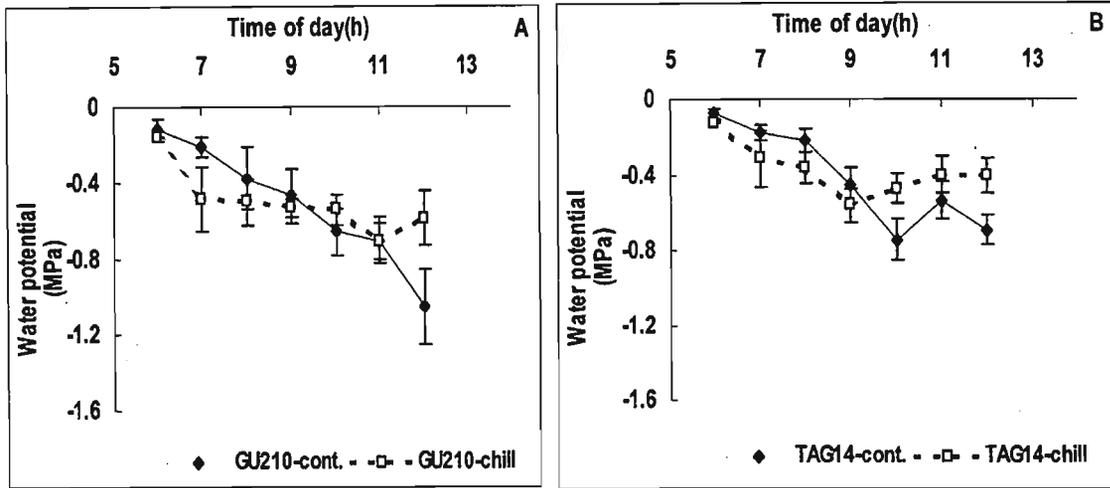


Fig. 6.4.3 Leaf water potential measured from morning to midday for GU210 (A) and TAG14 (B) clones that subjected to long-term soil-root-chilling with comparison controls. Error bars represent the \pm SEM ($n=5$ to 6).

6.4.3 Diurnal pattern of leaf gas exchange

The stomatal conductances (g_s) of the plants subjected to root chilling were low compared with those of the control plants (Fig. 6.4.4. A, B). The values of g_s in chilled TAG14 began to drop linearly from about 08.00 h to reach minimum values at midday, when control values were at their highest. In GU210 values of g_s of root-chilled plants were lower than controls and remained nearly constant throughout the morning. Statistical analysis revealed that the root chilling treatment significantly decreased the stomatal conductances between 08.00 h and midday in both clones ($P<0.004$). But, there were no clonal differences in g_s during both measured times ($P=0.220$). The reduction of g_s during the morning in plants subjected to root chilling was probably responsible for the response of leaf water potential during the morning (Fig. 6.4.3).

Associated with the reduction in g_s , the root chilling treatment consistently decreased net CO₂ assimilation rates (A) relative to controls in both clones throughout the morning (Fig. 6.4.4. C and D). A_{max} in plants subjected to root chilling occurred at 09.00 h, whereas in the control plants increases in A occurred after this time. (The high g_s recorded in the early morning in root-chilled plants of both clones, that was not associated with high net CO₂ assimilation rate, was probably a consequence of high relative humidity caused by the cold soil reducing air temperature in the immediate vicinity of the chilled plants on a still early morning.)

The relationship between net CO₂ assimilation and stomatal conductance measured from morning to midday was linear in all cases (Fig. 6.4.5.A & B, $P=0.000$), suggesting stomatal control of assimilation. However, the relationship for chilled TAG14 was weaker ($r^2 = 0.42$) than for the control of this clone ($r^2 = 0.66$), and for the GU210 clone under both chilled and control (r^2 ranging from 0.75 to 0.78), suggesting that chilled GU210 had better stomatal limitation compared with chilled TAG14.

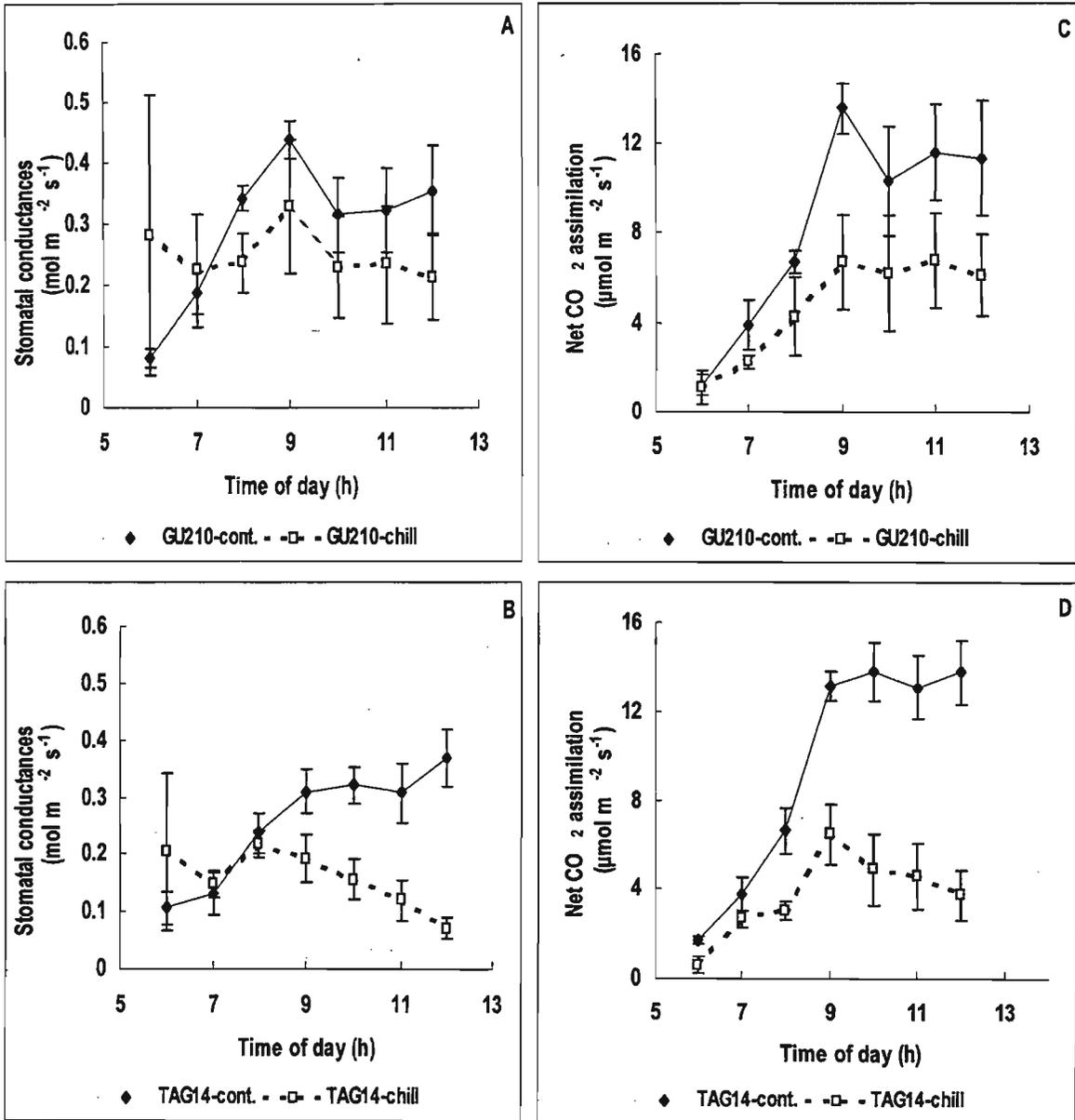


Fig. 6.4.4 Diurnal variation in stomatal conductances (A, B) and net CO₂ assimilation (C, D) for GU210 (A, C) and TAG14 (B, D) clones subjected to long-term soil-root-chilling with controls. Error bars represent the \pm SEM (n=5 to 6).

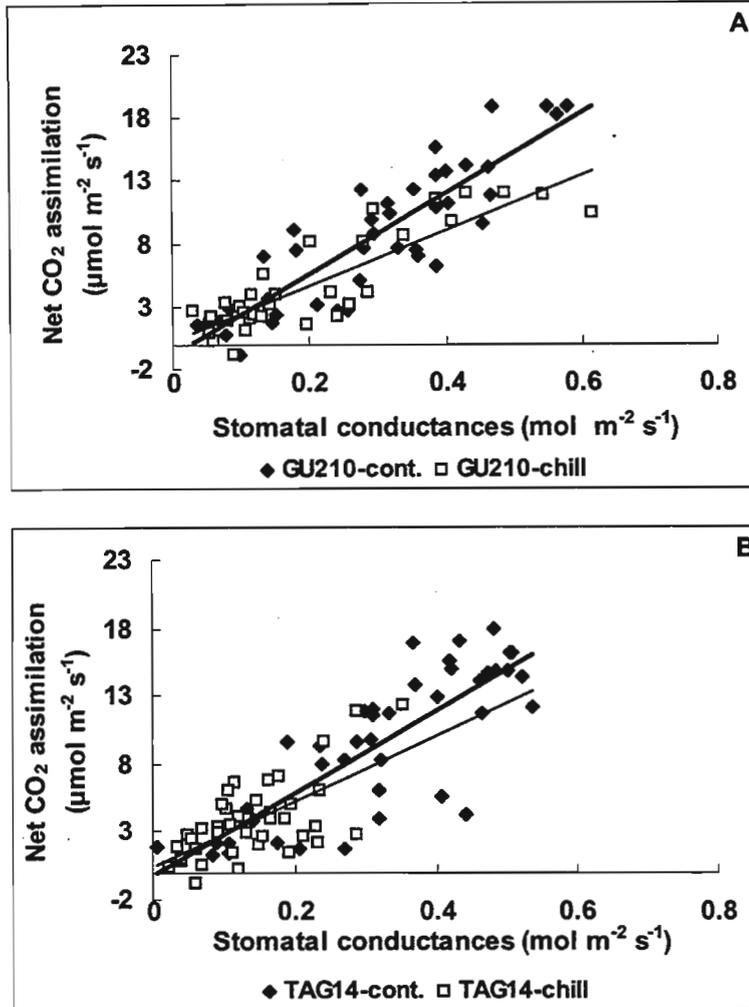


Fig. 6.4.5 The relationship between net CO₂ assimilation rate and stomatal conductances of the clones GU210 (A) and TAG14 (B) grown under root chilled and unchilled conditions. The fitted linear regression lines represent control (bold line) and chill (thin line).

6.4.4 Relationship between transpiration and the water potential

The fitted regression lines of leaf water potential against transpiration are shown in Fig. 6.4.6. The root-chilled plants of both clones had steeper declines in water potential than their corresponding controls, although this difference was significant only in TAG14. This is in keeping with stomatal conductances, where conductances in root-chilled GU210 were more similar to control counterparts than was the case for TAG14.

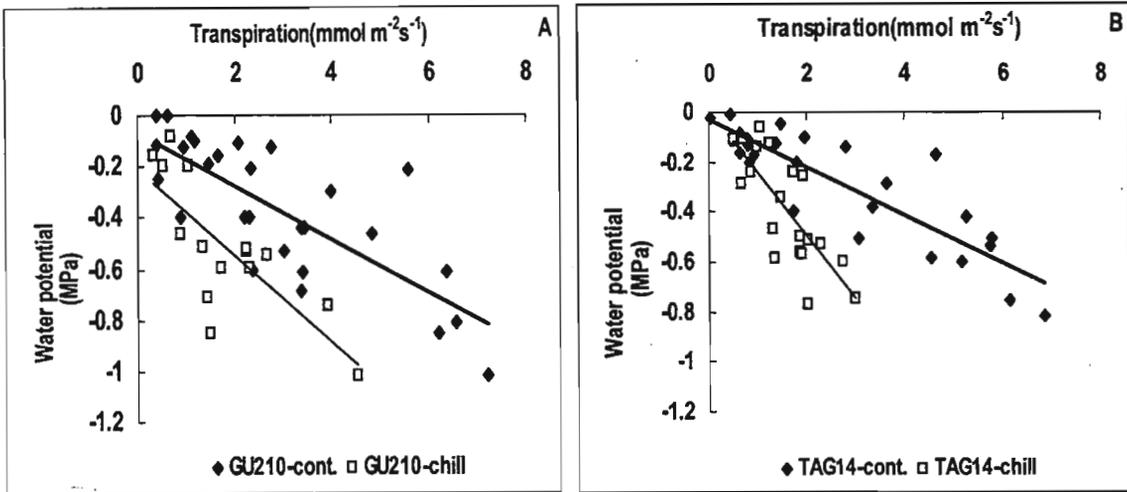


Fig. 6.4.6 The relationship between transpiration rate and the leaf water potential for GU210 and TAG14 clones subjected long-term root chilling (fine regression line) with their controls (bold regression line). Fitted regression lines extended to the maximum transpiration rate achieved by each clone with treatment.

The equations of the fitted regression lines are:

- a) Control clone of GU210 $-y = -0.10x - 0.065$, $r^2 = 0.61$ ($P = 0.000$)
- b) Chilled clone of GU210 $-y = -0.17x - 0.21$, $r^2 = 0.59$ ($P = 0.001$) (pair wise, a vs. b; $F = 2.37$, $P = 0.131$)
- c) Control clone of TAG14 $-y = -0.096x - 0.02$, $r^2 = 0.75$ ($P = 0.000$)
- d) Chilled clone of TAG14 $-y = -0.25x + 0.015$, $r^2 = 0.63$ ($P = 0.000$) (pair wise, c vs. d; $F = 13.88$, $P = 0.001$)

6.4.5 Root pressure

Positive root pressures of 6.10 and 15.50 kPa were measured overnight and midday, respectively, for different control set of GU210, with the corresponding values for TAG14 being 4.20 and 10.00 kPa, but these clonal differences were not significant ($F = 0.39$, $P = 0.550$ overnight, and $F = 0.58$, $P = 0.476$ at midday). Positive root pressures were not detected in plants of either clone subjected to root chilling.

6.4.6 Root, shoot and whole plant hydraulic conductances

Absolute hydraulic conductances of both clones subjected to both treatments are shown in Table 6.5.4. In both clones, plant subjected to root chilling had lower root ($F=32.32$, $P=0.000$) and shoot ($F=5.74$, $P=0.028$) conductances. Differences between clones were not significant.

Table 6. 4. 4 Absolute hydraulic conductances measured in two *Eucalyptus* spp. clones grown under root-chill condition and the control condition. Means are given \pm SEM (n = 5 to 6). Under each parameter, different capital (control) and small (chill) letters indicate significantly different values at $P<0.05$ separately for treatment. (Followed by One way-ANOVA).

Absolute Hydraulic Conductances ($\text{kg s}^{-1} \text{MPa}^{-1} \times 10^{-5}$)			
Clones	Treatments	K_r	K_s
GU210	Control	1.58 \pm 0.23 ^A	4.82 \pm 0.94 ^A
GU210	Chill	0.55 \pm 0.07 ^a	3.52 \pm 1.08 ^a
TAG14	Control	1.46 \pm 0.11 ^A	5.79 \pm 0.79 ^A
TAG14	Chill	1.08 \pm 0.11 ^b	3.62 \pm 0.35 ^a

When root hydraulic conductances were expressed per unit leaf area ($K_{r/LA}$), values for root chilled plants of GU210 were lower than those of their controls, but the decrease in leaf area brought about by root chilling in TAG14 was such that there were no differences between treatments in $K_{r/LA}$ in this clone (Fig. 6.4.7. A). For the treatments $F=6.04$, $P=0.024$, and for the clonal effect $F=3.11$, $P=0.095$. There was an interaction of $K_{r/LA}$ between treatment and the clone ($F=6.67$, $P=0.028$). When root conductances were expressed per unit root dry mass ($K_{r/trdw}$) (Fig. 6.4.7. C) essentially the same pattern was apparent; root chilling reduced $K_{r/trdw}$ of GU210, but not of TAG14 (for treatment $F=7.82$, $P=0.012$; for clone $F=0.28$, $P=0.606$; interaction of treatment x clone $F=2.78$, $P=0.113$).

Shoot hydraulic conductances expressed per unit leaf area ($K_{S/LA}$) are shown in Fig. 6.4.7. B. Adjustment of leaf area in response to chilling in both clones was such that neither treatment ($F=0.28$, $P=0.606$) nor clone had any significant effect ($F=0.01$, $P=0.922$). When shoot conductances were expressed per unit total shoot dry mass ($K_{S/tsdw}$) (Fig. 6.4.7. D) essentially the same pattern was observed; neither treatment ($F=0.14$, $P=0.713$) nor clone ($F=0.04$, $P=0.838$) had any significant effect.

Whole plant hydraulic conductances expressed per unit leaf area ($K_{P/LA}$) are shown in Fig. 6.4.8. Because roots constitute the major component of the overall plant resistance, the patterns observed were similar to those for root conductances expressed per unit leaf area. Root chilling reduced ($K_{P/LA}$) of GU210 but not of TAG14. Adjustment of leaf area by plants of TAG14 subjected to long term root chilling was such that the efficiency of the hydraulic pathway per unit leaf area was maintained, whereas adjustment of leaf area in GU210 was inadequate to achieve this. Interestingly, this is in contrast to the assessment of whole plant conductance indicated by the relationship between transpiration rate and leaf water potential (Fig. 6.4.6).

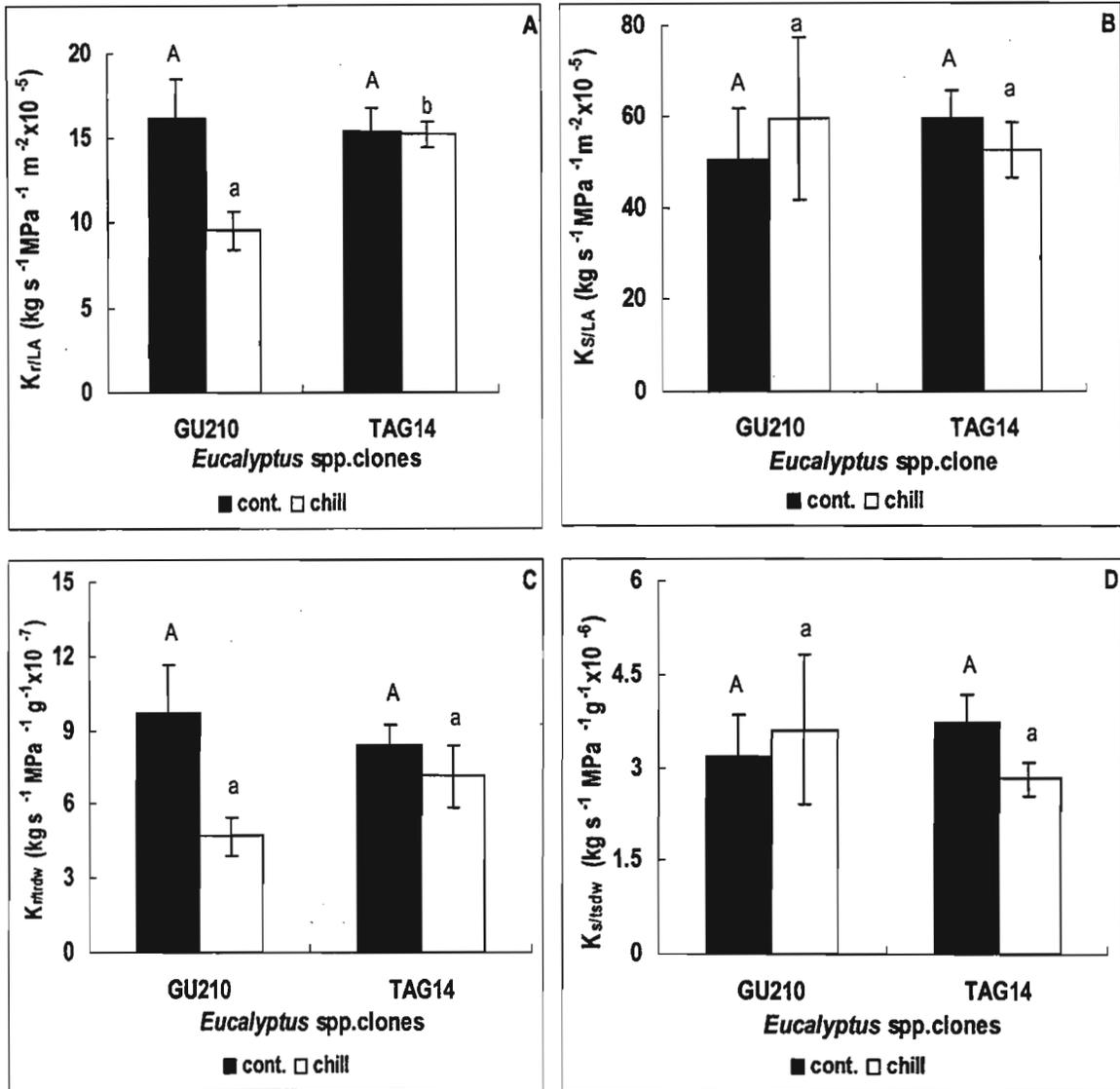


Fig. 6.4.7 Root and shoot hydraulic conductances measured in *Eucalyptus* spp. clones in long-term root chilled and control plants. K_r and K_s expressed per unit leaf area basis (A & B), K_r and K_s expressed per unit total root dry mass basis (C) and per unit total shoot dry mass basis (D). Error bars represent the \pm SEM of the mean (n=5 to 6). Different capital (control) and small (chill) letters indicate significantly different values at $P < 0.05$ separately for treatment.

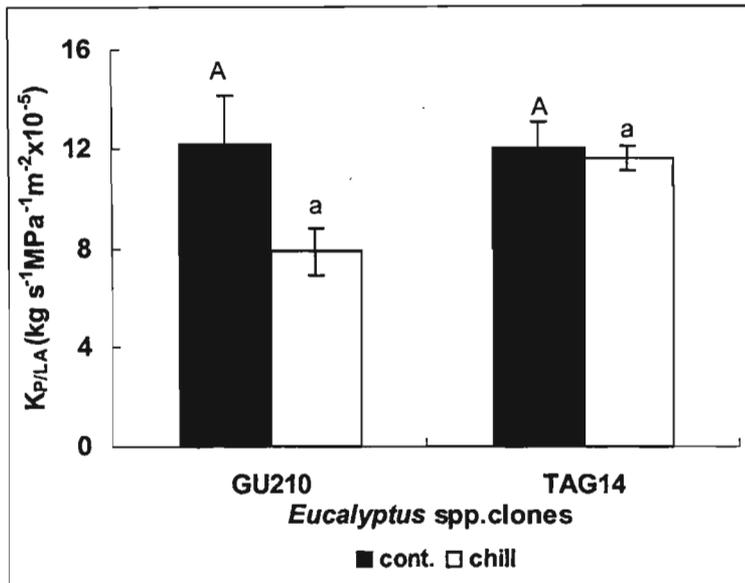


Fig. 6.4.8 Whole plant hydraulic conductances expressed per unit leaf area basis ($K_{P/LA}$) measured in *Eucalyptus* spp. clones in long-term root-chilled and control plants. Error bars represent the \pm SEM (n=5 to 6). Different capital (control) and small (chill) letters indicate significantly different values at $P < 0.05$ separately for treatment.

6.4.7 Relationship between whole plant hydraulic characteristics and the leaf physiology and productivity

The relationships between $K_{P/LA} / R_{Plant*LA} / K_P$ and the respective leaf physiological characteristics and plant biomass for control and chilled *Eucalyptus* spp. clones are shown in Fig. 6.4.9. There were no relationships between $R_{Plant*LA}$ and the minimum leaf water potential (Fig. 6.4.9. A), when data from both treatments and clones were pooled ($r^2=0.01$, $P=0.738$) or when treatments were analysed separately (control; $r^2=0.02$, $P=0.670$ and chilled; $r^2=0.00$, $P=0.903$).

There was no relationship between $K_{P/LA}$ and the maximum stomatal conductances ($g_{s,max}$) for both treatments (pooled data, Fig. 6.4.9. B; $r^2=0.00$, $P=0.837$) or when treatments were analysed separately (control; $r^2=0.01$, $P=0.744$ and chilled; $r^2=0.15$, $P=0.242$).

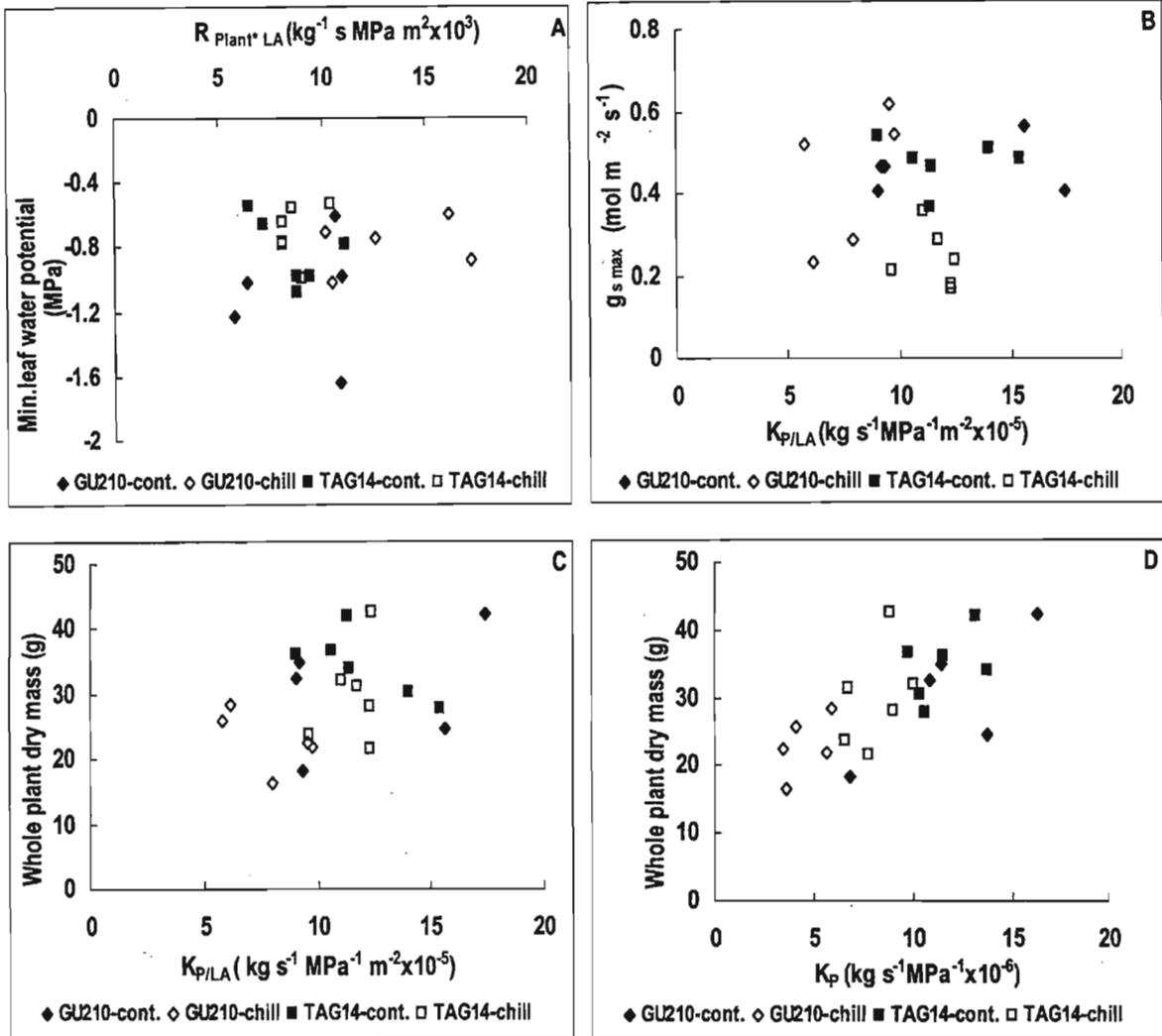


Fig. 6.4.9 Relationship between $R_{\text{Plant} \cdot \text{LA}}$ and minimum leaf water potential (A), $K_{P/LA}$ and maximum stomatal conductances (B), $K_{P/LA}$ and whole plant dry mass (C) and K_P and whole plant dry mass for two selected *Eucalyptus* spp. clones subjected to long-term chilling treatment with control.

The relationship between $K_{P/LA}$ and whole plant dry mass (all data for both clones and treatment pooled) was also not significant (Fig. 6.4.9. C, $r^2=0.10$, $P=0.147$). If the clones were analysed separately, the statistical values for TAG14 were $r^2=0.03$ and $P=0.561$, and for GU210 $r^2=0.20$ and $P=0.199$. There were no significant relationships if the data for treatments were analysed separately. There was a significant relationship between K_P (absolute value, not normalised by leaf area) and total plant dry mass (Fig. 6.4.8. D), when data from both clones and treatments was pooled

($r^2=0.47$, $P=0.001$). If the data from the clones were analysed separately, the relationship was also significant for GU210 ($r^2=0.57$, $P=0.011$), and that for TAG14 marginally so ($r^2=0.28$, $P=0.076$). However, the relationship between absolute whole plant conductances and the dry mass may have little physiological significance, as a larger plant body would be expected to be able to conduct more water.

6.4.8 The components total hydraulic resistance

Shoot hydraulic resistances expressed on a leaf area basis (R_{S*LA}), the components contributing to it, together with root resistance are shown in Table 6.4.5. The leaves constitute more than half of the shoot resistance, but there were no differences between clones ($F=1.44$, $P=0.245$) or treatments ($F=0.22$, $P=0.648$), although the leaf area was significantly higher for the control plants. Similarly, there were no significant effects of either clone ($F=0.00$, $P=0.955$) or treatment ($F=1.18$, $P=0.292$) in the resistances of stems plus petioles. Consequently, there were no differences in total shoot resistances (for clones $F=1.05$, $P=0.318$, and for treatment $F=0.03$, $P=0.857$).

Table 6.4.5 Contributions of hydraulic resistances of plant components to the whole-plant resistances in two *Eucalyptus* spp. clones grown under root chilling treatment and for the control plants. Means are given \pm SEM ($n = 5$ to 6). Under each parameter, different capital (control) and small (chill) letters indicate significantly different values at $P<0.05$ separately for treatment.

Hydraulic resistances expressed per unit leaf area ($\text{kg}^{-1} \text{ s MPa m}^2 \times 10^3$)					
Clones	Treatments	R_L	$R_{P+stems}$	R_S	R_r
GU210	Control	1.59 \pm 0.43 ^A	0.74 \pm 0.06 ^A	2.33 \pm 0.48 ^A	6.63 \pm 0.88 ^A
GU210	Chill	1.61 \pm 0.50 ^a	0.61 \pm 0.12 ^a	2.22 \pm 0.60 ^a	11.12 \pm 1.53 ^a
TAG14	Control	1.10 \pm 0.20 ^A	0.68 \pm 0.06 ^A	1.79 \pm 0.23 ^A	6.83 \pm 0.70 ^A
TAG14	Chill	1.37 \pm 0.20 ^a	0.66 \pm 0.07 ^a	2.03 \pm 0.25 ^a	6.69 \pm 0.38 ^b

R_r was much higher than R_S both in the control and root-chilled plants. There were significant effects on R_r of both clone ($F=6.75$, $P=0.018$) and treatment ($F=7.10$, $P=0.016$). Long term chilling of roots increased the resistance of roots of GU210 by

nearly 70%, but had no effect on root resistance of TAG14 (see also the data for conductances; Fig. 6.4.7. A).

The proportional contribution of the various components to total plant hydraulic resistance is shown Table 6.4.6. Roots contributed the major proportion in all cases, being as high as 83% in plants of GU210 subjected to root chilling. The proportional contribution of the components in TAG14 was unaffected by the root chilling and was similar to control GU210.

Table 6. 4. 6 Proportional contributions (expressed as a percentage) of plant components to the whole plant hydraulic resistances in two *Eucalyptus* spp. clones grown under root chilled and control conditions. Means are given \pm SEM (n =5 to 6).

Hydraulic resistances (%) expressed per unit leaf area					
Clones	Treatments	R _L	R _{P+B}	R _S	R _r
GU210	Control	16.61 \pm 2.58	8.71 \pm 1.10	25.31 \pm 1.99	74.70 \pm 1.99
GU210	Chill	11.94 \pm 3.46	4.67 \pm 0.93	16.62 \pm 4.28	83.38 \pm 4.28
TAG14	Control	13.01 \pm 2.22	7.97 \pm 0.42	20.99 \pm 2.52	79.01 \pm 2.52
TAG14	Chill	15.64 \pm 2.13	7.64 \pm 0.77	23.27 \pm 2.67	76.70 \pm 2.67

Chapter: 6.5 Discussion

This experiment was conducted in an attempt to establish whether reducing hydraulic conductance would reduce stomatal conductance, CO₂ assimilation and, in the long term, growth. The technique used to reduce conductance was to chill the roots; this approach was adopted because the roots constitute the major resistance in these plants, because the technique is non-invasive, and because the treatment can be applied in the long term. The experiment was conducted in two parts: short-term overnight chilling to establish that this treatment did influence root hydraulic conductance and leaf physiological characteristics, and long-term chilling to assess whether any physiological effects at the leaf level translated into effects on growth, and whether morphological and physiological adjustment to the treatment occurred.

Chilling of roots was achieved by chilling the soil in which the plants were rooted. There are two complications with this technique: firstly, observed effects on conductances may be an effect on the hydraulic properties of the soil itself, rather than the roots, consequent upon the increased viscosity of water at the reduced temperature employed. However, it is considered that the reduction of soil temperature to 15°C, as used here, would have only marginal effects on soil hydraulic conductivity, and would not be an important factor (personal communication, M. A. Johnston, Dept of Soil Science, University of Natal, Pietermaritzburg). Furthermore, in terms of the objective of the experiment – to assess the influence of increases in resistance in the soil-to-leaf hydraulic pathway – it is unimportant if the increase occurs in the soil or plant. The second complication is whether any effects on leaf physiology and growth observed were a direct effect of temperature *per se*, rather than a consequence of reduced hydraulic conductance. It is likely that reducing root temperature will reduce root metabolic rate and thus the strength of the roots as a sink, and so reduce partitioning of assimilate to the roots. However, root conductance data can be assessed in terms of root mass, so any differences in biomass partitioning consequent upon long term chilling should be accounted for.

6.5.1 Effects of long-term chilling on growth and allocation patterns

Long-term root chilling brought about substantial growth reductions in both clones (Table 6.4.1). There are few studies in the literature with which the data in this thesis can be compared. However, three patterns of response of biomass partitioning to the treatment could be envisaged: (i) no effect on relative allocation; (ii) the reduction in root temperature could have reduced the strength of the roots as a sink, and hence reduced proportional allocation to roots; (iii) reductions in conductivity, and hence the ability of the roots to supply water could have increased proportional allocation to roots. A decrease in leaf area has been recorded in *Acer saccharum* as a result of soil freezing (Bertrand *et al.*, 1994). Increased proportional allocation to roots under conditions of limited water availability is well known (Passioura, 1981) and has been observed in water stressed *Eucalyptus* species (Li, 2000; Li *et al.*, 2000a). In the experiment reported on here, in GU210 the proportional reduction in biomass was similar in all plant parts (Table 6.4.1), suggesting no effect of treatment on allocation patterns. In TAG14 there were indications of morphological adjustment to the chilling treatment: the reduction in root biomass (*c.* 10%) was less than the reduction in leaf mass (*c.* 20%) or area (*c.* 29%). However, the resultant increase in root: shoot ratio or decrease in the ratio of leaf area to root mass were not significant (Table 6.4.2).

6.5.2 Hydraulic conductances

As was to be expected, short-term root chilling had no effect on shoot hydraulic conductance expressed per unit leaf area. In the long-term root chilling experiment, because of the decreases in leaf area associated with chilling, differences in absolute shoot conductances disappeared when values were expressed per unit leaf area.

There was, however, considerable effect of root chilling on root conductance. In the both clones short-term root chilling reduced root hydraulic conductances to approximately half of control values, whether conductances were normalised to root dry mass or total leaf area (Figs 6.3.4. A and B). This is accordance with other reports

where root chilling decreased the hydraulic conductances (Markhart *et al.*, 1979; Cui and Nobel, 1994; Fennel and Markhart, 1998; Brodribb and Hill, 2000; Cochard *et al.*, 2000; Vernieri *et al.*, 2001). However, these studies were undertaken in the short-term only.

When root chilling was applied in the long-term differences between the clones became apparent. In GU210 the long-term effect was similar to the short-term: root hydraulic conductances, expressed either per unit root dry mass or unit leaf area, were reduced by approximately 50% by chilling. However, in TAG14, hydraulic conductances of long-term chilled roots expressed per unit root mass were only 15% (and non-significantly) lower than controls, and when expressed per unit leaf area were the same as controls (Fig. 6.4.7. A & C). Thus, in addition to the morphological adjustments (reductions in leaf area greater than reductions in root mass) there were more marked adjustments in root physiology. In both clones the roots made the major contribution to total plant resistance (>75%), so that when whole plant conductances normalised to leaf areas were calculated, the responses showed similar patterns to those of roots: a considerable reduction as a consequence of root chilling in whole plant conductance per unit leaf area in GU210, but no effect in TAG14 (Fig. 6.4.8). The response of TAG14 is consistent with the observation of Jaquish and Ewers (2001) that freezing did not affect the root conductances under natural conditions in some ring-porous temperate woody plants.

The major resistance to water flow through roots is the resistance to radial flow (from the root surface to the stele) rather than axial flow up the root (Steudle and Peterson, 1998). Thus root surface area per unit root dry mass will have a major effect root resistance or conductance expressed per unit dry mass. Roots produced by plants subjected to chilling were longer and thicker, but fewer in number than their control counterparts, and the mass of fine roots normally observed was absent from chilled roots. (This is similar to the structure of the roots of chilled maize seedlings reported by Kiel and Stamp (1992)). Such a morphology would strongly influence surface

area: mass ratios and reduce root conductance in plants subjected to long-term root chilling.

Water moving in the radial pathway must cross membranes to enter the stele. The aquaporins (water channel proteins in membranes) are thought to mediate this transport and can respond rapidly to a number of stresses (reviewed by Javot and Maurel, 2002). It is possible that the increase in root hydraulic conductance brought about by long term chilling was a result of changes in the aquaporins (Fennel and Markhart, 1998; Vernieri *et al.*, 2001). The physiological adjustment shown by roots of TAG14 on long-term chilling (maintenance of high root conductance per unit root dry mass) could also have been a consequence of changes in aquaporins, or it might have been resultant on anatomical alterations, such as reductions in the thickness or density of casparian strips in endo- or exodermis. Unfortunately, no anatomical observations were made to assess this possibility.

The inverse of the slope of the relationship between transpiration rate and leaf water potential can also be taken as a measure of the whole plant conductance per unit leaf area (the 'evaporative flux method', EFM). The influence of root chilling on conductances assessed this way was not always in agreement with that assessed by measuring conductances directly with the HPFM. In the case of short-term chilling, in GU210 both methods showed a similar proportional decrease (51%, HPFM; 46% EFM) in conductance caused by root chilling, whereas in TAG14, the HPFM indicated a 39% reduction on root chilling, but the EFM showed no effect (Figs 6.3.4.C and 6.3.3). With long term chilling the reverse was the case: in GU210 root chilling showed a 35% reduction in whole plant conductance measured by the HPFM, and although the reduction assessed by the EFM was similar (41%) the effect was statistically non-significant. In TAG14 the HPFM showed no effect of root chilling, but the EFM indicated a 62% decline (Figs 6.4.8 and 6.4.6). The HPFM measures maximum (fully hydrated) conductances, and Tsuda and Tyree (1997 & 2000) have shown that the HPFM and the EFM yield comparable values of whole plant conductance per unit leaf area, when compared on well watered plants. A possible

explanation for the discrepancy between the results of the two methods from the long-term study is that chilling the roots of TAG14 caused considerable cavitations, reducing root (and hence whole plant) conductances considerably below the maximum value measured by the HPFM, whereas this effect was not apparent in GU210. (The field studies (Chapter 3) indicated that the main stem of TAG14 was more vulnerable than that of GU210, although there were no differences between the clones in the pot studies (Chapters 4 and 5)). No explanation can be offered for the discrepancy between the results using the two methods in short-term root chilled TAG14.

6.5.3 Effect of hydraulic conductances on leaf physiology

Reductions in hydraulic conductance brought about by short-term root chilling could be expected to reduce either leaf water potential or transpiration rate, or both, as has been shown by Cui and Nobel (1994), Bunce (1999) and Brodribb and Hill (2000). If transpiration is reduced sufficiently water potential may remain nearly constant, as has been shown by Cochard *et al.* (2000). However, in the experiment reported here, the reduction in root hydraulic conductance brought about by chilling had very little effect on leaf physiology in the short term. The reductions in leaf water potential were slight (GU210) or inconsistent (TAG14) (Fig. 6.3.1), and the reductions in stomatal conductance relative to controls (Fig. 6.3.2. A & B) were apparent only at midday such that only the maximum transpiration rates achieved were affected (Fig. 6.3.3). Net CO₂ assimilation rates were not significantly affected (Fig. 6.3.2. C & D). It is possible that if root temperatures had been reduced below 15°C, more marked effects on leaf physiology would have occurred, but preliminary experiments showed that even short term exposures to temperatures below 10°C caused root damage. Thus, despite the limited effect on leaf physiology brought about by root temperatures of 15°C, it was considered to be adequate to undertake the long-term study.

After long-term root chilling, leaf water potentials did not decline regularly as the day progressed; rather, after an initial decline values remained fairly constant, and did not decline below the values exhibited by control plants (Fig. 6.4.3). This is consistent with the results of other experimental reductions of hydraulic conductance (Sperry *et al.*, 1993; Sperry and Pockman, 1993; Saliendra *et al.*, 1995 and Hubbard *et al.*, 2001). The maintenance of high water potentials was probably a consequence of the reduced stomatal conductances (Fig. 6.4.4. A & B), which led to lower maximum transpiration rates (Fig. 6.4.6), indicating good stomatal control of leaf water status. Associated with the lower stomatal conductances of plants subjected to long-term root chilling were lower net CO₂ assimilation rates (Fig. 6.4.4. C & D) of plants subjected to long-term root chilling; the relationship between CO₂ assimilation and stomatal conductance was linear (Fig. 6.4.5), suggesting some stomatal control of photosynthesis. These patterns were apparent in both clones, suggesting that the effect of long-term root chilling on whole plant hydraulic conductance indicated by the EFM (a reduction) was more realistic than the effect measured using the HPFM (maintenance of conductance).

6.5.4 Root to shoot signalling: a mechanism for stomatal control

The ability of the stomata of plants subjected to root chilling to reduce transpiration to the extent that leaf water potential did not decline below control values raises questions concerning the mechanism by which stomata sense plant water status. Both hydraulic signals (decreasing water potential) (Bunce, 1999; Brodribb and Hill, 2000; Cochard *et al.*, 2000; Salleo *et al.*, 2000; Yao *et al.*, 2001) and chemical signals such as like abscisic acid (ABA) (Liang *et al.*, 1996; Whitehead, 1998; Croker *et al.*, 1998) have been implicated in stomatal response. According to Saliendra *et al.* (1995) root to shoot non-hydraulic signalling is an inefficient mechanism for rapid stomatal control due to lengthy transport time in woody plants. However, the fact that water potentials of root chilled plants were actually higher than those of control plants 09.00 h (Fig. 6.4.3) suggests that maintenance of low stomatal conductance in the root chilled plants was not simply an hydraulic response. It is likely that this maintenance

of low stomatal conductance was in response to a non-hydraulic signal, such as ABA. Chilling-induced water stress induced ABA in sugar maple (Bertrand *et al.*, 1994) and in *Phaseolus vulgaris* L. (Pardossi *et al.*, 1992; Vernieri *et al.*, 2001), and flood-induced water stressed *Ricinus communis* also produced ABA (Else *et al.*, 2001) may promote the stomatal closure. However, Else *et al.* (2001) suggested that the continued stomatal closure that permitted recovery of xylem water potential could be in response to increased intercellular CO₂ concentrations arising from lower photosynthesis, rather than as a response to ABA.

6.5.5 Conclusion

It has been shown that short term reductions in $K_{P/LA}$ induced by root chilling caused decreased transpiration in *Quercus robur* L. (Cochard *et al.*, 2000), and in some conifers and angiosperms (Brodrribb and Hill, 2000). Recently, Hubbard *et al.* (2001) reduced stem hydraulic conductivity in ponderosa pine seedlings by an air-injection technique and found a reduction in whole plant hydraulic conductances that was associated with reductions in transpiration, g_s and A, while leaf water potential remained constant. In the short term, reductions in $K_{P/LA}$ occurred in both clones, although the response of leaf physiology was somewhat damped compared with the work cited above.

There are two potential responses to long term root chilling. Reduced root, and hence whole plant hydraulic conductance could lead to reduced water potentials and/or reduced stomatal conductances, reduced CO₂ assimilation and consequently reduced growth. Alternatively, physiological adjustments (e.g. changes in aquaporins) could maintain hydraulic conductivity, and so maintain leaf level physiological characteristics. However, low root temperatures could reduce sink strength, and so root growth, which in turn would reduce the leaf area that could be supported. In this case reduction in growth rate would not be a direct consequence of hydraulic limitations. In GU210 the short term root chilling-induced reduction in $K_{P/LA}$ was still apparent after long term exposure, and this was associated with reduced stomatal

conductances and CO₂ assimilation. Furthermore, the proportional reduction in growth was approximately equal in all plant parts (Table 6.4.1), and root: shoot ratios were unaffected (Table 6.4.2). These data are consistent with the hypothesis that hydraulic conductances can place limitations on growth. Although the relationship between $K_{P/LA}$ and total plant biomass of individual plants was not significant ($r^2=0.20$, $P=0.199$), it was stronger than this relationship for TAG14 ($r^2=0.03$, $P=0.561$), and it may have become stronger if the experimental growth period had been extended. TAG14 appears to have responded in a less clear manner. There was a suggestion morphological adjustment, in that the reduction in root growth was less than the reduction in shoot growth, leading to an increase in root: shoot ratio, although the effect was not significant (Tables 6.4.1 and 6.4.2). There are conflicting data concerning physiological adjustment: the reduced root hydraulic conductance measured with the HPFM observed in the short term (Fig. 6.3.4. A &B) was not carried through in the long term, there being no differences between chilled and control plants in root conductances (expressed either per unit dry mass or leaf area) or whole plant hydraulic conductances (Figs 6.4.7 and 6.4.8). This is at variance with the evaporative flux method, which indicated higher whole plant resistances in the plants subjected to root chilling (Fig. 6.4.6). Stomatal conductance and CO₂ assimilation were reduced by root chilling, but there was no relationship between whole plant hydraulic conductance and biomass of individual plants, and so it is not clear whether the reduction in growth is a consequence of reduced hydraulic conductance, or of a direct response to reduced root temperature.

Chapter 7: General Discussion and Conclusion

The objectives of this study were outlined in Chapter 1 (Section 1.8) and in Chapter 4 (Section 4.1), and they can be summarized as follows: (a) to assess the impact of water availability on growth and leaf physiological characteristics, and any relationships between them, (b) to assess the effects of treatments on hydraulic characteristics, including vulnerability to cavitation, (c) to assess whether there are any relationships among hydraulic characteristics, leaf physiological properties and growth, and (d) to assess whether any relationships identified in (c) are causative. Differences among genotypes could impact on assessments of relationships, and so the experiments were conducted on three clones. In this chapter the results of the various experiments will be assessed in terms of the objectives listed above, referring to published data where appropriate.

7.1 Growth and leaf physiological characteristics

As was to be expected, water availability had a major impact on growth, whether measured as height, overbark diameter or total biomass. Perhaps coincidentally, reductions in total biomass as a result of low watering were similar in the 14 and 21 month studies (about 47%). In neither study were there clonal effects on biomass accumulation under either watering treatment. In the 14 month study there were clonal differences in total leaf area and leaf area ratio for plants subjected to high watering (lower in GC550), but these were not apparent under low watering treatment. Clonal differences in above ground biomass were also apparent in the 3 month field study, but they were absent in the 21 month study. It appears from previous studies of potted *Eucalyptus* spp. clones that interclonal differences apparent in young specimens disappear with age (February *et al.*, 1995; Osorio *et al.*, 1998; Pita and Pardos, 2001), as was observed in this study. Reductions in the various components of the plants in the 14 month study were such that watering treatment had no effect on the root:shoot ratio. Surprisingly, in the 21 month study, the reduction in root growth caused by low water was greater than the reduction in shoot growth, such that low water decreased the root:shoot ratio in all the clones. This is in contrast to

other studies with *Eucalyptus* spp. clones (Li, 2000; Li *et al.*, 2000a), and contrary to what one would expect (Passioura, 1981).

An error was made when estimating the water supply for the 14 month study, and even the 'high' water plants suffered considerable water stress. Stomatal conductances and photosynthetic rates were considerably lower than those of plants growing in the field or those measured in the 21 month study. The plants were also considerably smaller than the plants in the 21 month study when they were 14 months old. The degree of stress was such that there was actually no treatment effect on leaf physiological characteristics (although there was a treatment effect on total biomass).

In the 21 month study low watering led to significant declines in leaf water potential, stomatal conductance and net CO₂ assimilation rates. (Although gas exchange rates were higher than in the 14 month study, they were lower than those recorded in the field). This could be an age effect, or a pot effect; minimum leaf water potentials measured on high watered plants in the 85 l pots were slightly lower than those measured in the field, suggesting a pot effect. There was a clonal effect on water potential (GU210 operated at slightly lower values) but no clonal effect on the other physiological characteristics. Questions arise concerning the cause of the decline in stomatal conductance, and whether the reduced g_s and A were responsible for the reduced growth. The reduction in g_s under low watering could have been an hydraulic effect, with low water potential leading to partial stomatal closure, or it could have been a response to high intercellular CO₂ concentration (c_i) caused by low CO₂ assimilation rates that arise as a consequence of water stress affecting the mesophyll processes of photosynthesis. Unfortunately, the response of A to c_i could not be measured (there was severe competition among researchers for the instrument at that time) and so direct estimates of photosynthetic capacity were not made. However, in low watered plants stomatal conductances declined from early in the morning (08.00 h) when light intensity was still increasing. This is a pattern that would be expected if g_s was responding to declining water potential; if g_s was being controlled by A through c_i , a continued increase in g_s as A increased with PAR would be more likely (although some effect of water stress on photosynthetic capacity cannot be ruled out).

Net CO₂ assimilation rate was a linear function of stomatal conductance, although in high watered TAG14 the relationship was not as strong as in the other clones and treatments. Although rates were much lower in the 14 month study, linear relationships between A and g_s also occurred, and, similarly, 'high' watered TAG14 showed a poor relationship. This linear response suggests that g_s limits A (assuming that g_s is not simply a direct response to c_i). There were no differences in the slopes of the response between watering treatments, in keeping with the concept that the major limitation on A was stomatal rather than mesophyll. The suggestion of stomatal limitation of photosynthesis under both treatments is supported by the fact that leaf level WUE was the same for both treatments; reductions in g_s influenced A and E equally in both treatments. Whether the signal to which the stomata responded was purely hydraulic (leaf water potential or turgor pressure) or chemical (ABA from the roots in response to dry soil) is immaterial. The crux is whether stomata limit CO₂ assimilation. The poor fit of the high watered TAG14 data to a linear relationship suggests that factors other than g_s were important in determining A in this clone.

Although there are factors other than maximum photosynthetic rates that influence growth (e.g. sink strength; see Farrar, 1999), prolonged reductions in net CO₂ assimilation rate must reduce the photoassimilate available for growth. Thus the data from this experiment are consistent with the hypothesis of a long-term water stress-induced reduction in stomatal conductance giving rise to long-term reduced CO₂ assimilation, leading to reduced growth. Although there were minor interclonal variations in some physiological characteristics, this pattern is consistent across the clones.

7.2 Hydraulic properties

There has been considerable recent interest in plant hydraulic characteristics, and particularly, relationships between hydraulic characteristics and growth. It is a common concept that higher hydraulic conductances, both root and shoot, lead to less leaf water stress, a decrease in xylem cavitations, and an increase leaf gas exchange, which in turn promotes growth, and high K_{P/LA} has been suggested to favour fast

growth (Tyree and Ewers, 1996; Tyree *et al.*, 1998; Nardini and Tyree, 1999). Low values of $K_{P/LA}$ can lead to low water potentials and early stomatal closure, preventing further water loss, and so could be considered to be an adaptation to drought conditions. There are numerous reports in the literature relating some measure of conductance with leaf physiological characteristics and/or growth, and short-term reductions in conductance, induced by notching, air injection or root chilling, generally reduce leaf water potential, stomatal conductance and photosynthesis (see Section 1.6.2). However, a problem with some of these studies is that conductance was measured on isolated stem segments and did not include the effects of roots or leaves.

Long-term water stress can reduce hydraulic conductance, particularly of the roots. The major resistance in the roots is the radial flow from the surface to the stele (e.g. Steudle and Peterson, 1998), and long-term stress can increase this resistance by the development of the endodermis (Kramer, 1983; Lo Gullo *et al.*, 1998,) the formation of exodermis (Perumalla and Peterson, 1986; Lo Gullo *et al.*, 1998) or similar anatomical modifications. However, root hydraulic conductances are frequently measured using an HPFM and so are maximum values, excluding any embolisms. The major resistances to water flow can be located in the soil, or at the soil-root interface (Faiz and Weatherly, 1978 & 1982), and so data obtained with the HPFM may not reflect the resistance actually experienced.

An important aspect of the hydraulic characteristics of the *Eucalyptus* spp. clones measured in the present study was the considerable contribution of the roots to total plant hydraulic resistance, being 72 to 75% in high watered plants in the 21 month study. This means that whole plant conductances largely reflect those of the roots, and any variation induced by water stress in shoots will have minimal effect of total soil-to-leaf resistances.

Root hydraulic conductances can be expressed either per unit leaf area supplied ($K_{r/LA}$) or per unit root dry mass ($K_{r/trdw}$). Conductance expressed per unit leaf area is a measure of the sufficiency of the roots to supply the leaves; expressed per unit root

mass is a measure of the efficiency of the roots; how much carbon is invested in roots to achieve that sufficiency. $K_{r/LA}$ will be strongly influenced by the leaf area carried, and a common response to water availability is adjustment of leaf area to maintain the sufficiency of the roots. $K_{r/trdw}$ will be influenced by root morphology (surface area per unit mass), anatomy (extent of suberization) and physiology (activity of aquaporins).

In the 14 month experiment neither watering treatment or clone effected $K_{r/trdw}$, and proportional reductions in leaf area and root mass in response to low water were similar, such that no treatment or clonal effects were apparent in $K_{r/LA}$. Whole plant conductances expressed per unit leaf area ($K_{P/LA}$) were likewise not affected by watering treatment, although there was a slight difference among clones under low water conditions. The responses of hydraulic characteristics in the 21 month experiment were more complex. There was a clonal effect on $K_{r/trdw}$, with TAG14 having higher values than the other clones. Although the water effect was not significant, the efficiency of the roots of GC550 and GU210 was lower under low water, while that of TAG14 increased under low water. Although the low water treatment reduced root growth more than leaf area carried, leading to decreases in root:shoot ratio, this effect was similar across clones such that the patterns in $K_{r/LA}$ were similar to those in $K_{r/trdw}$. As roots constituted the major resistance, these patterns were also apparent in $K_{P/LA}$; whole plant conductance was reduced by low water in GC550 and GU210, and increased in TAG14.

The caveat concerning the use of the HPFM (maximum values only measured) remains. However, the slope of the relationship between leaf water potential and transpiration rate can be used as an estimate of whole plant conductance or resistance (the evaporative flux, EF, method). In the field studies the slopes of the relationship for the two clones were not different, in keeping with the HPFM measurements of $K_{P/LA}$ (perhaps coincidentally, although differences were not significant, both estimates showed TAG14 to have a higher conductance than GU210). In the 21 month study, the EF method indicated a decline in conductance in all three clones with low water. This is at variance with the HPFM method, which showed an increase in

TAG14. Using the EF method, increases in soil to leaf hydraulic resistances with reduced water availability have been shown in *Pinus sylvestris* (Irvine *et al.*, 1998) and in seedlings of loblolly pine (Wakamiya-Noborio *et al.*, 1999).

Another important aspect of hydraulic conductance is that of vulnerability to cavitation. Embolisms will block xylem conduits and increase hydraulic resistance and there are number of reports indicating good correlation between vulnerability to cavitation and drought tolerance or provenance (e.g. Franks *et al.*, 1995; Cochard *et al.*, 1992a; Alder *et al.*, 1996; Hacke and Sauter, 1996; Kavanagh *et al.*, 1999; Brodribb and Hill, 1999; see also Section 1.5.2). However, high vulnerability may not be a disadvantage under conditions of high evaporative demand as the water released by cavitation events is available for transpiration (Tyree *et al.*, 1991).

In the present study cavitation events were detected acoustically on main stems, and vulnerability was assessed as either the leaf water potential corresponding to maximum rates of acoustic emissions ($\psi_{L,EPH_{max}}$), or the mean water potential of data points between 5 and 10% of total cumulative emissions ($\psi_{cav,cUAE,\%}$). The methods yield different estimates of vulnerability, with $\psi_{cav,cUAE,\%}$ indicating greater vulnerability (initiation of extensive cavitations at higher water potentials) than maximum rates of acoustic emissions. In the 14 month study watering treatment influenced vulnerability as assessed as $\psi_{cav,cUAE,\%}$, but not as $\psi_{L,EPH_{max}}$ but had no effect, assessed by either technique, in the 21 month study. Both techniques (and the line intersection method in plots of cUAE against water potential, where this could be done) indicated that GC550 was more vulnerable than the other clones. At 21 months there was a linear relationship between vulnerability assessed by either method and minimum leaf water potentials developed, and the relationship was almost 1:1 with $\psi_{L,EPH_{max}}$. This implies stomatal control to reduce transpiration rates to prevent extensive cavitation occurring, and supports the suggestion that assimilation is limited by stomatal conductance. However, it should be pointed out that reductions in g_s were initiated at water potentials higher than the minimum developed.

7.3 Relationship between hydraulic characteristics and leaf physiology and growth

A positive correlation between stomatal conductance and hydraulic conductance of the soil to leaf pathway (assessed by the EF method) has been observed in sugar cane (Meinzer and Grantz, 1990) and in some Brazilian cerrado woody species (Meinzer *et al.*, 1999). Similarly, a good correlation was also shown between stomatal conductance and leaf specific hydraulic conductivity (of excised stems, though) in *Vitis vinifera* (Lovisolo and Schubert, 1998). In a recent study Brodribb and Field (2000) demonstrated a highly significant relationship between photosynthetic capacity (measured as the quantum yield of photosystem II) and leaf specific hydraulic conductivity (also of excised stems) in a range of conifers and angiosperms. However, none of these studies directly investigated the influence of water supply.

In the 21 month experiment in present study, reduced watering caused reductions in leaf water potential, stomatal conductance, net CO₂ assimilation and growth, and also reduced whole plant hydraulic conductance in two of the clones (measured by the HPFM; this reduction occurred in all three clones when assessed by the EF method). The relationship between whole plant resistance and water potential was not significant, although this is not surprising as the reduced transpiration rate would maintain water potentials close to those of the high watered plants. Across all treatments and clones there was a weak, but statistically significant relationship between maximum stomatal conductance and whole plant hydraulic conductance. The relationship between growth (biomass production) and $K_{P/LA}$ was marginally significant ($P=0.066$), but removal of two outlier points improved this to a P value <0.001 (these outlier points were a consequence of considerably lower leaf areas than average; the reason for this is not known). The response of $K_{P/LA}$ of TAG14 was different from the other clones and also at variance with the EF method, so two further regression analyses were undertaken: biomass against $K_{P/LA}$ with the data for low watered TAG14 and the additional outlier removed, and biomass against whole plant conductance estimated as the inverse of the slopes of the water potential vs. transpiration plots. These analyses yielded similar r^2 values (0.49 and 0.42,

respectively), both having a P value < 0.001. These data are consistent with the hypothesis of low hydraulic conductances giving rise to low assimilation and growth rates. (Some authors have undertaken regression analyses of mean values of hydraulic conductance against mean values of physiological parameters. However, it is felt that it is statistically sounder to use the entire data set in this study, even though this gives lower correlation coefficients and significance levels, as using averages contravenes the requirement that all data points in a regression analysis are independent.)

7.4 Is the relationship between hydraulic conductance and leaf physiology and growth causative?

There are two problems with the hydraulic limitation hypothesis as it stands. The first of these concerns the nature of the signal perceived by stomata that brings about closure; inherent in the hypothesis is the assumption that the signal is hydraulic. There has recently been considerable debate about hydraulic vs. chemical signals; Davies and Zhang (1991), Bano *et al.* (1993), Tardieu and Davies (1993) and Fort *et al.* (1997) invoke the involvement of ABA produced by the roots in controlling stomatal conductance, while Bunce (1999), Salleo *et al.* (2000) and Cochard *et al.* (2000) suggested the involvement of hydraulic signals, instead of chemical signals. This thesis provides no data that are pertinent to this debate, so the matter will not be discussed further.

The other problem is that the evidence supporting the hydraulic limitation hypothesis is purely correlative, and although this evidence may be persuasive, direct experimental evidence of a causative relationship between hydraulic conductance and growth is lacking. There have been a number of studies where manipulations of hydraulic conductance have been shown to effect leaf level physiology (e.g. Sperry *et al.*, 1993; Sperry and Pockman, 1993; Saliendra *et al.*, 1995; Brodribb and Hill, 2000; Cochard *et al.*, 2000; Hubbard *et al.*, 2001; Vernieri *et al.*, 2001). However, all these experiments were conducted in the short-term only, and long-term consequences of these manipulations of conductance have not been demonstrated. In an attempt to

address this problem the effect of long-term root chilling on hydraulic characteristics, leaf physiology and growth on two of the clones was undertaken.

The long-term response to reduced root temperatures can be complicated by morphological and physiological adjustments that could mask the effects of temperature on hydraulic conductance, and this seems to have occurred to some extent in TAG14. In GU210, long-term exposure to low root temperature caused a reduction in growth, all plant parts being reduced by about the same proportion. There was a reduction in $K_{r/trdw}$, and, because of the equivalent reduction in biomass of plant parts, this reduction translated into lower $K_{r/LA}$ and $K_{P/LA}$. The EF method of assessing soil-to-leaf resistance similarly indicated a decrease in whole plant conductance. These data are indicative of a direct long-term effect of temperature on hydraulic conductance, rather than an indirect effect of temperature through root sink strength. These reductions in hydraulic sufficiency were associated with reductions in stomatal conductance, net CO₂ assimilation and growth. Within the GU210 clone, the relationship between $K_{P/LA}$ and biomass was not significant ($P=0.199$), but the data set was small, and the relationship may have become significant if the experiment had been conducted for longer so that the differences in biomass between the treatments had become bigger. TAG14 seemed to undergo adjustment to long-term root chilling. The decrease in root growth was less than that in shoot growth, suggesting morphological adjustment, and the reduced $K_{r/trdw}$ observed in short-term chilling disappeared in the long term, suggesting physiological adjustment. The lack of effect on $K_{r/trdw}$ translated into lack of long-term chilling effect of $K_{r/LA}$ and $K_{P/LA}$. In keeping with this, the reduction in growth brought about by long-term chilling in TAG14 (13%) was less than that in GU210 (34%). (However, it must be pointed out that the EF method of assessing soil-to-leaf conductance indicated an effect of long-term chilling on TAG14; the same discrepancy was found with this clone in the 21 month study.) It should also be noted that root chilling did bring about reductions in stomatal conductance and assimilation rates, and a small reduction in growth. It is not clear whether this reduction in growth was a consequence of reduced hydraulic conductance, or some other response to reduced root temperature. Because of the morphological and physiological adjustments to root chilling exhibited by TAG14,

the results from this clone are unsuitable for testing the hypothesis of hydraulic limitation.

7.5 Conclusion

This study was undertaken to investigate the relationships between hydraulic characteristics, leaf physiology and growth. Different watering treatments were imposed to generate differences in growth. The studies were undertaken on three genetically closely related clones, to avoid complications in data interpretation that comparing material with different growth forms and growth patterns would create.

The material grown for 14 months was inadvertently severely water stressed, such that treatment differences on leaf physiology were not shown. In the material grown for 21 months in larger pots, plants exposed to reduced watering showed lower whole plant hydraulic conductances, lower stomatal conductances and net CO₂ assimilation rates, and reduced growth. Interclonal variations, where significant, were minor. There was a significant relationship between whole plant hydraulic conductance and plant growth. These data are consistent with the hypothesis that hydraulic conductance effects plant growth

Using two of the clones an experiment was conducted to directly test the hypothesis of hydraulic limitation, root chilling being used as the technique to manipulate hydraulic conductance. In one of the clones root chilling decreased hydraulic conductance, stomatal conductance and net CO₂ assimilation rate. There was an associated decrease in growth, and the data can be taken to support the hypothesis. In the other clone, there appeared to be morphological and physiological adjustments to long-term root chilling. The data are not such as to reject the hypothesis; rather, they are unsuitable to assess the hypothesis.

As a final caveat it should be borne in mind that growth is a complex process controlled by a number of factors, and so even under 'optimum' conditions it is unlikely that growth will be linked to a single character. A problem also arises when it

is attempted to control a character such as hydraulic conductance, as other properties are likely to be influenced as well. However, the data presented in this thesis, together with those from a number of other authors, do provide evidence that whole plant conductance is an important parameter influencing physiological status and growth.

A number of further studies could be undertaken. The plants investigated in this thesis showed an unusually high contribution by the roots to total plant hydraulic resistance. What is the basis for this: are root resistances in *Eucalyptus* spp. clones particularly high relative to other species (if so, why?), are shoot resistances lower than other species (if so, why?), and what are the ecological consequences of this high contribution by the roots to total plant resistance? In this study root conductances were measured using the HPFM, which gives maximum values: it would be interesting to measure the actual root conductances and thus make some estimate of the vulnerability of roots to cavitation. Both reduced water supply and low temperature decreased root hydraulic conductance per unit root dry weight: to what extent is this an anatomical response (development of the endodermis or formation of an exodermis) or a physiological response (activity of aquaporins)? The conductivity of stems was high, possibly indicating a high stem water content (if the trunks of felled trees are cut into 1 m lengths, water has been observed to drain from these segments under gravity): would cavitation events in stems make this water available to the transpiration stream, and what influence would this have on the overall water budget of tree, and the response to water stress? The clones studied here have been bred specifically for the production of pulp: to what extent have the hydraulic properties, particularly of the stems, been affected by this? This could be extended to a detailed comparison of fast versus slow growing trees.

Chapter: 8 References

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