Broad crowned trees and the hydraulic limitation hypothesis

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Abstract

The hydraulic limitation hypothesis (HLH) provides a physiological explanation of what limits height in trees. It states that resistance to water flow increases with pathway length, causing water potential to decrease and, as a consequence, the premature closing of stomata thus limiting photosynthesis and growth. The existence of broad crowned trees, however, appears to present a challenge to the HLH as vertical growth is more limited than that of longer horizontal shoots. This suggests that pathway length may not be the main factor leading to height limitation, because water is travelling a longer distance in the horizontal stems than in the vertical ones. In this thesis I investigated the HLH and factors influencing tree shape and height in *Acacia papyrocarpa* Benth, a broad crowned tree from south-eastern Australia.

Mature, isolated *A. papyrocarpa* trees from two different sites were found to have asymmetric crowns with a non-random, northerly orientation. This orientation could not be explained by wind direction, or loss of branches due to mistletoe infection. The most likely explanation is that the northerly orientation maximises light interception during the Southern Hemisphere winter.

At two sites with contrasting water availability, trees were taller at the more mesic site whereas phyllode $\delta^{13}C$ at the top of the canopy was similar in trees from both sites. These results are in agreement with a water limiting mechanism. However, in trees with longer horizontal pathways than vertical ones, phyllode $\delta^{13}C$ of the longest horizontal stems was lower than that at the top of the tallest vertical stems. Thus, longer path length did not result in more conservative water use as has been argued for the HLH. Because there were no differences in light environment or in hydraulic conductivity between branches sampled at the two canopy positions, the difference in phyllode $\delta^{13}C$ suggests that the effects of gravity on water transport could be more important than pathway resistances.

Following these results, I had planned to quantify some effects of gravity on water status in small trees, however, preliminary measurements of xylem pressure potentials in fully hydrated leaves showed a large variability that overcame the intra-canopy differences that gravity would be predicted to generate. In attempting to account for this variability I measured balance pressure (BP) on fully hydrated, non-transpiring detached leaves from 4 different species. BP in such leaves should be close to 0 kPa, however, it ranged from 3 kPa to 200 kPa or higher, despite a calculated measurement error of only 2 kPa. The variability in BP could not be solely accounted for by differences in species, hydration time, plant water status, light history, or leaf position on the plant. Leaf area and LMA, however, did explain up to 61% of BP variability in some species. The negative non-linear relationships between these leaf characteristics and BP suggest that leaf growth was causing part of the disequilibrium. In order to reduce confounding factors during pressure chamber measurements, leaves need to be selected carefully to avoid the large variability that may be associated with leaf growth.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except were due reference has been made in the text.

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Chapter 1) Introduction and literature review

1.1 Introduction

It is widely accepted that vertical growth in trees is an evolutionary consequence of competition for light (Iwasa et al. 1984, King 1990, Falster and Westoby 2003). However, within a species tree height is limited not only by genetic constrains, but also by environmental and physiological processes. An upper limit on the maximum height that a tree can reach is determined by the mechanical properties of its wood but in nature, trees rarely achieve this maximum. Some years ago the hydraulic limitation hypothesis (HLH) was proposed to explain the physiological limits to tree height (Ryan and Yoder 1997). It states that resistance to water flow increases with pathway length, causing water potential to decrease, which triggers the premature closing of stomata limiting photosynthesis and thus growth. Since then, a number of researchers have provided evidence in support of the HLH (e.g. Ryan and Yoder 1997, Koch et al. 2004, Ryan et al. 2006), however, it has also been criticized because most trees have mechanisms that compensate for increased resistances to water flow related to path-length (Becker et al. 2000b). The original mechanism of the HLH was based more on the effects of water pathway length than on the effects of gravity, in contrast, there is an alternative mechanism that is based on the effect that gravity has on cell turgor and therefore cell growth (Koch et al. 2004, Woodruff et al. 2004). In order to have a deeper understanding of the hydraulic limits to tree height it is relevant to know whether the original source of hydraulic limitation is water pathway length or gravity. The shape of broad crowned trees may be useful in this regard. For example, the HLH in its original form fails to explain why growth of vertical stems in broad-crowned trees ceases while growth of longer horizontal shoots continues. In this case, length per se cannot account for the limit in height because growth continues in the horizontal stems, even though the path length for water transport is greater than that through the vertical stems of such trees. Thus, if hydraulic factors are influencing the shape of broad crowned trees, they may be a consequence of gravity rather than path length resistances.

In this thesis I focused on the crown architecture of a broad crowned tree, *Acacia papyrocarpa* Benth (western myall). A number of hypotheses have been proposed to explain height and crown shape of broad crowned trees, but hydraulic limitation is not one of them (e.g. Iwasa et al. 1984, Midgley et al. 2001, Archibald and Bond 2003). Therefore, I first investigated whether maximum height in these trees is influenced by hydraulic limitations, regardless of the mechanism(s) responsible for the hydraulic limitation (Chapter 2). Then, after having found evidence supporting hydraulic limits to tree height, I compared effects caused by length and height in the same trees to test if pathway length or gravity effects were responsible for the limits in tree height (Chapter 3).

The hydrostatic gradient is the original source for the effects of gravity on water transport. However, this gradient has been considered too small in comparison with the high xylem tensions recorded in plants (e.g. Passioura 1982, Ryan and Waring 1992). In addition, even the existence of a continuous water column, as proved by the gravitational hydrostatic gradient, has been questioned, challenging the main theory of water transport: the cohesion-tension theory (Zimmermann et al. 1993, 1994, 2004). Therefore, studies testing the hydrostatic gradient are particularly needed as are those dealing with sources of error of the techniques used to measure it.

In this thesis I tried to test the hydrostatic gradient using the pressure chamber in small plants. However, due to the level of accuracy that was needed to assess the hydrostatic gradient in small plants, I focused first on the precision and accuracy of the pressure chamber technique. I found sources of variation in measurements that prevented me pursuing the original objective of studying the hydrostatic gradient. Instead I attempted to isolate the sources of variability in these measurements (Chapters 4 and 5).

1.2 Factors influencing tree height

Each tree species has its own genetic characteristics for growth, branching pattern and wood strength, among others, that will determine its maximum possible height. Plants also respond to their environment through physiological processes that cause individuals with the same genetic makeup, but growing in different conditions, to reach different maximum heights. Although some hypotheses have been suggested to explain these physiological limitations it is only recently, with the postulation of the hydraulic limitation hypothesis (HLH; Ryan and Yoder 1997) that discussion of this theme has re-awakened (e.g. Becker et al. 2000b, Mencuccini and Magnani 2000, Ryan et al. 2006). In this review I will first discuss the range of hypotheses put forward to explain maximum height, and then, I will discuss how crown shape in some arid and semiarid trees may help refine the mechanistic basis of the HLH.

1.2.1 Maintenance Respiration Hypothesis

One of the earliest explanations of what limits tree height is the maintenance respiration hypothesis (MRH). Yoda et al. (1965) reasoned that total respiration relative to photosynthesis would increase with tree size because the living portion of woody biomass (the sapwood) increases more than leaf area. Because photosynthesis is proportional to leaf area, higher sapwood:leaf ratios will yield greater respiration costs relative to photosynthesis. The increased respiration costs use carbon that, in a smaller, younger tree, would contribute to further wood production. This hypothesis has been questioned because the respiration of woody biomass has been estimated to be too low to account for the limit in plant height (Ryan and Waring 1992, Mencuccini and Grace 1996). In addition, the MRH cannot account for the variation in maximum tree height, within a species, for trees growing in similar climates (Ryan and Yoder 1997), nor why growth in trunk diameter continues long after growth in height has ceased (e.g. Bullock 2000), or why horizontal branches in broad crowned trees continue growing after vertical growth has stopped. In fact, broad crowned trees decrease their sapwood:leaf ratios when growth in height decreases and lateral growth continues (e.g. Phillips et al. 2003).

1.2.2 Mechanical Limitation Hypotheses

The maximum height that a plant can reach has also been explained in terms of biomechanical constraints (e. g. McMahon 1973, Becker et al. 2000b). McMahon (1973) found that none of 576 tree species studied was higher than the critical buckling height, and concluded that size proportions (i.e. height relative to stem diameter) are limited by elastic or mechanical factors. Niklas (1994) found that plant heights in 111 species examined were only one-fourth the maximum theoretical height that stems could reach before they undergo predicted elastic-mechanical-failure. He argued that this “safety factor” is mechanically desirable because stems typically sustain additional loadings (*e.g.* wind pressure, impacts from falling branches, the biomass of epiphytes, snow or ice accumulations). However, in a later paper, Niklas and Spatz (2004) presented a growth-hydraulic model that provided more accurate and realistic predictions of height than the mechanical model of McMahon (1973). They argued that the previously estimated “safety factors” were speculative because mechanical constraints on plant heights had not been tested. In contrast, Sperry et al. (2008) argued that the scaling of stem diameter with height is a consequence of mechanical constraints rather than hydraulic ones. They argued that these mechanical constraints prevent top-heavy trees that would otherwise be more hydraulically efficient. Evidence in support of this is the scaling of stem diameters and length in vines which is quite different from that of self-supporting trees. Recently, Niklas (2007) showed with theory and empirical relationships that maximum tree height is not constrained mechanically, however, he also argued that both mechanical and hydraulic constraints may together limit maximum tree height.

Evolution of self-supporting trees may have been driven by both mechanical and hydraulic constraints (Niklas 2007). According to the principle of equalization of marginal returns on alternative expenditures, natural selection would result in many traits limiting height simultaneously and not a single one (Westoby et al. 2002). However, under natural conditions the relative importance of the different factors changes, for example, while wind may be very strong and limitative in some environments, it may be insignificant in others. Thus, under particular conditions some plants could be more limited in height by catastrophic biomechanical failure than by other process. For example, Putz and Milton (1982) found that snapped trunks caused 60% of tree deaths in a tropical forest. This could be explained by weaker wood anatomy and architecture of some tropical trees in comparison with species from other environments (Bullock 2000) in combination with greater exposure to hurricanes. In contrast, in tropical forest with a strong dry season the higher parts of the canopy may be shed during the dry season because of water stress (Bullock 2000). This results in trunk diameter continuing to grow long after growth in height has ceased (Bullock 2000), making stronger trees with reduced mechanical limitations. In parallel with environmental factors, plant developmental patterns may also constraint plant height and influence whether mechanical or hydraulic factors may be more limiting to height. For example, palms may be limited mechanically because they have little capacity to increase the girth of stems. Thus, the safety factor against elastic buckling steadily declines as palms continue to grow vertically, so that stems of older individuals often bend under their own weight, limiting height (Rich 1987, Niklas 1993).

1.2.2.1 Wind effects

There are two different, but additive, mechanical factors that can influence trees. One is gravity-loading, i.e. the weight of a plant that may produce elastic or tensile/compressive failures at the base of the plant (Niklas 2007). The other mechanical factor is wind-induced drag forces that can cause mechanical failure before plants reach their theoretical critical buckling heights (Niklas 2007). However, wind may exert different kind of effects over plants and not all of them are mechanical. Therefore, it is important to outline them. First, wind may cause a direct mechanical failure or damage to leaves, branches, limbs or the whole plant. Secondly, wind may indirectly cause mechanical abrasion. For example, moving branches may have an abrasive effect on each other (Telewski 1995). Other sources of abrasion are substances carried by wind, such as dust, salt, ice or chemicals (Boyce 1954, Ogden 1980, Thomas 2000, Raventos et al. 2001, Nicolotti et al. 2005). Thirdly, wind may also cause developmental changes, a response called thigmomorphogenesis that usually includes a reduction in height growth while diameter growth is increased, resulting in stronger plants (Telewski 1995). Finally, wind may also induce atmospheric stress around leaves (Telewski 1995). This stress is not mechanical and is fully considered in the hydraulic limitation hypothesis described below. All these effects, individually or combined, may affect not only tree height but also crown orientation. The “flag” shape of exposed or isolated trees in extreme conditions (e.g. Wade and Hewson 1979, Noguchi 1979, Backhouse and Pegg 1984) is the best example that, in some regions, wind may be the main factor limiting tree height. Wind can not only limit height in some isolated trees, but also in more crowded conditions, for example the elfin forests of tropical environments (Lawton 1982, Cordero et al. 2007).

1.2.3 Hydraulic Limitation Hypothesis (HLH)

The hydraulic limitation hypothesis (HLH) was originally proposed by Friend (1993), and by Ryan and co-workers (Ryan and Waring 1992, Yoder et al. 1994, Ryan and Yoder 1997) to explain hydraulic limits to tree height. Currently, there are two proposed mechanisms for this hydraulic limitation: one is stomatal limitation of photosynthesis and the other is turgor limitation to cell growth (Koch and Fredeen 2005).

Stomatal limitation of photosynthesis was the only mechanism proposed by Ryan and co-workers in their original HLH. Their aim was to explain not only declines in forest productivity with age, but also site-to-site differences in tree growth, maximum height and productivity (Ryan and Waring 1992, Yoder et al. 1994, Ryan and Yoder 1997). They proposed that resistance to water flow increases with xylem path length in tall trees and large branches, causing a decline in leaf water potential and, eventually, stomatal closure early in the day. Because stomatal closure also limits carbon dioxide influx, any reduction in stomatal opening would also decrease daily (and eventually seasonal) carbon assimilation by the canopy. Similarly, Friend (1993), argued that due to gravity and resistances to water flow, leaf water potential at the top of a 100 m tall tree could fall below -1.6 MPa and that values as low as this have been related to a decline in stomatal conductance and photosynthesis in many species. This mechanism of the HLH can be examined using a hydraulic model that combines stomatal conductance to water vapour and hydraulic conductance along the path from soil to leaf (Hubbard et al. 1999, Barnard and Ryan 2003, Delzon et al. 2004, Franks and Brodribb 2005):

$$G_s = K_{\mathrm{L}}(\Psi_{soil} - \Psi_{leaf})/\mathrm{D} \qquad (1.1)$$

where,

G_s is the foliage stomatal conductance,

K_{L} is the leaf specific hydraulic conductance from soil to leaf,

D is the leaf to air vapour pressure deficit,

Ψ_{soil} is the soil water potential and

Ψ_{leaf} is the leaf water potential.

They argued that a reduction in K_{L} as trees increase in height would result in a proportional reduction in G_s if D and $\Delta\Psi$ (i.e. $\Psi_{soil} - \Psi_{leaf}$) remained constant.

The second mechanism associated with the HLH, turgor limitation, was proposed by Friend (1993) and further developed and tested by Koch et al. (2004) and Woodruff et al. (2004). It states that gravity effects on the water potential would reduce cell turgor with tree height if the osmotic potential remains constant along the tree. Because cell turgor is necessary for cell growth, if it diminishes then eventually leaf and bud expansion will cease at treetops. If the osmotic potential does not remain constant, i.e. when osmotic adjustment occurs, then, the cost of producing osmolytes would compete with growth for resources (Woodruff et al. 2004). Reduced hydraulic conductance may also directly affect tissue growth, limiting the size of distal xylem conduits and further increasing hydraulic limitation (Sperry et al. 2008). In addition, slower growth with increased height would lead to smaller annual increments in photosynthetic leaf area at treetops, further affecting carbon assimilation and allocation.

1.2.3.1 Compensating mechanisms for hydraulic limitations

Plants may have mechanisms that partially compensate for the hydraulic limitations of greater height, i.e. plants could acclimate as height increases (Barnard and Ryan 2003, Ryan et al. 2006). For example, it is argued that xylem conduit tapering should permit that total resistance of a tube running from trunk to petiole to remain constant, regardless of path length (West et al. 1999, Becker et al. 2000a). This could ensure an equal water supply to all leaves, both at different heights within a plant, and in plants of different sizes growing in similar environments. However, empirical data indicate that trees approaching their maximum height have non-optimal conduit tapering, which does not fully compensate for increased length (Anfodillo et al. 2006). Furthermore, this tapering model does not take into account the total number of parallel conduits per growth increment, which may make whole tree conductance length dependent (Sperry et al. 2008). Another possible compensatory mechanism is that sapwood area relative to leaf area (As:Al) generally increases with tree height (McDowell et al. 2002b), thus compensating for increased path length resistance as height increases (McDowell et al. 2002b, Barnard and Ryan 2003). Other factors that may offset hydraulic limitations are an increase in water storage capacity in stems (Goldstein et al. 1998), and an increase in allocation to fine roots as trees grow in height (Magnani et al. 2000). Both may compensate for the increased contribution of stems to total hydraulic resistance with size. All these structural changes in xylem anatomy and biomass allocation help ameliorate any reduction in hydraulic conductance (K_L in eq. 1.1) with path length. In addition to structural changes there also seems to be some physiological acclimations to tree height. For example, osmotic adjustment may partially compensate for the effects of height on cell turgor (Woodruff 2004). There is also a physiological adjustment in the minimum water potential supported by plants (affecting $\Delta\Psi$ in eq. 1.1) that compensates for tree height. In some species, taller trees have been shown to have greater $\Delta\Psi$ than small trees and because an increase in $\Delta\Psi$ increases the driving force for water flow, this may compensate for tree height (McDowell et al. 2002a, Barnard and Ryan 2003). However, lower xylem pressure potential would also affect xylem cavitation and cell growth (Sperry et al. 2008).

Initially, these compensations were taken as evidence against the HLH (e.g. Becker et al. 2000b). However, it was argued that the fact that these compensations occur is evidence that hydraulic limitations must be important for the overall fitness of woody plants (Bond and Ryan 2000). It was also proposed that the HLH should include the costs of all the compensation processes, i.e. the cost of acclimation to height (Mencuccini and Magnani 2000).

1.2.3.2 Evidence supporting the Hydraulic Limitation Hypothesis

The HLH has attracted much attention and is currently the best supported hypothesis for explaining limitations to tree height (e.g. Meinzer et al. 2001, Midgley 2003, Koch et al. 2004, Ryan et al. 2006). It has been shown that tall trees have lower transpiration rates and total hydraulic conductance (Ryan et al. 2000) and lower net photosynthetic rate and stomatal conductance than shorter trees (Kolb and Stone 2000). Stomatal conductance and photosynthesis also decline when plant hydraulic conductance is reduced experimentally (Sperry 2000, Hubbard et al. 2001). Hydraulic conductance is also related to photosynthetic capacity (Brodribb and Feild 2000). In general it is well established that hydraulic conductance by itself correlates with stomatal conductance and photosynthesis (see Tyree 2003 for a review). Grafting studies have also shown that it is size, and not age, that reduces growth at treetops in tall trees (Bond et al. 2007, Mencuccini et al. 2007). However, there have also been results no consistent with the HLH especially during instantaneous measurements, for example leaf level gas exchange and xylem pressure potential (see review in Ryan et al. 2006). In contrast, when more weighted indicators are used, for example stable carbon isotope composition ($\delta^{13}C$), the data are more consistent with the HLH (see review in Ryan et al. 2006).

Stable carbon isotope composition ($\delta^{13}C$) of plant tissues has often been used to test the hydraulic limitation hypothesis. C_3 photosynthesis preferentially uses the lighter ^{12}C isotope and discriminates naturally against the heavier ^{13}C isotope, however, when stomatal conductance declines discrimination also decreases (Farquhar et al. 1989). Thus the ratio of $^{13}C/^{12}C$ ($\delta^{13}C$) in plant tissue provides a more integrated measure of stomatal conductance than instantaneous measurements because it reflects long-term effects. For this reason it has often been used to test the hydraulic limitation hypothesis (Yoder et al. 1994, McDowell et al. 2002a, Koch et al. 2004). It has been found that $\delta^{13}C$ in trees is consistent with increased stomatal closure with tree height, i.e. $\delta^{13}C$ values increase with increasing height in the same tree (Ryan and Yoder 1997, Koch et al. 2004). Similar patterns were found in the upper canopy of young and old trees, i.e. higher values of $\delta^{13}C$ on taller trees in comparison with shorter ones (Yoder et al. 1994, McDowell et al. 2002a). The isotopic ratio also increases with branch length (Waring and Silvester 1994). These results suggest lower stomatal conductance in tall trees relative to short trees and with increasing height in the same tree.

Although there have been attempts to determine the relative contribution of length and height on the isotopic ratio, both parameters are confounded because they are usually measured in vertically oriented trees (e.g. Koch et al. 2004). In addition, nitrogen variability seems also to add a confounding factor in forest trees (Duursma and Marshall 2006).

Most studies testing the HLH have focused on stomatal limitation to photosynthesis. There have been very few studies testing the effect of turgor limitation on growth (Koch et al. 2004, Woodruff et al. 2004). It has been shown that turgor decreases with increasing tree height, both at midday and also predawn when conditions are almost hydrostatic (Koch et al. 2004, Woodruff et al. 2004). Although a vertical gradient in osmotic adjustment has also been demonstrated for some trees, this has not been high enough to fully compensate for the vertical gradient in leaf water potential (Woodruff et al. 2004). Vertical trends in leaf size and LMA have also been shown to be consistent with reduced growth with increasing height (Marshall and Monserud 2003, Koch et al. 2004, Woodruff et al. 2004). In addition, branch elongation also reduces with increasing tree height (Woodruff et al. 2004). All these lines of evidence seem to suggest that turgor limitation could work simultaneously with stomatal limitation (e.g. Koch et al. 2004). The involvement of both mechanisms can be better examined by substituting $\Psi_{leaf} = P_{leaf} + \Psi_{\Pi leaf} + \rho_w gh$, and by using $R_{soil\ to\ plant}$ instead of K_L in Equation 1.1:

$$G_s = (\Psi_{soil} - P_{leaf} - \Psi_{\Pi leaf} - \rho_w gh)/R_{soil\ to\ plant}D \qquad (1.2)$$

where,

P_{leaf} is leaf pressure potential,

$\Psi_{\Pi leaf}$ is leaf osmotic potential,

$\rho_w gh$ is the gravitational potential term (with a magnitude of 0.01MPa per metre in height),

ρ_w is the density of water,

g is the acceleration due to gravity,

h is the height of water from soil to leaf (i.e. plant height, from root to leaf) and,

$R_{soil\ to\ plant}$ is the hydraulic resistance of the flow path from soil to leaf.

$R_{soil\ to\ plant}$ can be further separated into two components: $R_{soil} + R_{plant}$ where R_{soil} is soil resistance and R_{plant} is plant resistance from root to leaf. In addition, because P_{leaf} is the parameter related to turgor it is more convenient to isolate it from Equation 1.2.:

$$P_{leaf} = \Psi_{soil} - \Psi_{\Pi leaf} - \rho_W gh - (R_{soil} + R_{plant})\ G_s\ D \tag{1.3}$$

Equation 1.3 indicates that an increase in R_{plant} and/or h as trees increase in height would result in a proportional reduction in P_{leaf} if the rest of the parameters remain constant. However, as mentioned before, G_s and $\Psi_{\Pi leaf}$ are also reduced with height (e.g. Koch et al. 2004, Woodruff et al. 2004), which would allow further increase in tree height. Nevertheless, empirical data show that P_{leaf} is also reduced with height (e.g. Koch et al. 2004, Woodruff et al. 2004), which indicates that there could be a minimum for P_{leaf}, G_s and $\Psi_{\Pi leaf}$ and this may eventually stop growth. It may be possible that many traits contribute to limiting tree height, which is consistent with the principle of equalization of marginal returns on alternative expenditures (Westoby et al. 2002).

1.3 Broad crowned trees

1.3.1 Small and isolated trees

Most studies testing the HLH have used tall trees, making these studies difficult because of the specialized equipment needed for canopy access (Ryan et al. 2006). In contrast, small trees are logistically more accessible and may be an inexpensive alternative. However, very few studies testing the HLH have been performed on trees smaller than 15m (see Ryan et al. 2006). The cause of this bias has not been discussed before but I suspect that it may be related to the low resolution of some techniques (e.g. pressure chamber technique) in comparison with the high intra-canopy variability of the parameter being measured (see next section). Sufficient tree height would help to overcome such variability. However, by studying intra-canopy variability of some key parameters, and their causes, the HLH could be tested on a greater range of trees.

Some of the smaller trees that grow in the arid and semiarid environments have broad crowns. They usually grow in open environments, i.e. isolated, which is another advantage for studies testing the HLH, because studies performed in crowded environments sometimes confound the effect of tree size with that of the light environment (Duursma and Marshall 2006). A third advantage of using broad crowned trees is that the effects of height and pathway length on water transport can be examined separately in the same canopy (see section 1.3.3). Despite all these advantages, only one study testing the HLH in broad crowned trees has been published to my knowledge (Phillips et al. 2003). However, there have been many other studies and hypotheses, not related with the HLH, explaining height and shape of broad crowned trees.

1.3.2 Hypotheses explaining height and crown shape of broad crowned trees

In arid and semiarid environments many trees produce broad crowns particularly when they grow isolated from other trees (e.g. Lange and Purdie 1976, Midgley et al. 2001, Archibald and Bond 2003). Genetically fixed bud development determines branching patterns and therefore growth form (Hallé et al. 1978, Schulze et al. 1986). Genetic constraints, however, are not the only determinants of plant shape. Bud dormancy and release is influenced by hormonal, nutrient and environmental factors (Arora et al. 2003). Hormonal control is important (e.g. Leyser 2005) and is the means by which plants respond to environmental cues (Arora et al. 2003). Environmental factors by themselves seem to play a very important role in crown shape (Hallé et al. 1978, Wilson 2000). Broad crowned trees have a decurrent growth with lack of control of a terminal shoot leader (i.e. low apical control), this results in lateral branches growing as fast as, or even outgrowing, the terminal shoot producing a rounded tree top (Brown et al. 1967, Wilson 2000). This growth habit allows an extreme individual plasticity in tree shape. It has been argued that the benefits of spreading canopies outweigh the benefits of tall crowns (Midgley et al. 2001, Archibald and Bond 2003). Because resources may become limited with tree size, then the same factor that favours spreading would also limit plant height. Several factors have been proposed to explain this trade-off. For example, if vertical growth is limited because there is no benefit in being tall due to the lack of competition for light, then horizontal growth can be selected because it maximizes sunlight exposition (Iwasa et al. 1984). Other hypotheses suggest that trees trade height for spread because the great shade that is formed in those trees attracts grazers to below canopy sites; grazers fertilise the site, disperse fruits and help to prevent fires by removing under canopy vegetation (Midgley et al. 2001). Archibald and Bond (2003) suggested that in areas of frequent fire trees grow tall enough to overcome flame height (but see Balfour and Midgley 2006), in contrast, in areas free of fire trees grow shorter and laterally. They also argued that a wide, laterally spreading tree restricts access by large herbivores to the inside of the tree (Archibald and Bond 2003). Another explanation suggests that a short spreading canopy is a response to wind loading (Thomas 2000). A tree in the open may be exposed to more wind than in the forest, wind also increases with height, therefore shorter trees with spreading canopies would diminish wind load, sail area and lever arm effects without limiting their photosynthetic area. Wind may also increase the VPD between leaves and the atmosphere (Telewski 1995) and it is argued that flat-topped canopies help to resist the drying winds by allowing leaves to shelter each other (Horn 1971, Thomas 2000). In this thesis, I used the western myall, an Australian broad crowned tree, as a model for testing the HLH and most of the hypotheses described here.

1.3.3 Western Myall and the HLH

Acacia papyrocarpa Benth (Western Myall) is distributed in the arid and semi-arid parts of South Australia and Western Australia (Whibley and Symon 1992). *A. papyrocarpa* can develop a dense, broad canopy that can spread so much that older individuals frequently have branches that become procumbent. The change of crown shape as trees get older has been used to identify age classes in this species (Lange and Purdie 1976, Ireland 1997). This growth habit produces trees with horizontal branches that can be longer than the vertical height of the tree (Chapter 3). This tree can be useful to demonstrate that some of the hypotheses described above (section 1.3.2) do not provide a general explanation for the shape of broad crowned trees. For example, *Acacia papyrocarpa* grow in environments free of fires. Therefore two explanations related to fires (trees grow tall to avoid fires and crowns spread to attract grazers that then eliminate fuel) do not apply here. The chenopod shrublands where *Acacia papyrocarpa* grows seldom, if ever are subject to fires. Additionally, the absence of spines and other defences against browsing in Acacias in Australia suggests that herbivory by large animals may not have been an important selection pressure (Brown 1960). Therefore, the argument that crown spreading protects from browsing is not a sufficient explanation in all circumstances. Furthermore, horizontally oriented crowns are not compatible with the respiration hypothesis (section 1.2.1) because growth in height has stopped but investment in longer horizontal stems continues. Testing the HLH in broad crowned trees could weaken the other hypotheses. For example, if water transport limits height in broad crowned trees then there should be a relationship between maximum height and water availability. Such a relationship has been observed in at least one species of broad crowned tree, *Prosopis velutina* (Stromberg et al. 1992). The other hypotheses are not supported by the observation that trees can vary in height within a site because it is improbable that flame height, wind or herbivore height would vary within a site. However, water availability could vary because of topography, soil differences or distance to a water source.

If water transport were the main factor limiting height in *Acacia papyrocarpa* then the crown shape of this tree may be useful for identifying more specific mechanisms of the HLH. So far the HLH has been tested on very tall trees with vertical crowns. There has been no attempt to compare water pathway length versus tree height because both parameters are confounded in studies performed on vertical crowns (e.g. Koch et al. 2004, Barnard and Ryan 2003, McDowell et al. 2002a). A broad crowned tree is a good model for such studies because the effects of height and pathway length on water transport can be examined separately. Comparisons can be made between two parts, one with less length but more height (i.e. the top mainly vertical stems) and the other with more length but less height (i.e. the mainly horizontal branches). An HLH mechanism based on the increased resistances caused by pathway length cannot account for the limit in height in these trees because in the horizontal stems water is actually travelling a longer pathway but horizontally. Thus, this mechanism does not explain why these trees stop growing in height, because growth of longer horizontal shoots continues. However, longer horizontal branches could be explained by structural differences between vertical and horizontal branches and/or by the effect of gravity on water transport. Structural changes that affect hydraulic conductivity are not only related to path length but also to branch growth (Rust and Roloff 2002), position (Protz et al. 2000), and orientation (Schubert et al. 1995, Schubert et al. 1999). Therefore, it is possible that broader crowns have resulted from structural changes that improve hydraulic conductivity in horizontal branches and/or reduce it in vertical ones. On the other hand, an alternative explanation is that the effects of gravity on water transport are more important than path length resistances.

1.4 Water transport and possible effects of gravity

1.4.1 Cohesion-Tension theory

For centuries researchers have asked how water can be transported to the top of tall trees. In 1895 Dixon and Joly proposed the Cohesion-Tension (C-T) theory (Dixon and Joly 1895), which has been the most widely accepted explanation for water movement in trees during the 20th century (Scholander et al. 1965, Zimmermann 1983, Nobel 1991, Tyree 1997, Steudle 2001). According to this theory, when water evaporates from leaves a tension is created through the xylem and the water column is pulled upwards. The strong cohesive forces between water molecules allow an appreciable tension to exist in an uninterrupted water column from roots to leaves. One of the main assumptions of the theory is that the water column is not broken or interrupted (e.g. Scholander et al. 1965). The C-T theory has remained almost unchanged since its inception but new discoveries like the common occurrence of cavitation and its recovery on a daily basis (Salleo et al. 1996, Canny 1997b, McCully et al. 1998, McCully 1999, Tyree et al. 1999, Brodribb and Holbrook 2004), the hydraulic connection between the parenchyma tissue and xylem vessels (Schneider et al. 1999, Thurmer et al. 1999, Wistuba et al. 2000), and the involvement of aquaporins in water transport (Tyerman et al. 2002), suggest a role for living tissue in water transport in plants, a possibility not previously considered by the original C-T theory. The C-T theory has also been questioned because of possible overestimation of xylem tensions by the pressure chamber (e.g. Zimmermann et al. 1993, Zimmermann et al. 1994, Canny 1997a).

1.4.2 Gradients in water potentials

The best known effect of gravity on water transport is a water potential gradient at hydrostatic conditions. When water is not flowing through vessels there must be a gravity induced water potential gradient of 0.01 MPa per metre in height (Scholander et al. 1965, Hellkvist et al. 1974, Legge 1985, Nobel 1991). This gravitational hydrostatic gradient should be evident when the system is at equilibrium during the night and at dawn, when there is no transpiration. Indeed, gradients close to 0.01MPa per metre have been found under these conditions, (Scholander et al. 1965, Connor et al. 1977, Baurle et al. 1999, Koch et al. 2004). Smaller gradients (Hellkvist et al. 1974, Connor et al. 1977, Legge 1985, Zimmermann et al. 1994, Benkert et al. 1995) or lack of a gradient (Zimmermann et al. 2002) have also been reported which questions the existence of a continuous water column (Zimmermann et al. 1993, 1994, 2004). Nevertheless, it is accepted that the hydrostatic gradient may be the original source for the turgor limitation mechanism, therefore contributing to the limits to tree height (Koch et al. 2004, Woodruff et al. 2004).

Following transpiration during the day the former hydrostatic gradient becomes a hydrodynamic gradient which is steeper as a result of the resistance to water flow throughout the plant (Hellkvist et al. 1974, Connor et al. 1977, Baurle et al. 1999). Gradients larger than 0.04 MPa m^{-1} have been found during the day in tall plants (Hellkvist et al. 1974, Connor et al. 1977, Legge 1985). The steepness of the gradients seems also to be related to the evaporative demand of the leaves (Hellkvist et al. 1974, Connor et al. 1977, Legge 1985, Baurle et al. 1999). However, some researchers have found a gradient smaller than the gravitational pressure gradient even in transpiring plants (Tobiessen et al. 1971, Zimmermann et al. 1994, Benkert et al. 1995, Zimmermann et al. 2002), which has been taken as evidence against the Cohesion-Tension theory (Zimmermann et al. 1993, Zimmermann et al. 1994, Zimmermann et al. 2004). Nevertheless, the hydrodynamic gradient has been proposed to be the main source for the carbon limitation mechanisms (i.e. the stomatal limitation of photosynthesis) in the original hydraulic limitation hypothesis (Ryan and Waring 1992, Yoder et al. 1994, Ryan and Yoder 1997).

When Ryan and Waring (1992) first formulated the hydraulic limitation hypothesis they discarded the effect of gravity because “the -0.1 MPa difference expected from a 10-m difference in tree height would not likely affect stomatal conductance and photosynthesis”. Regarding the contribution of gravity to plant water potential, several authors have expressed a similar view, for example: “except in tall trees, it is usually safe to ignore gravitational potential” (Passioura 1982), or “in short plants the difference in gravitational potential is negligible” (Koch and Fredeen 2005). This view may be because “gravity reduces the absolute value of leaf water potential more in taller trees” (Phillips et al. 2003) in comparison to small ones (see also Koch and Fredeen 2005). However, the importance of gravity in small trees could be underestimated. Minimum leaf water potential reflects the balance between soil water supply and atmospheric evaporative demand, together with plant hydraulic characteristics (Bhaskar and Ackerly 2006). Equation 1.3 shows that soil water supply (which affects Ψ_{soil} and R_{soil}) and atmospheric evaporative demand (vapour pressure deficit, D) may be analogous to the effect of R_{plant} and/or h. For example, higher soil moisture (which increases Ψ_{soil} and reduces R_{soil}) may compensate for a higher R_{plant} and/or h (i.e. a taller plant) while the rest of the factors are held constant (see Chapter 2). In the other extreme, reduced soil moisture (which reduces Ψ_{soil} and increases R_{soil}) is always related with a shorter tree height (lower R_{plant} and/or h). Consequently, taking into account separately both soil and plant factors, it may be concluded that gravity reduces the absolute value of leaf water potential more in tall plants than in short trees, but also that soil moisture reduces the absolute value of leaf water potential more in short trees than in tall trees. Accordingly, arguing that the effects of gravity are not important in limiting height in short trees is analogous to contending that soil moisture is not important in limiting height in tall trees. Both factors are important in reducing the absolute value of leaf water potential in both short and tall trees.

Another possible source of misunderstanding is that the effects of gravity and pathway resistance have not been properly defined when analysing the hydraulic limitation hypothesis. The effects of gravity are often compared with resistances across the entire soil-plant continuum, but the comparison should only be against internal plant resistances because the studied effect is a longer or shorter water pathway in the plant, not effects due to soil moisture. Short trees are found in dry environments, and under dry conditions most resistances may be located in the bulk soil and at the soil-root interface (Cruizat et al. 2002). Root hydraulic resistance also increases with drought (Vandeleur et al. 2009), and the majority of the root resistance is located between the soil and the root stele (e.g. Amodeo et al. 1999). Therefore, when comparing the hydrostatic gradient in small trees against the entire soil-plant continuum it is logical that the effects of gravity would be very small in comparison with all resistances, but the majority of them would be related to soil drought and not to plant length. A proper comparison should take into account only of resistances from the root stele up to the top of the canopy.

The effects of gravity are exactly the same for every meter in height in both short and tall trees. If we accept that a tall tree can be appreciably affected by the effects of gravity in water transport then we are accepting that those trees are affected by only 0.01MPa for every meter in height and that their cells are sensitive to those small changes in water potential. The same consequences can be expected for small trees because there is no current hypothesis or evidence suggesting physiological differences between cells from short and tall trees.

1.4.3 Water tensions in plants

Most researchers accept that water tensions as high as –10 MPa can be found in some plants (Kolb and Davies 1994, Tyree 1997, Williams et al. 1997, Wei et al. 1999a, Pockman and Sperry 2000, Steudle 2001, Tyree and Zimermann 2002). When these values are compared with the gravity effect of only 0.01 MPa m^{-1}, it might has been logical to conclude that gravity is not important (Ryan and Waring 1992) or perhaps only for taller trees (Passioura 1982, Koch and Fredeen 2005) given that the highest tensions have been found in shrubs (e. g. Kolb and Davis 1994, Williams et al. 1997). However, if the maximum tension that could be supported by plants is much lower than currently accepted, the relative importance of the gravitational effect increases.

The maximum tension that could be supported by plants is still unresolved and this is an issue of current debate (see Tyree 1997, Meinzer et al. 2001, Zimmermann et al. 2004). Although most researchers accept as valid the high water tensions (-10 MPa) registered with the pressure chamber, there are still many discrepancies between this technique and other methods for measuring xylem tensions, especially in the range between –1.5 and -10 MPa. For example, comparisons between the pressure chamber and thermocouple psychrometer have shown the best agreement in the range 0 to -1.5 MPa with increasing discrepancies at higher tensions (Hardegree 1989). Comparison of the pressure chamber against the calculated rotational tension in a centrifuge showed good agreement at tension as high as –1.7 MPa, but there were no data at higher tensions (Holbrook et al. 1995a). Pockman et al. (1995), using centrifuged pressure to induce tension, found water potentials between –0.5 and -3.5 MPa. However, their results show that the best agreement between different techniques was at tensions lower than –2.0 MPa, with higher discrepancies between –2.0 and -3.5 MPa. More controversial have been the comparisons between pressure probe and pressure chamber techniques. One research team found agreement between both techniques over the range 0 to –0.4 MPa (Melcher et al. 1998) and another between 0 and –0.8 MPa (Wei et al. 1999b). In both studies, tensions bigger than -1.0 MPa were never measured with the pressure probe, but both teams interpreted the results in different ways. One team argued that this was because such high tensions do not exist, and the pressure chamber overestimates tensions (Melcher et al. 1998, Zimmermann et al. 2000). The other team argued that the pressure probe technique cannot measure tensions higher than -1.0 MPa and that pressure chamber measurements are valid (Wei et al. 1999a, Wei et al. 1999b). Clearly more research is needed to establish the highest tension that plants can support, but it seems to lie between -2 MPa to -10 MPa.

1.4.4 Pressure chamber

As discussed before, most comparisons between techniques are made against the pressure chamber which has been the most frequently used technique for measuring xylem pressure potential in plants and the only one used to test the HLH. In addition, much of the evidence for the Cohesion-Tension theory also depends on data obtained using pressure chambers (Tyree 1997). Thus, experimentally testing the assumptions associated with the pressure chamber remains critical. One of the assumptions, that after being cut water is held at the end of vessels in a transpiring leaf, was falsified by Canny (1997a) but positively tested by Tyree et al. (2003).

Most studies that have used the pressure chamber to study the hydrostatic gradient have been made in tall trees (e.g. Scholander et al. 1965, Tobiessen et al. 1971, Connor et al. 1977, Koch et al. 2004, Woodruff et al. 2004), very few in trees smaller than 15m (e.g. Hellkvist et al. 1974, Zimmermann et al. 2002) or in small non woody plants (e.g. Begg and Turner 1970, Turner and Begg 1973). This predisposition to tall plants may be related to the accuracy and/or natural variability during pressure chamber measurements because an accuracy better than 10 kPa is needed to distinguish a gradient of only 10 kPa per meter in small plants. In contrast, in larger trees enough tree height would help to overcome some measurement variability. This possibility was suggested by Scholander et al. (1965) when they wrote: “obviously the taller the tree the better our chances of success”. It should be noted that the precision during that pioneering work was of ~100 kPa. Although modern studies have shown that the technique can be as precise as ~5 kPa (e.g. Wei et al. 1999b), there seems also to be a high intra-canopy variability of more than 100 kPa for leaves at hydrostatic conditions and at the same canopy position (e.g. Zimmermann et al. 2002, Brooks et al. 2003). Therefore, it is important to study precision, bias and accuracy of the pressure chamber technique and the origin of the intra-canopy variability, in order to be able to measure gravity head in small plants, which are easier to manipulate and less expensive to research. The understanding of this variability during pressure chamber measurements may also help to resolve the contradictory data obtained for the hydrostatic gradient.

1.4.5 Xylem cavitation

The other mechanism where gravity may be important is in the refilling of cavitated vessels. Cavitation is a sudden change from liquid to vapour phase within normally water-filled xylem conduits (Tyree and Sperry 1989); its environmental causes include water stress and winter freezing. Water stress-induced cavitation occurs when xylem pressure becomes sufficiently negative to overcome the capillarity forces of water in pit membrane pores and air is aspirated into the vessels via those pores from adjacent air spaces (Zimmerman 1983, Sperry and Tyree 1988). As the percentage of vessels that are cavitated increases in a stem, the hydraulic conductance decreases triggering water stress and stomatal closure, and if the vessels are not refilled with water again (cavitation recovery) the ultimate consequences can be reduction of growth and dieback (Zimmerman 1983, Tyree and Sperry 1989).
Cavitation is a far more common phenomenon than had been assumed in the past. It has been found in several species growing in natural conditions (e.g. Langan et al. 1997, Williams et al. 1997, Pockman and Sperry 2000). It also has been shown to occur in roots, stems, branches, petioles and blades. Although diurnal changes in water content of vessels have been recognized for some time (Brough et al. 1986), it was not until recently that diurnal variation in cavitation and recovery of cavitated vessels was demonstrated (Salleo et al. 1996, Canny 1997b, McCully et al. 1998, McCully 1999, Tyree et al. 1999, Brodribb and

Holbrook 2004). Cavitation begins in the morning and recovery (water refilling) can begin early in the afternoon. One important observation is that some cavitation occurs before the stomata are completely closed (Brodribb and Holbrook 2003, Brodribb et al. 2003). The closure of stomata does not prevent cavitation from occurring, although it could prevent runaway cavitation. If cavitation is common, then recovery from cavitation should be a very important process in plants, because the longer it takes to recover the longer photosynthesis is impeded. The efficiency of the recovery mechanism may be an important factor in determining tree height.

1.4.6 Cavitation recovery

Cavitated vessels can be restored to their functional state after they are refilled with water. It had been thought that cavitation could be repaired during the night, especially after rain, when plant water potential is at its maximum, but a number of studies have shown that refilling of cavitated vessels can occur concurrently with transpiration, i.e. under conditions of water tension in the xylem (Salleo et al. 1996, Canny 1997b, McCully et al. 1998, McCully 1999, Tyree et al. 1999); although this is not universal across species (Hacke and Sperry 2003). Holbrook and Zwieniecki (1999) suggested that due to the overarching walls of the bordered pit and the contact angle of water it was possible that the positive pressures required for gas dissolution could be contained within refilling vessels by the formation of a convex gas-water interface within each pit chamber. In this way, there would be no contact between water under pressure in the pit channel and water under tension in the pit membrane. In a subsequent study they measured contact angle and inter-vessel pit geometry in six species and found good agreement with their model (Zwieniecki and Holbrook 2000).

There are at least three hypotheses for the refilling process. The earliest hypothesis involves the mechanism that had been suggested to explain root pressure. That is, the active loading of solutes into the root xylem would produce a very negative osmotic potential that would cause the movement of water into the xylem creating a positive hydrostatic pressure, forcing water up through the xylem into the stem (Lambers et al. 1998). However, some discrepancies have been found when the osmolality of the exudate and the external solution have been compared (see Pickard 2003 for a review). Pickard (2003) proposed an alternative hypothesis that could explain both root pressure and refilling of cavitated vessels. The main idea is that cell membranes may be different at two interfaces around the symplast: semi-permeable, with many aquaporins, at the soil-symplast interface, but permeable, with aquaporins plus pores larger than aquaporins (probably plasmodesma), at the symplast-xylem interface. This would result in two different fluxes: osmotically driven water uptake from soil towards symplast, and pressure driven water flux from symplast towards the xylem. The energy expended would be that of the osmolyte uptake pumps that would maintain the cell osmotic content and thus the cell wall pressure. A third hypothesis is the pit membrane osmosis hypothesis (Hacke and Sperry 2003) that proposes that contact parenchyma cells release solutes of large molecular size that are trapped in vessels by the pit membrane, acting as an osmotic membrane. The osmotic gradient drives water from cells and other vessels. The refilling vessel remains hydraulically connected to the transpiration stream.

All the proposed refilling mechanisms involve active processes driven by living tissue associated with the xylem. Different sources of evidence support the involvement of parenchyma cells in water transport and cavitation recovery, for example, this is suggested by the location, structure and function of plasma membrane aquaporins (Tyerman et al. 2002). Aquaporins have been found around the vascular tissue (Barrieu et al.1998, Otto and Kaldenhoff 2000), and girdling or the application of mercuric chloride (an aquaporin inhibitor) to the transpiration stream both reduce rates of recovery from cavitation

(Zwieniecki et al. 2000). It has also been found that parenchyma cells that accompany xylem vessels are hydraulically connected with the vessels (Schneider et al. 1999, Thurmer et al. 1999, Wistuba et al. 2000). Because parenchyma cells are interconnected (Chaffey and Barlow 2001) and because there is radial and axial water flow through the symplast (Anisimov 1993), then an osmotic gradient could be expected to occur along parenchyma cells adjacent to vessels in order to compensate for gravity effects on the water continuum throughout the symplast. Witsuba et al. (2000) found that in upright plants there was no longitudinal turgor pressure gradient, whereas a base to apex directed turgor pressure gradient developed during the night in horizontally placed plants. This was due to an increase in turgor pressure towards the apex. They also found that the cellular osmotic pressure was roughly 50 kPa higher in the upper part of a 5m plant than at the base, which is sufficient to overcome the gravitational hydrostatic gradient. They suggested that the unbalanced osmotic pressure of adjacent cells could be important in the refilling of cavitated vessels. Refilling of cavitated vessels of tobacco plants occurred simultaneously with an increase in turgor of the adjacent cells, indicating the important role of the "unbalanced" osmotic pressures of the tissue cells. If this osmotic gradient exists in all plants and is involved in the recovery of cavitated vessels it might be expected that lower branches are cheaper to repair than higher ones. There are two alternative consequences of gravity for cavitation repair. First, if an osmotic gradient that overcomes the effect of gravity can be maintained (e.g. Wistuba et al. 2000) then higher branches would have more investment in osmolyte production and/or active osmolyte uptake than lower branches. Second, if the osmotic gradient cannot be maintained (similar to what was found in leaves in Woodruff et al. 2004) then lower branches would have greater water availability than higher branches. Whatever the case, it makes the refilling after cavitation more costly in higher branches than in lower branches. Because cavitation decreases hydraulic conductance and triggers stomatal closure, then, I suggest, that gravity effects on the refilling mechanism may be more important than path-length resistances as the source for the carbon limitation mechanisms (i.e. the stomatal limitation of photosynthesis) in the hydraulic limitation hypothesis. This gravity effect on water transport could help to explain the shape of broad crowned trees.

1.5 Conclusions and research aims

During the last decade the HLH hypothesis has been the most studied and supported hypothesis explaining the limits in heights for trees (Ryan et al. 2006). However, in recent reviews it has been concluded that HLH may not be universal (Ryan et al. 2006) and that hydraulic factors may not be the only ones limiting tree height (Westoby et al. 2002, Niklas 2007, Sperry et al. 2008). Therefore, when studying limits in tree height different factors should be taken into account and their specific predictions should be tested. The HLH states that in tall trees increased path length creates resistances to water flow, which reduce water potentials, eventually causing stomatal closure early in the day. This results in a limitation of daily and seasonal carbon assimilation, and therefore growth. Other hydraulic mechanisms have also been proposed that may be alternative or additional to the one originally proposed (Koch et al. 2004, Woodruff et al. 2004, Sperry et al. 2008). To date, most studies testing the HLH have confounded tree length with tree height when using tall vertical trees. In this thesis I used a broad crowned tree as model system in which to compare gravity and path length effects in the same tree. This approach is relevant not only for the HLH but also for basic knowledge of water transport in order to differentiate between effects caused by path-length resistances and those caused by gravity. It is possible that gravity effects on cavitation and its refilling mechanism could be more important than path-length resistances in limiting stomatal conductance and tree height.

Using tall trees to test the HLH is logistically difficult and expensive because tall structures are needed (Ryan et al. 2006). In contrast, testing the HLH in small trees could be less expensive and logistically simpler. However, one of the limitations in studying small trees may be the accuracy and variability of pressure chamber measurements, especially when measuring the hydrostatic gradient in water potential. The hydrostatic gradient is the origin of many consequences that gravity can have on water transport and plant growth. Although it is widely recognized, some studies have recently questioned the existence of a continuous water column (Zimmermann et al. 1993, 1994, 2004). An initial objective of the project was to study the gravitational hydrostatic gradient in small plants and also intra-canopy differences in water potential, using the pressure chamber technique. To achieve this it would have been necessary to have an accuracy with the pressure chamber of less than 10 kPa (the effect of gravity per metre in height). However, extensive preliminary work showed variations in the measurements that were larger than 10 kPa, and that were difficult to control. This prevented me from conducting some of my proposed experiments. Instead, I studied precision, bias and sources of variability during pressure chamber measurements under controlled conditions; I also tested some assumptions of the pressure chamber technique (Chapters 4 and 5).

The main aims of this thesis were to:

1.- Characterise the architecture of *Acacia papyrocarpa* Benth (Western Myall) with respect to tree height, branch length and orientation. (Chapters 2 and 3)

2.- Test whether height in *A. papyrocarpa* is limited by water transport. Since there are alternative hypotheses that could explain height and shape of broad crowned trees, it is necessary to test the predictions of a hydraulic limitation mechanism. (Chapter 2)

3.- Use *A. papyrocarpa,* as a model to test the effects of tree height and pathway length on carbon isotope composition ($\delta^{13}C$) and hydraulic conductivity. This would be the first time that such comparisons have been made in the context of the HLH. (Chapter 3)

4.- Determine precision and bias during pressure chamber measurements and test the equilibrium assumption during xylem pressure potential determinations. The equilibrium assumption implies that water potentials between a non-transpiring leaf and the medium to which the leaf is attached reach equilibrium. It also implies that a non-transpiring leaf should have constant xylem pressure potential. (Chapter 4)

5.- Using controlled conditions, study the variability in balance pressure that occurs with pressure chamber measurements and search for possible causes of this variation. This would help to reduce variability during determinations and may help in solving some controversies related to water transport theory. (Chapter 5)

Chapter 2) Factors affecting height and crown shape in the broad crowned tree *Acacia papyrocarpa* Benth.

2.1 Abstract

In arid lands many trees develop low, widespread canopies. Lack of competition for light, mechanical stability against wind load, and interaction with fire and herbivores have been proposed for limiting height and promoting crown spread in broad crowned trees. In contrast, there is also a general hypothesis for the limits to tree height that suggests that height is limited hydraulically. Using western myall (*Acacia papyrocarpa* Benth, Fabaceae) as a model, I investigated which factor may impose the major limit on height in these broad-crowned trees. I measured tree height and carbon isotopes ratios ($\delta^{13}C$) at the tops of trees growing on a steep hillslope and on the adjacent base of the hill. Crown horizontal radius was measured every 90 degrees in 93 isolated trees from different sites. The same parameter plus foliage height was measured every 30 degrees in 10 young and 15 mature trees. Trees were significantly taller at the base of the hill where water availability is greater. In contrast, carbon isotopes ratios ($\delta^{13}C$) were similar for trees from both sites. Crowns were consistently oriented towards the equator at all sites. Foliage height increased from leeward to windward portions of the individual canopies, contrary to the expected consequences of a wind effect. The results suggest that even though trees may reach different maximum heights because of differences in water availability, they may have similar physiological limits. This supports a hydraulic limitation mechanism while contradicting other hypotheses because it is improbable that flame height or herbivore height would change accordingly at the same site. Competition for light was absent while the wind factor could not explain crown shape. I suggest that broad-crowned trees spread in the open because there are no neighbors, it

maximizes light interception and diminishes wind load, but a major physiological limit on height could be a hydraulic mechanism.

2.2 Introduction

There is large variability in the shape and size of tree canopies. Part of this variability is due to genetically fixed branching patterns and therefore growth form (Hallé et al. 1978, Schulze et al. 1986). Environmental factors, however, also play a very important role in shaping trees (Hallé et al. 1978). In arid and semiarid environments many trees produce broad crowns, particularly when they grow in open locations, isolated from other trees (e.g. Lange and Purdie 1976, Midgley et al. 2001, Archibald and Bond 2003). It has been argued that a number of factors favour a spreading canopy over tall crowns in open situations (Midgley et al. 2001, Archibald and Bond 2003). For example, while competition for light in forests can favour vertical growth, horizontal growth in open situations maximizes light interception (Iwasa et al. 1984). It has also been suggested that a spreading canopy is favoured in open situations because the shade that is formed attracts grazers that fertilise the site, disperse fruits and help to prevent fires by removing under-canopy vegetation (Midgley et al. 2001). Archibald and Bond (2003) suggested that, in areas where fire is frequent, trees grow tall enough to overcome flame height (but see Balfour and Midgley 2006), while in areas free of fire, trees grow shorter and laterally. They also argued that a wide, laterally spreading canopy restricts access by large herbivores to foliage inside the canopy (Archibald and Bond 2003). Another explanation is that a short spreading canopy is a response to wind loading (Thomas 2000). A tree in the open may be exposed to more wind than one in a forest, and wind load also increases with height, therefore shorter trees with spreading canopies would diminish wind load, sail area and lever arm effects without limiting their photosynthetic area. Wind not only causes direct mechanical damage, but can also reduce growth and increase leaf to air vapour pressure deficit (VPD) (Telewski 1995). It has been argued that flat-topped canopies help trees to resist drying winds by allowing leaves to shelter each other (Horn 1971, Thomas 2000). In addition to affecting height, wind can also affect crown orientation. It is well known that trees exposed to strong winds usually have a crown with a lower and smaller windward side, even presenting a “flag” shape in extreme conditions (Noguchi 1979, Wade and Hewson 1979, Backhouse and Pegg 1984).

In addition to all these factors, water availability may also influence tree height. The hydraulic limitation hypothesis (HLH) states that resistances to water flow increase with xylem path length, causing a reduction in leaf water potential with tree height and, eventually, stomatal closure early in the day (Ryan and Yoder 1997, Barnard and Ryan 2003, Ryan et al. 2006). Stomatal closure will result in daily, and eventually seasonal, reductions in carbon gain leading to limitations in growth. There is also another recently proposed mechanism that states that gravity effects on the water potential would reduce cell turgor with tree height and because cell turgor is necessary for cell growth, then leaf and bud expansion will eventually cease at treetops (Koch et al. 2004, Woodruff et al. 2004). While there is growing evidence supporting the HLH (e.g. Ryan and Yoder 1997, McDowell et al. 2002a, Koch et al. 2004, Woodruff et al. 2004, Ryan et al. 2006), it has also been criticised (e.g. Becker et al. 2000b) because plants may have mechanisms that compensate for the increase in resistance that occurs with increased path length (Becker et al. 2000b, McDowell et al. 2002a, Ryan et al. 2006). Nevertheless, it is accepted that the effect of gravity on water transport will always be present (Koch et al. 2004, Woodruff et al. 2004, Ryan et al. 2006). Therefore, a hydraulic limitation, may also explain height limits of broad-crowned trees (e.g. Phillips et al. 2003).

Testing the specific predictions of the HLH in broad crowned trees enables me to determine whether hydraulic limitation is a major determinant of shape and height, relative to other possible factors. Whole-tree performance can be examined with respect to HLH using a hydraulic model that combines stomatal conductance to water vapour with hydraulic conductance of the flow path from soil to leaf (Hubbard et al. 1999, Barnard and Ryan 2003, Delzon et al. 2004, Franks and Brodribb 2005):

$$Gs = (\Psi_{soil} - \Psi_{leaf})/(R_{soil} + R_{plant})D$$

(2.1)

where Gs is the foliage stomatal conductance, Ψ_{soil} is soil water potential, Ψ_{leaf} is leaf water potential, R_{soil} is soil hydraulic resistance, R_{plant} is plant resistance from root to leaf (per unit leaf area), and D is VPD. One of the main tenants of the HLH is that stomata must close to maintain Ψ_{leaf} above a minimum threshold (Barnard and Ryan 2003) and this stomatal closure leads to a growth cessation in height. According to Equation 2.1, a reduction in Gs, to maintain Ψ_{leaf} constant, can be a result of increased R_{soil}, increased R_{plant}, increased D, or reduced Ψ_{soil}. The gravitational component of water potential also diminishes Ψ_{leaf} as plants become taller. This contribution can be better examined by substituting $\Psi_{leaf} = P_{leaf} + \Psi_{\Pi leaf} + \rho_w gh$ in Equation 2.1:

$$Gs = (\Psi_{soil} - P_{leaf} - \Psi_{\Pi leaf} - \rho_w gh)/(R_{soil} + R_{plant})D \qquad (2.2)$$

where $\Psi_{\Pi leaf}$ is leaf osmotic potential, P_{leaf} is the pressure potential, $\rho_w gh$ is the gravitational potential term (with a magnitude of 0.01MPa per metre in height), ρ_w is the density of water, g is the acceleration due to gravity and h is plant height, from root to leaf.

It may be that P_{leaf} is a key trait for the limits height in plants (e.g. Koch *et al.* 2004; Woodruff *et al.* 2004) because a lack of turgor prevents cell growth (Cosgrove 1986, 1997a). Therefore isolating P_{leaf} from Equation 2.2:

$$P_{leaf} = \Psi_{soil} - \Psi_{\Pi leaf} - \rho_w gh - (R_{soil} + R_{plant})\, GsD \qquad (2.3)$$

Equation 2.3 predicts that high Ψ_{soil} and low R_{soil} would compensate for high R_{plant} and/or high h (i.e. taller plants), whereas low Ψ_{soil} and high R_{soil} would be related to low R_{plant} and/or low h (i.e. shorter plants), when the rest of the factors are held constant. Thus differences in tree height could be caused by differences in soil moisture (which is related to Ψ_{soil} and R_{soil}) without any difference in plant or leaf physiology. It should be noted that although osmotic adjustment (change in $\Psi_{\Pi leaf}$ with height, e.g. Koch et al. 2004, Woodruff et al. 2004) and compensations for R_{plant} (Ryan et al. 2006) may occur, they may not be high enough to avoid a reduction in P and Gs. In addition, a reduction in Gs may not completely prevent a reduction in P (Koch et al. 2004). The combined effect of R_{plant}, h, R_{soil} and Ψ_{soil} may be the origin of the strong relationship between maximum tree height and water availability observed in many species (e.g. Ladiges and Ashton 1974, Rambal and Leterme 1987, Stromberg et al. 1992, Holbrook et al. 1995b).

Plants reaching maximum height would have, at the top of the canopy, the lowest stomatal conductance possible (i.e. the one related with growth reduction and minimum turgor), and this physiological limit should not vary among trees with the same genetic constraints. Therefore, it is expected that plants reaching maximum height but growing in sites with different water availability would have the same Gs at the top of the canopy (Koch et al. 2004). Such prediction will be best detected through an indicator of long-term stomatal conductance such as stable carbon isotope composition ($\delta^{13}C$). Plants discriminate naturally against the heavy ^{13}C in comparison with ^{12}C, however, when stomatal conductance is lower the discrimination is also lower (Farquhar et al. 1989). Many studies testing the HLH have used $\delta^{13}C$ (Yoder et al. 1994, McDowell et al. 2002a, Koch et al. 2004). Some studies have found that $\delta^{13}C$ values vary from the base to the top of trees, a pattern consistent with increased stomatal closure with tree height (Ryan and Yoder 1997, Koch et al. 2004). Similar patterns were found when comparing the upper canopies of young trees with old ones (Yoder et al. 1994, McDowell et al. 2002a, Phillips et al. 2003). This technique can also be used to test whether trees reach the same physiological limit to maximum height on sites with different water availability (Koch et al. 2004). In theory, if trees from sites with different water availability reach their maximum height, and if they belong to the same population (i.e. with the same genetic constraints), then they should have similar $\delta^{13}C$ at the top of the canopy regardless of actual tree height (Koch et al. 2004).

In this study I used western myall (*Acacia papyrocarpa* Benth, Fabaceae, Fig. 2.1), an Australian species found in arid and semi-arid environments that produces very broad canopies, to investigate whether hydraulic limitation is a major determinant of tree shape and height, relative to other possible factors. Our specific aims were to: a) test if trees growing in sites with greater water availability are taller on average, b) test if trees reaching maximum height have similar carbon isotope composition at treetops regardless of the height itself, and c) quantify crown shape and orientation as it may indicate if wind is a major determinant of height in these trees. Because crown orientation could be related to other factors we also analysed mistletoe infection and sun exposition.

2.3 Methods

2.3.1 Study site

Acacia papyrocarpa Benth (western myall) is a broad crowned tree with a height varying from 4 to 11m and a dense spreading canopy (Fig. 2.1). The crown can spread so much that older individuals often have branches that become procumbent. This results in an array of very long, low lying branches. The characteristic crown shape has been used to identify age classes (Lange and Purdie 1976, Ireland 1997). The species is distributed in the arid and semi-arid parts of South Australia and Western Australia (Whibley and Symon 1992). This evergreen species has a seasonal flush of canopy growth in the driest part of the year, between November and February (spring-summer), followed by a period of increased phyllode mortality in winter (Maconochie and Lange 1970).

The study was conducted in South Australia at Middleback Field Centre (32º57'S, 137º24'E), 16 km NW of Whyalla, and at Nectar Brook station (32º42"S, 137º58'E), 26 km SE of Port Augusta (Fig 2.2). At both sites the climate is arid with average yearly rainfall of 223 mm (Middleback) and ~300 mm (Nectar Brook) with large inter-annual variability.

Summers are hot (mean daily maximum for January is 29.2ºC at Middleback and 31.9ºC at Nectar Brook) and winters mild (mean daily minimum for July is 7.2ºC at Middleback and 7.6ºC at Nectar Brook). At Middleback the dominant vegetation is an open woodland of *A. papyrocarpa* with a chenopod shrub understorey. *A. papyrocarpa* is mainly located in low lying areas, while other plants occupy more xeric sites on the slopes of rockier hills. The vegetation is similar at Nectar Brook, except that *A. papyrocarpa* is also found on hills as well as in valleys.

2.3.2 Crown shape and orientation

A survey was conducted using 43 isolated trees at Middleback station in January 2002. I chose isolated individuals whose canopies were at least 15 m from other canopies to avoid the influence of shading effects. Individuals of two age classes were chosen: newly mature trees in which the crown had begun to spread (width to height ratio > 1) and old mature trees with extensive crown spreading. I avoided very old individuals with branches resting on the ground and/or with canopy gaps in the middle of the crown. Crown radius over each of the four cardinal points (north, east, south and west) was measured in every tree. To do this, I localized the edge of the crown (i.e. its vertical projection) with a plumb line and then measured the straight distance to the base of the trunk (Fig 2.1). These four radii were used to calculate the average radius of individual crowns. The difference between each sector radius and the average radius was then calculated for each tree to give a measure of canopy asymmetry.

Another survey was conducted at Middleback in September and October 2001, and April 2003. Isolated trees, as described above, were used. I measured 10 young individuals with a rounded crown (width to height ratio = 1), and 15 mature individuals with a fully developed broad crown. Young individuals were mainly mistletoe-free whereas some of the mature trees had mistletoes. Horizontal branch length was measured at 30^{o} intervals (i.e. 12 crown sectors) around each tree as well as maximum tree height. I also measured the height of the lowest part of the canopy in each 30^{o} sector. Tree height was measured with an extendable measuring pole. One person held the pole while another one assessed from a distance the maximum height of the crown (Fig 2.1).

In order to assess whether mistletoe infection affects crown shape and orientation I counted the number of mistletoes in each quarter of the canopy for every tree during the first survey. The horizontal diameters of each mistletoe were also measured and then calculated their projected basal area. These areas were summed for each canopy quarter to give a cumulative area per quarter.

The impact of wind on canopy shape and orientation was assessed by analysing wind speed and direction data from the closest weather stations to the two sites (Middleback and Nectar Brook). Wind data were obtained from the Australian Bureau of Meteorology for Whyalla (closest meteorological station to Middleback), and for Port Augusta and Port Pirie (closest stations to Nectar Brook).

2.3.3 Tree height and stable carbon isotope ratios

A survey was also conducted at Nectar Brook in November and December 2002 where *A. papyrocarpa* is found growing in various topographic positions. To minimize any orographic effect on rainfall a small hill (around 120 m in height) was chosen for the study site. The hill included a steep hillslope (slope of 21 - 29%) and a very gently inclined slope at the base of the hill (slope of 1.8 - 9.6%). Soil on the hillslope had a calcrete horizon near the soil surface (~20cm) while at the base of the hill no calcrete was found down to a depth of 60cm (pers. obs.). The presence of calcrete could limit even further water availability for plants on the hillslope relative to those growing at the base of the hill. Because the gradient in water availability was manifested over quite small distances, relatively homogenous meteorological variables (rainfall, temperature, etc.) are expected throughout the site. Soils at the base of the hill may be richer in nutrients than soils on the hillslope; this interaction between moisture and nutrients is almost unavoidable and has been acknowledged since the conception of the HLH (e.g. Ryan and Yoder 1997). I ran a 500 m transect through the *A. papyrocarpa* population, beginning at the base of the hill (at ~35 masl) and finishing on the hillslope (at ~115 masl) about 200m before reaching the crest of the hill. I established 5 points, one every 100 m; 2 points on the base of the hill and 3 on the steep hillslope. The 10 tallest trees closest to each of the five points were used in the survey. In this survey the effect of shading on crown shape could not be removed because there were not enough isolated trees and most canopies were within few meters of other canopies. However, I again measured horizontal branch length at 90^o intervals around each tree (i.e. four crown sectors) and maximum tree height as in the previous surveys. Changes in hill slope along the transect were measured with surveying instruments (theodolite and levelling rod).

During the survey at Nectar Brook, the five tallest trees from each of three positions along the transect were selected: two on the steep hillslope and one on the base of the hill. From each tree I took one phyllode sample from the top of the highest vertical branch. Samples were oven dried at 60ºC for 72 h before being ground to a fine powder with a mortar and pestle. They were analysed for carbon isotope composition ($\delta^{13}C$) with a precision of ±0.10‰ in the West Australian Biogeochemistry Centre at the University of Western Australia.

2.4 Results

2.4.1 Crown shape and orientation

Isolated trees from Middleback had asymmetric crowns with non-random orientation (N=43, ANOVA $p<0.05$; Fig 2.3a, Table 2.1). North-facing horizontal branches were consistently longer than south-facing ones, and east- and west-facing branches were of intermediate lengths. Trees from Nectar Brook, in spite of being less isolated than Middleback trees, had a similar crown shape and orientation (N=50, ANOVA $p<0.05$; Fig 2.3b). Circular statistics (Batschelet 1981, Zar 1999) indicated that the mean angle for the shortest branch was 187º for trees beginning to spread (mature trees) and 142º for older trees at Middleback (Table 2.1). At Nectar Brook, the mean angle was 184º for trees on the hillslope while the position of the shortest branch was random for trees at the base of the hill. These results show similar mean angles between trees on the hillslope of Nectar Brook and trees at Middleback. The oldest sampled trees at Middleback had a tendency to shift their crown orientation (Table 2.1) which may be related to crown damage from mistletoe infection. The lack of significant directedness of the shortest lateral branch of trees at the base of the hill in Nectar Brook may be related to the size of the tree. These trees were selected because of their height (i.e. the tallest trees at each position) and some did not have complete canopies.

Measurements every 30º (azimuthal orientation) on mature trees with complete canopies at Middleback showed that there was a gradual change from the longest branches around north to the shortest branches on the south sides of trees (Fig. 2.4a). The same pattern of crown orientation was found in young trees without mistletoes (Fig. 2.4b). The average difference between the largest and shortest branches was around 1 m in mature trees while only 0.5 m in young trees (Fig. 2.4). The mean angles of the shortest branches for mature and young trees were 190º and 218º, while the mean angles of the longest branches were 21º and 7º, respectively.

Heights of the lower part of the canopy varied with azimuthal orientation of the crown sector (Fig. 2.5). Lower canopy height was non-random. Isolated trees with complete crowns had the N-NE part of the crown (mean angle 24º) closer to the ground than any other part of the canopy, while the S-SW part of the crown (mean angle 189º) was highest from the ground (Fig. 2.5, Table 2.1). Heights of the lower part of the canopy were negatively related to the length of the branches in each section (Fig. 2.6). Branches towards S-SW not only are the shortest in lateral length but also the highest from the ground (Fig. 2.4, 2.5 and 2.6, Table 2.1). The upper part of the canopy presented the same pattern, in height from the ground, as the lower part of the canopy (pers. obs.).

There was no difference in frequency of mistletoes or cumulative area between each of the four crown orientations (ANOVA p=0.2 and p=0.13 respectively). Similarly, there was no difference in the ratio of mistletoe area to crown area between each of the four orientations (ANOVA p=0.41). However, cumulative area of all mistletoes was positively correlated with the size of tree crowns (r=0.65).

Wind direction was mainly bimodal at the three weather stations (Fig 2.7). Southerly winds predominated in Port Augusta, south to south-easterly in Whyalla and south to south-westerly in Port Pirie. All three wind directions are clearly related with the presence of the Spencer Gulf (see Fig. 2.2), and could represent sea breeze, i.e. offshore winds that go from sea to land (see Simpson 1994). The second peak in the distribution was northerly for Whyalla and Port Augusta, and NW for Port Pirie, and could represent onshore winds going from land to sea. Based on the obvious relationship between wind direction and the presence of the Spencer Gulf, it is expected that in Middleback wind would be similar to that of Whyalla (i.e. predominantly south to south-easterly) whereas in Nectar Brook wind would be mainly from the Southwest. The whole area is dominated by light and moderate winds with almost no strong winds except for Port Augusta, in the upper part of the gulf, which seems to be the main passage for most of the channelled wind (Fig. 2.7).

2.4.2 Tree height and stable carbon isotope ratios

At Nectar Brook, trees growing on the steep hillslope were shorter (5.95±0.6m overall average and standard deviation [SD]) than trees growing at the base of the hill (7.5±1.4m overall average and SD; ANOVA $p<0.01$; Fig. 2.8). There was no difference in tree height between the three sites on the steep hillslope, or between the 2 sites at the base of the hill. Importantly, phyllode $\delta^{13}C$ at the top of the canopy was the same for trees from the steep hillslope (-24.7±0.7‰: both hillslope sites combined) and from the base of the hill (-24.8±0.8‰; ANOVA $p=0.247$; Fig. 2.9), despite the differences in tree hight (6.4±0.5m and 8.6±1.7m) between trees from the two topographic positions (ANOVA $p<0.01$; Fig. 2.9). Crown area, calculated as a circular area from the average of 4 crown radii, was not different among trees from the three sampling points (46.2±23.8m^2 and 56.4±13m^2 in the hillslope, 51.9±25.4m^2 in the base of the hill; ANOVA $p=0.76$).

2.5 Discussion

2.5.1 Crown shape and orientation

My data show that crowns of *A. papyrocarpa* at the study sites are asymmetric and non-random with a north to north easterly orientation. Among several factors that could explain this crown shape I investigated the impact of mistletoe infection, wind load and light interception. Mistletoes may contribute to the loss of branches (Tennakoon and Pate 1996) but no difference was found in mistletoe infection between the four canopy quarters of *A. papyrocarpa*, thus it is unlikely that mistletoe infection influences the N-NE crown orientation in this species. Furthermore, the fact that small trees without mistletoes had a similar orientation to older trees makes it unlikely that this N-NE crown orientation could have been caused by mistletoe infection.

Wind can also influence exposed tree crowns, depending on its strength and direction (Noguchi 1979, Wade and Hewson 1979, Backhouse and Pegg 1984, Telewski 1995). In the present study there was no evidence to suggest that wind was the major factor determining the N-NE orientation of *A. papyrocarpa* at the study sites. Wind speeds at both sites are generally light to moderate (<30km h^{-1}) which is likely to cause motion only in small branches but not major twig damage (Cullen 2002). Additionally, wind directions at the study sites seem to be controlled by the presence of the Spencer Gulf (see Simpson 1994), which results in wind blowing from different directions at the two study sites. Despite this difference, crown orientation was similar in trees from both sites. Finally and more importantly, the distribution of branch heights is not as expected for a broad crown affected by wind or salt. Wind would cause windward branches to have lower height than leeward branches (e.g. Noguchi 1979) or as Boyce (1954) stated regarding salt effects: "the surface of the crown commonly presents an even slope upward from the seaward side giving a peculiarly espalier aspect and indicating that the inhibition of branch development is diminished landward." However, branches in the studied trees were higher at the windward side than at the leeward side of the crown, i.e. the slope of the crown surface is downward from the seaward side. This crown shape is more related to avoiding self-shading than to consequences of wind and/or salt damage.

The consistency of the crown shape across different tree sizes and site conditions, as well as the negative relationship between branch length and height, suggests that the N-NE orientation may be related to maximising light interception during winter and not to damage by wind or mistletoes. This would be consistent with a general pattern in desert plants of foliage being oriented towards the equator. For example, in the shrub *Larrea tridentata* it was found that most plants had their branches oriented toward the equator in a way that would favour light capture in the mornings and in spring (Neufeld et al. 1988). The distribution of the rosettes in *Yucca brevifolia,* an arborescent monocot, were also predominantly towards the equator (Rasmuson et al. 1994). Similarly, *Pachipodium namaquanum,* a columnar succulent, showed a characteristic nodding of the terminal 20-

60cm of the apex at an inclination of between 45 and 65 degrees towards the equator (Rundel et al. 1995). Cladodes of *Opuntia spp*. have also been shown to be orientated towards the equator, especially when they develop during winter (Nobel 1986). Most of these studies have explained this characteristic orientation as an adaptation for more efficient interception of light when the angle of the sun is low, for example in the morning or afternoon and in winter or early spring which often coincides with the development of new foliage in desert species. To my knowledge, these data are the first to report evidence of crown orientation towards the equator in woody trees in an arid environment. The phyllodes in *A. papyrocarpa* are long and narrow (4-8 cm long, 1-3 mm broad, (Jessop and Toelken 1986) and mainly vertically oriented (pers. obs.). This would reduce radiation loads around midday in summer months but would enhance interception of light during morning and afternoon hours, and in seasons when the sun"s angle is low (Ehleringer and Werk 1986).

Crown orientation towards equator could be caused not only by differences in growth rate but also by differential loss of twigs, branches and big limbs. The evergreen *A. papyrocarpa* gains and loses phyllodes every year; gains occur in spring and summer whereas net losses occur from late summer until the next spring (Maconochie and Lange 1970). *A. papyrocarpa* also loses big limbs after stresses (Lange and Sparrow 1992, pers. obs.). I observed more evidence of branch mortality from the southern part of the crown, where more self-shading occurs. The distribution of branch heights in the lower part of the canopy was also consistent with avoidance of self-shading.

2.5.2 Limits to tree height and broad crowned trees

The data from the Nectar Brook survey suggest that tree height in *A. papyrocarpa* may be limited by water transport. If water transport is involved in limiting tree height then trees growing in sites with greater water availability should be taller on average, as I found for *A.*

papyrocarpa. Similar results have been reported in other species. For example, maximum height in *Prosopis velutina*, a broad crowned tree, varies as function of groundwater depth (Stromberg et al. 1992). A positive relationship between height and annual rainfall was found for *Eucalyptus viminalis* (Ladiges and Ashton 1974) and for the shrub *Quercus coccifera* (Rambal and Leterme 1987). In dry tropical forests there is a strong negative relationship between tree height and the duration of drought and a positive relationship with moisture availability (Holbrook et al. 1995b). These results represent further evidence supporting hydraulic limits to tree height. However, part of the differences in height in those studies could also be explained by genetic differences among different plant populations (e.g. Ladiges and Ashton 1974). In my study with *A. papyrocarpa*, I reduced genetic differences by sampling within a single population at the Nectar Brook site. I sampled the tallest trees at the base and further up the hill, and in each case only sampled mature trees with spreading canopies (see Lange and Purdie 1976). Spreading canopies indicate that trees have switched from predominantly vertical growth to lateral growth. Thus, the observed differences in height between trees at the base of the hill and those at the top, indicate that growth in height is limited at both sites, but at different heights.

I also compared the carbon isotope composition from the treetops and found an average value of -24.7 ‰. This value is high relative to other values for the same species (-25.7 ‰ at treetops and -26.4 ‰ at 2.5 m height; Chapter 3) or to the average from several central Australia trees (-26.9 ‰; including *A. papyrocarpa*; Ehleringer *et al.* 1985) and may be indicative of some water stress. The carbon isotope composition from the treetops was similar for trees from the steep hillslope and the base of the hill (Fig 7), as would be predicted by the HLH if water limitation at the top of the tree were similar at both sites. That is, in the two sites *A. papyrocarpa* had reached the same physiological limit to further vertical growth, but at different heights because of differences in water availability. Koch et al. (2004) reported a similar observation in redwoods growing with different conditions of water availability. Like *A. papyrocarpa* in this study, the redwoods also had different heights (~80m and ~110m), but similar carbon isotope composition (-22‰) at treetops. Koch et al. (2004) also argued a similar "physiological ceiling" for both groups of trees.

Our data suggest that tree height in *A. papyrocarpa* is limited by hydraulic mechanisms. In other study I used the broad crowned architecture of *A. papyrocarpa* to compare the contribution of both gravity and the pathway length to limiting tree height (Chapter 3). That study suggested that gravity may be more important than plant pathway length as the source of hydraulic limitation and supports other studies that have found gravity effects on water transport as being important in limiting tree height (e.g. Koch et al. 2004, Woodruff et al. 2004). If gravity effects on water transport are important in limiting tree height then they could drive the shape of broad crowned trees. Lower horizontal branches may be facilitated because they are less limited hydraulically than higher vertical branches. In the case of *A. papyrocarpa* horizontal branches could grow until weight causes them to fall, explaining the presence of procumbent branches in older individuals (Lange and Purdie 1976, Ireland 1997).

Other hypotheses proposed to explain height and crown shape in broad crowned trees may not hold as general explanations, or at least can be refuted in the case of *A. papyrocarpa*. First, having trees with different maximum heights at the same site (Nectar Brook) contradicts the other hypotheses because it is improbable that flame height or herbivore height would change accordingly at the same site. The difference in tree height was also independent of competition for light because this factor was practically absent in both topographic conditions on the hill at Nectar Brook. Secondly, there are some broad crowned trees growing in environments free of fires, such as *A. papyrocarpa* at my study sites. Therefore two explanations related to fires, namely, that trees grow tall to avoid fires and that crowns spread to attract grazers that eliminate fuel, do not apply here. In fact, more leaf litter accumulates under the crowns of *A. papyrocarpa* than in areas beyond the tree (Facelli unpublished data). In addition, it was recently found that even for sites with frequent fires the important parameter for avoiding death by fire was stem diameter rather than tree height (Balfour and Midgley 2006). Thirdly, in Australia tree browsing appears not to have been a major selective pressure as it is in other continents, as is attested by the lack of spines and other defences against browsing animals in Australian Acacia trees (Brown 1960). Therefore, the argument that crown spreading is an adaptation to protect foliage from browsing does not apply in this situation. Finally, wind can also be discarded as major factor limiting height in *A. papyrocarpa* at my field sites. Wind has direct mechanical effects on trees and may cause growth reduction (Telewski 1995), however, in isolated or exposed trees, it is expected that wind would affect the whole crown not only the tallest part of the canopy. Therefore, it is expected that protected leaves and branches attain larger heights than windward branches (e.g. Boyce 1954, Noguchi 1979). However, trees at my study sites had higher branches at the windward side than at the leeward side of the crown, contrary to the expected consequences of wind or salt affectation (e.g. Boyce 1954, Noguchi 1979).

In conclusion, I suggest that height in broad crowned trees, as well as crown spreading, may be explained by hydraulic mechanisms. Providing that trees have meristematic plasticity to produce horizontal stems and there are no interfering shadows of neighbours, trees could produce longer horizontal stems simply because those stems may be less limited hydraulically than taller vertical stems. Broad crowned trees have a crown shape that maximizes light interception and diminishes wind load, but the physiological limit for tree height could be hydraulic. Finally, asymmetry in the crowns of the *A. papyrocarpa* trees here studied, which were oriented to the north-northeast (towards the equator), is likely to be related to maximising light interception during the southern hemisphere winter.

2.6 Tables and figures

Table 2.1. Mean angle of the shortest, longest, highest and lowest branch of Western Myall, *Acacia papyrocarpa*, in Middleback and Nectar Brook. The mean angle and confidence intervals (CI) were obtained using circular statistics (Batschelet 1981, Zar 1999). The Rayleigh test was used to test whether the data differed significantly from randomness (Batschelet 1981, Zar 1999) and its critical level or probability value (p) is shown.

Site	Tree age class or position	Crown sectors	n	Mean angle ± 95% CI	p	Mean angle ± 95% CI	p
				Shortest branch		Longest branch	
Nectar Brock	Base of the hill	4	20	176 ± NS	0.27	22 ± 47	0.03
Nectar Brock	Hillslope	4	30	184 ± 21	<0.001	21 ± 29	<0.001
Middleback	Mature	4	23	187 ± 31	0.001	7 ± 50	0.04
Middleback	Old	4	20	142 ± 39	0.01	315 ± 27	<0.001
Middleback	Young	12	10	218 ± 40	0.01	7 ± 30	0.001
Middleback	Mature	12	15	190 ± 50	0.04	21 ± 53	0.05
				Highest branch		Lowest branch	
Middleback	Young and mature trees	12	21	190 ± 18	<0.001	24 ± 30	<0.001

Fig. 2.1. A photograph of Western Myall, *Acacia papyrocarpa*, showing how measurements of canopy dimensions were performed. a) Tree height, b) lower crown height, c) lateral length.

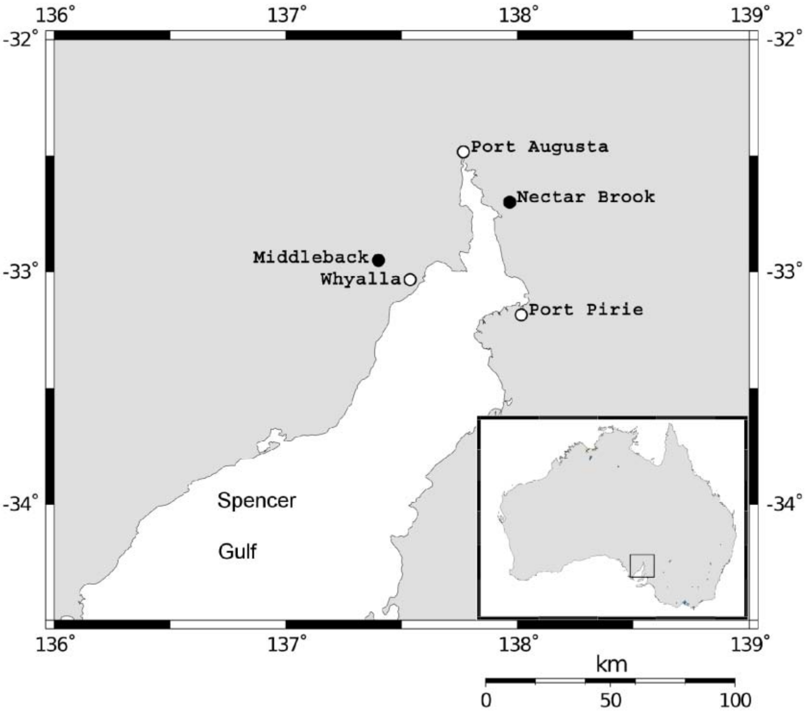

Fig. 2.2. Location of the two study sites in South Australia (filled circles). The closest weather stations to the study sites are also shown (diamonds).

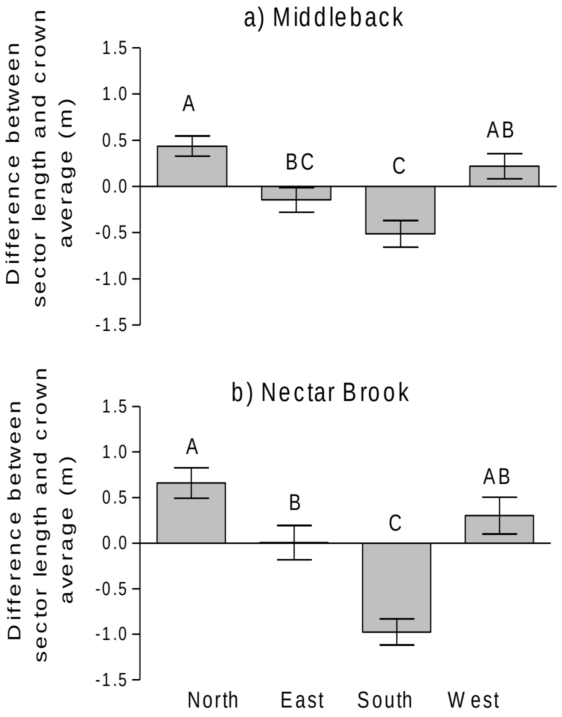

Fig. 2.3. Mean length of Western Myall, *Acacia papyrocarpa*, crowns in 90º sectors for a) isolated trees from Middleback (N=43), and b) trees from the hill at Nectar Brook (N=50). The y axis represents the average ± SE of the individual differences between the horizontal length of each sector (N, E, S, W) and the average radius of the individual crown.

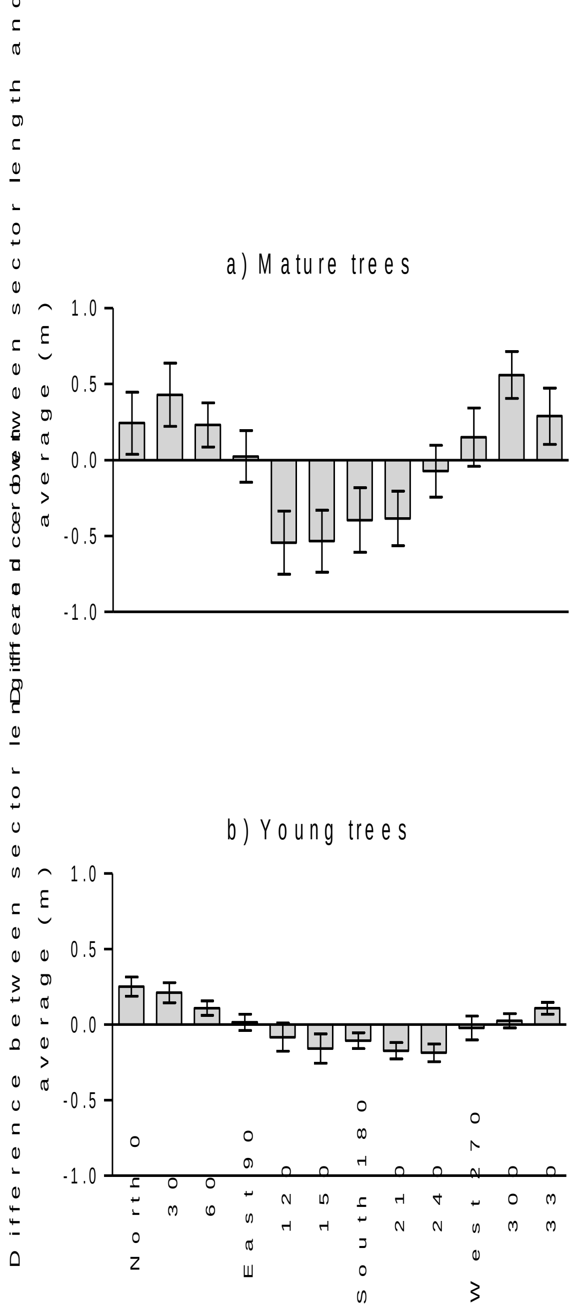

Fig. 2.4. Mean length of *Acacia papyrocarpa* crowns in 30º sectors for a) mature, non-procumbent trees (N=12), and b) young, non-spreading trees (N=10) at Middleback. The y axis represents the average ± SE of the individual differences between the horizontal length of each 30º sector and the average length of the twelve sectors of the individual crown.

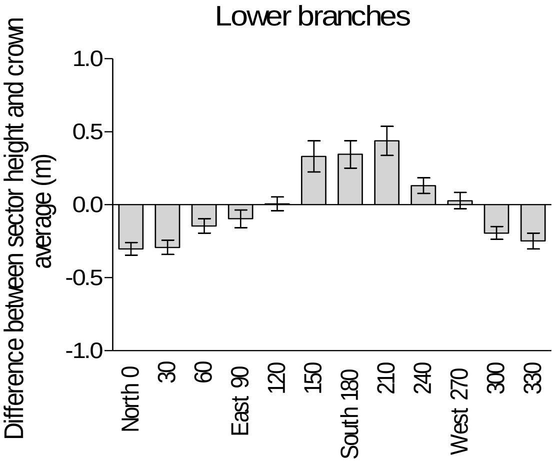

Fig. 2.5. Height from ground to the lower part of the canopy for *Acacia papyrocarpa* in 30º sectors of the crown, from isolated trees at Middleback (N=21). The y axis represents the average ± SE of the individual differences between the height of each 30º sector and the average height of the twelve sectors of the individual crown.

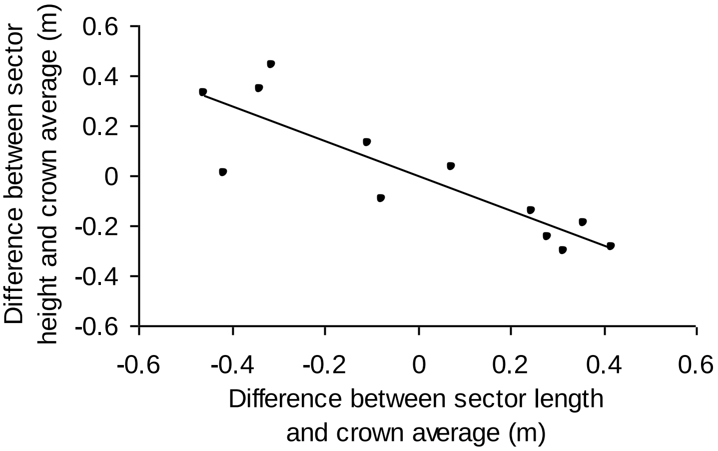

Fig. 2.6. Relationship between height from ground to the lower part of the canopy and length of *Acacia papyrocarpa*, from isolated trees at Middleback (N=21). Each point represents the same averages described in Fig 4 and 5, i.e. the average of the individual differences between the height, or length, of each 30º sector and the average height, or length, of the twelve sectors of the individual crown (r^2=0.75, p<0.001).

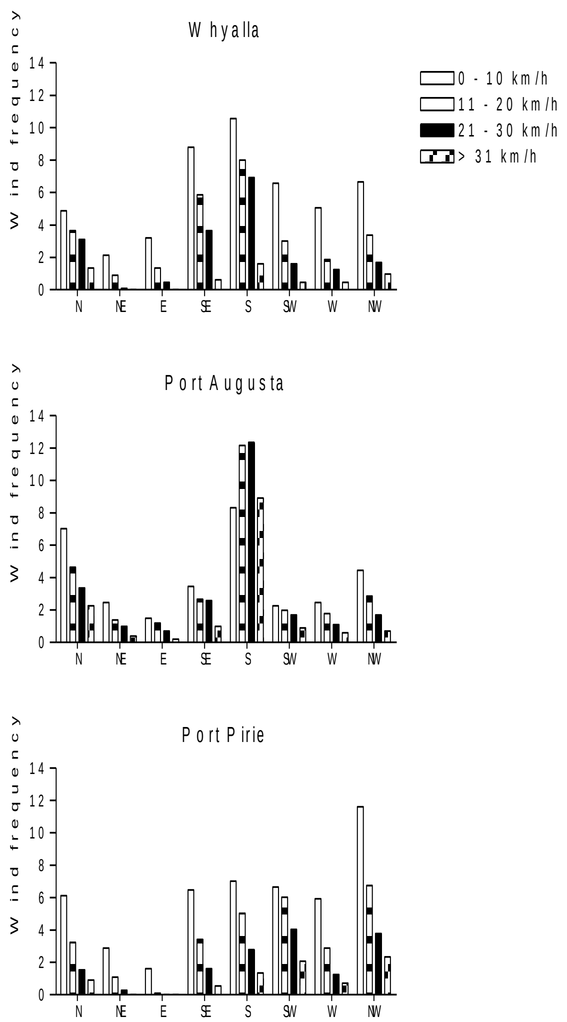

Fig. 2.7. Average wind frequency on 12 azimuthal sectors at Whyalla (closest weather station to Middleback), and Port Augusta and Port Pirie (the two closest weather stations to Nectar Brook). Data represents the average frequency over 44 y at Whyalla, 35 y at Port Augusta and 46 y at Port Pirie.

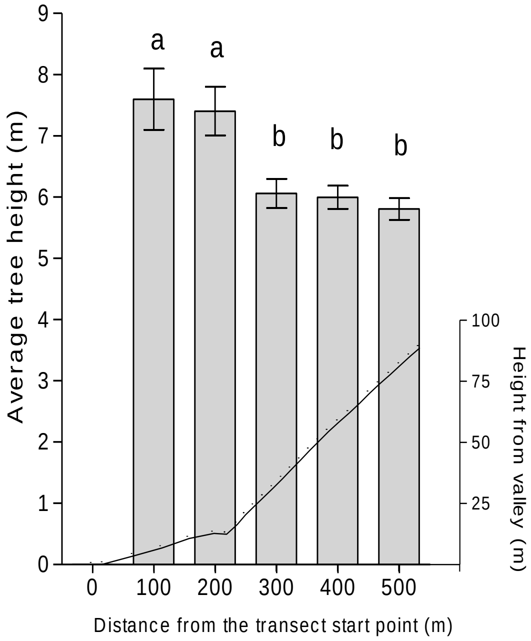

Fig. 2.8. Average tree height of *Acacia papyrocarpa* (N=10), at Nectar Brook in relation to position on the 500m transect up the hillslope (height of hill is indicated by closed squares). Note that three sites were on similar slopes at different heights on the hill, whereas the remaining two were at the base of the hill. Different letters indicate where statistically significant differences occur.

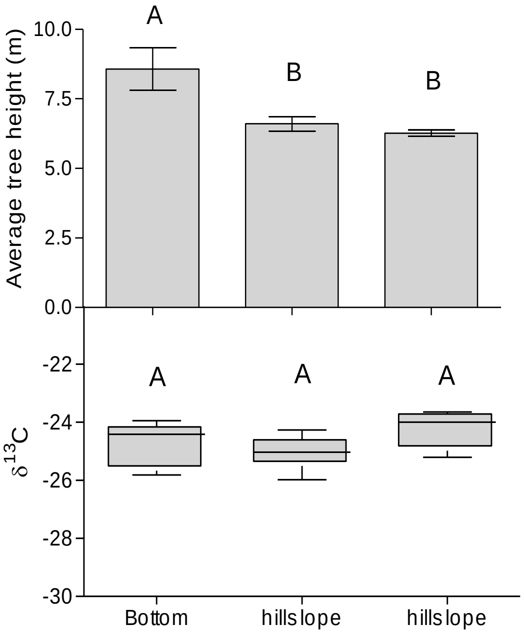

Fig. 2.9. Tree height, location on hillslope and carbon isotope composition ($\delta^{13}C$) for the five tallest trees at three different positions on the hill at Nectar Brook. Different letters indicate where statistically significant differences occur.

Chapter 3) **Carbon isotope composition is affected more by height than pathway length in the broad crowned tree, *Acacia papyrocarpa* Benth.**

3.1 Abstract

According to the hydraulic limitation hypothesis, as trees grow taller gravity and the increasing pathway, which increases resistances to water flow, cause water potential to decrease that triggers the premature closing of stomata limiting photosynthesis and therefore height growth. The relative contribution of pathway length and gravity to this hydraulic limitation, however, is difficult to ascertain because they are usually confounded in studies using vertically-oriented trees. I used a broad-crowned tree, *Acacia papyrocarpa*, to separate the effects of height (i.e. gravity) and pathway length to determine their relative importance to the hydraulic limitation to height growth. Vertically oriented branches in the centre of *A. papyrocarpa* canopies were taller, but had shorter pathlengths, than horizontally oriented branches on the north-facing sides of isolated trees. Phyllode carbon isotope ratio ($\delta^{13}C$) was lower on the longer, north-facing branches than on the vertical ones, suggesting that water was more limited in the taller, but shorter branches. On the other hand, there were no differences in hydraulic characteristics between the two types of branches. I also found no difference in foliar nitrogen concentration or leaf mass per unit area (LMA). The difference in height between canopy parts seems to be the only factor that could explain the difference in $\delta^{13}C$. I suggest that the effects of gravity on water transport could be more important than pathway resistances in limiting height even in these small trees.

3.2 Introduction

The hydraulic limitation hypothesis (HLH) was proposed to explain physiological limits to tree height (Ryan and Yoder 1997, Ryan et al. 2006). This hypothesis states that resistances to water flow increase with pathway length, causing water potential to decrease and, as a consequence, the premature closing of stomata, thus limiting photosynthesis. The effect of gravity on the water column also contributes to the water potential reduction and its consequences. The hypothesis has received wide support and some of its predictions have been confirmed by empirical observations (e.g. McDowell et al. 2002a, Koch et al. 2004, see a review in Ryan et al. 2006). However, it has also been challenged (Becker et al. 2000b) because of the existence of mechanisms in plants that compensate for the increased resistance with path length (Becker et al. 2000b, see also McDowell et al. 2002a, Ryan et al. 2006). Nevertheless, it is accepted that the effects of gravity on water transport are always present (Koch et al. 2004, Woodruff et al. 2004, Ryan et al. 2006). In fact, it has been suggested an additional height-limitation mechanism based on the effect that gravity have on cell turgor and therefore cell growth (Koch et al. 2004, Woodruff et al. 2004). The available evidence suggests that this turgor limitation work simultaneously with the stomatal limitation of the HLH (e.g. Koch et al. 2004). An equation that may show the relationship between stomatal limitation and turgor in whole tree performance is (see Chapter 2):

$$P_{leaf} = \Psi_{soil} - \Psi_{\Pi leaf} - \rho_w g h - (R_{soil} + R_{plant})\, Gs\, D \qquad (3.1)$$

where P_{leaf} is the pressure potential (i.e. turgor when is positive), Ψ_{soil} is soil water potential, $\Psi_{\Pi leaf}$ is leaf osmotic potential, $\rho_w gh$ is the gravitational potential term (with a magnitude of 0.01MPa per metre in height), ρ_w is the density of water, g is the acceleration due to gravity, h is plant height (from root to leaf), R_{soil} is soil hydraulic resistance, R_{plant} is plant hydraulic resistance (per unit leaf area), Gs is the foliage stomatal conductance and D is leaf to air vapour pressure deficit. Equation 3.1 also shows that both path length (i.e R_{plant}) and gravity ($\rho_w gh$ term) contributes to limit P_{leaf}.

The proponents of the HLH have recently reviewed several papers testing the HLH and have found some conflicting results (Ryan et al. 2006). They suggested that water potential may be related to changes with tree height but there could be different response pathways. Therefore, more research and different approaches are needed to fully understand the detailed mechanisms of hydraulic limits to tree height. One approach is to determine the relative contribution of the effects of gravity on water transport, in comparison with path length resistances, in limiting tree height.

There have been several studies comparing the magnitude of the gravity head with that of the pathway resistances using water potential measurements (e.g. Baurle et al. 1999, Hubbard et al. 1999, Koch et al. 2004). It is assumed that during the night plants reach equilibrium with soil water (but see Donovan et al. 2001), that predawn water potentials would represent such equilibrium and would show the gravity head along the height of a tree. The gravity head contributes -0.01 MPa for every meter of vertical height, and this has been confirmed by predawn measurements of water potential (e.g. Baurle et al. 1999, Koch et al. 2004). The predawn water potential or the calculated gravity head is then compared directly with midday water potentials (Baurle et al. 1999, Koch et al. 2004) or with the calculated hydraulic conductance (Hubbard et al. 1999). With these approaches, it has been found that the gravity head determines about 70% of the measured water potential in 110m tall trees of *Sequoia sempervirens* (Koch et al. 2004) but only 10% of the hydraulic conductance for 12m tall trees of *Pinus ponderosa* (Hubbard et al. 1999). From this estimation, it has been assumed that gravity affects, with the same magnitude, other parameters such as stomatal conductance and carbon isotope composition (e.g. Hubbard et al. 1999, Koch et al. 2004). However, using these approaches may underestimate the importance of gravity effects in small trees. Minimum leaf water potential reflects not only plant hydraulic characteristics but also the balance between soil water supply and atmospheric evaporative demand (Bhaskar and Ackerly 2006). Short trees (lower R_{plant} and h in equation 3.1) usually develop in environments with reduced soil moisture (which reduces Ψ_{soil} and increases R_{soil} in equation 3.1) and high vapour pressure deficit, and these environmental factors reduce more the absolute value of leaf water potential than the combined effects of plant resistances and gravity. Therefore, it would be strongly biased to try to compare gravity and path length resistances based on a comparison between the gravity term and midday water potential. A related problem is that the effects of gravity are often compared against resistances of the entire soil-plant continuum; however, a proper comparison would be against internal plant resistances, because the HLH relates to a longer or shorter water pathway in the plant, not effects due to soil moisture. Short trees are found in dry environments, and under dry conditions most resistances may be located in the bulk soil and at the soil-root interface (e.g. Cruizat et al. 2002). Root hydraulic resistance also increases with drought (Vandeleur et al. 2009), and the majority of this resistance is located between the soil and the root stele (e.g. Amodeo et al. 1999). Therefore, comparing the hydrostatic gradient in small trees against the entire soil-plant continuum would result in a very small effect of gravity in comparison with all resistances, but the majority of them would be related to soil drought and not to plant length. The comparison should take into account only resistances from the root stele up to the top of the canopy. However, to date there is no direct comparison between the effects of height and pathway length on the carbon isotope composition of leaves. This is essential because, except for water potentials, all measurements usually taken to test the HLH clearly confound both height and length in vertically-oriented trees.

Leaf carbon isotope composition (leaf $\delta^{13}C$) has been used as an index of long term water stress in a number of studies of the hydraulic limitation hypothesis (Yoder et al. 1994, McDowell et al. 2002a, Barnard and Ryan 2003, Koch et al. 2004). In C_3 plants the ratio between the abundance of stable isotopes ^{13}C and ^{12}C in plant tissue is commonly less than in the air due to discrimination against ^{13}C (Farquhar et al. 1989). This discrimination is directly related to intercellular partial pressure of CO_2 and therefore to stomatal conductance. Because $\delta^{13}C$ is a more weighted value than stomatal conductance it has been often used in testing the hydraulic limitation hypothesis (Yoder et al. 1994, McDowell et al. 2002a, Barnard and Ryan 2003, Koch et al. 2004). Not only have higher values of $\delta^{13}C$ (less negative in relation to an international standard) been reported for taller trees than for shorter ones (Yoder et al. 1994, Hubbard et al. 1999, McDowell et al. 2002a, Phillips et al. 2003, but see Barnard and Ryan 2003) but also increasing values have been related to increasing height in the same trees (Ryan and Yoder 1997, Barnard and Ryan 2003, Koch et al. 2004). These results suggest that water stress is greater toward the top of trees, and also in taller trees relative to shorter ones. Other studies have also found higher values for longer branches than for shorter ones (Walcroft et al. 1996, Warren and Adams 2000). However, there are alternative explanations for gradients in $\delta^{13}C$ into the same trees. Foliage $\delta^{13}C$ is determined not only by the CO_2 supply (stomatal conductance) but also by CO_2 demand (assimilation rate), indicating that the photosynthetic capacity of the leaves should also be taken into account (Farquhar et al. 1989, Livingston et al. 1998, Duursama and Marshall 2006). It has been shown that vertical gradients of $\delta^{13}C$ in trees are related to gradients in nitrogen concentration of the leaves, an indicator of photosynthetic capacity (Livingston et al. 1998, Duursama and Marshall 2006). Livingston et al. (1998) suggested that nitrogen concentration may be determined by the distance to the leader branch, i.e. the apical one. Therefore, if trees are not completely isolated, and if photosynthetic capacity is not accounted for, the interpretation of gradients in $\delta^{13}C$ may be confounded. In addition, no proper tests have been made to compare length versus height because both parameters are clearly confounded in vertically-oriented trees. In contrast, a broad crowned tree provides an ideal model for such studies because comparisons can be made between two parts of the same tree, one with shorter path length but greater height (i.e. the vertical stems) and the other with more length but less height (i.e. the horizontal branches).

Broad crowned trees have already been studied in relation to the HLH and has been demonstrated that they may be limited in height by hydraulic mechanisms (Chapter 2, Phillips et al. 2003). In an oak species (*Quercus garryana*) it was found that carbon isotope ratios ($\delta^{13}C$) were consistent with greater time-integrated stomatal resistance in 25 m tall trees compared to 10 m trees that were growing under the same soil and atmospheric conditions (Phillips et al. 2003). In another study, I have shown that for *Acacia papyrocarpa,* there was a direct relationship between maximum tree height and water availability, and trees with different maximum heights across a water availability gradient had similar carbon isotope ratios ($\delta^{13}C$) as would be expected if they had similar hydraulic limitations (Chapter 2). The crown shape of broad crowned trees may reflect a situation where gravity is the main source of the hydraulic limitation in height, because the path length that water must travel to reach the top of a tree may be shorter than the path length of lateral branches. However, it is also possible that broader crowns have resulted from structural changes that improve hydraulic conductivity in horizontal branches and/or reduce it in vertical ones. For example, there are structural changes affecting hydraulic conductivity that are related to branch growth (Rust and Roloff 2002), position (Protz et al. 2000) and orientation (Schubert et al. 1999, Schubert et al. 1995), and they may influence canopy shape. This possibility also needs to be tested.

I used the broad-crowned tree, *A. papyrocarpa,* to test the effect of both aboveground path length and height of individual branches, on the carbon isotope composition ($\delta^{13}C$) of the foliage. I also assessed phyllode nitrogen content and LMA, as well as hydraulic conductivity of both horizontal and vertical branches from the outer canopy of isolated trees.

3.3 Methods

3.3.1 Field site and sampling

Measurements were conducted on *Acacia papyrocarpa* Benth (Western Myall) growing near Middleback Field Centre, 16 km NW of Whyalla, South Australia (32º57'S, 137º24'E). *A. papyrocarpa* is a broad crowned tree with height ranging from 4 to 11m and a dense spreading canopy with the longest horizontal stems usually oriented toward north, i.e. toward the equator (Chapter 2). The crown can spread so much that older individuals frequently have branches that become procumbent. The evergreen species is distributed in the arid and semi-arid parts of South Australia and Western Australia (Whibley and Symon 1992). Climate at the Middleback site is arid with average annual rainfall of 223 mm (average for 1923-2005), but with a large inter-annual variability. Summers are hot (mean daily maximum for January = 29.2° C) and winters mild (mean daily minimum for July = 7.2° C). The dominant vegetation of the area is open woodland of *A. papyrocarpa* with a chenopod shrub understorey.

A preliminary comparison between height and a proxy of horizontal pathway length was made on 25 trees. I used data collected during a survey conducted at Middleback in September and October 2001, and April 2003. Isolated individuals were chosen whose canopies were at least 15 m from other canopies to avoid the influence of shading effects. I measured 10 young individuals with a rounded crown where spreading was just beginning and 15 mature individuals with a fully developed broad crown. Tree height was measured with an extendable measuring pole. One person held the pole while another one assessed from a distance the maximum height of the crown (Fig 3.1). I measured the straight crown radius toward north. To do this, the edge of the crown was marked (i.e. its vertical projection) with a plumb line and then I measured the straight distance to the base of the trunk (Fig 3.1). The height of the point at which the horizontal stem branched from the main trunk was also measured (Fig 3.1). This height was added to the north radius as a proxy of lateral pathway length. The actual distance that water would travel is longer than this because branches are sinuous, so these preliminary measurements represent an under-estimate of water transport pathways.

A more detailed analysis was made of six completely isolated trees. The canopies of neighbouring trees were more than 15 m from the canopy of the target tree in all cases. Therefore, the target individuals were not exposed to shadows from neighbouring trees. The individuals selected were mature trees with crowns broader than tall and without procumbent branches. In each individual, the three longest north-facing branches and the three tallest vertical branches from the centre of the crown were selected. In each tree, all branches selected for sampling originated from a different stem or limb. I estimated the internal pathway that water would travel into each branch by measuring the outside sinuous length (Fig. 3.1). This sinuous pathway length was measured with a flexible tape from the base of the stem at the soil level, following contortions and ramifications of each branch to the most distal part of the branch. Height of both vertical and horizontal branches was measured with an extending pole (Fig. 1). I also measured incident photosynthetic photon flux density (PPFD) (AccuPAR 80 Ceptometer, Decagon Devices, Inc.; Pullman, Washington, USA) at midday in winter for the exposed northern and top parts of the canopy of each tree (Fig. 3.1).

3.3.2 Hydraulic conductivity

Hydraulic conductivity measurements were made on the same six trees used for the detailed measurements of vertical and horizontal path length. Branches for hydraulic conductivity were the same ones used for measuring pathway distance and height (Fig. 3.1), and were collected in November 2003. Branches were cut at a point where stem diameter was approximately 1.5 cm. The resulting samples were between 60 and 100 cm in length and from that a section of about ~40 cm from the cut point was taken. Stems were wrapped in moist paper, put into a plastic bag in a cooler and transported to the lab for measurement of hydraulic conductivity. Measurements were made over five consecutive days. Stems were stored in a cold room at 2.5 ºC before measurements. A final segment of ~10 cm was re-cut under water from the middle part of the original segment. Hydraulic conductivity measurements were made with two custom-built systems as described in Sperry et al. (1988). In each case, the upper reservoir was a plastic, intravenous drip bag and the lower reservoir was a plastic beaker. The lower reservoir was placed on an electronic balance that was connected to a computer that logged changes in mass. A thin layer of oil was added to the lower reservoir to avoid water evaporation. I used deionized, filtered (2 µm) and acidified water (pH 2 with HCl). Cavitation was eliminated with a 100 kPa flush of 20 min as suggested by Sperry et al. (1988). Water flow was measured over 15 min under a pressure of 9 kPa, before and after the 100 kPa flush. Stems from the two canopy positions were alternated between the two systems throughout the study. Stem diameters were measured with vernier callipers after removing the bark. Maximum and minimum diameters were measured at both extremes of the stem and the average was calculated.

Hydraulic conductivity (K) was calculated as:

$$K = F(L/\Delta P),$$

where F = mass flow rate (kg s^{-1}), L = length of the stem and ΔP = pressure gradient.

Hydraulic conductivity was also normalized to phyllode area (leaf specific hydraulic conductivity, K_{LS}) and to branch cross-sectional area (shoot specific hydraulic conductivity, K_{SS}). The Huber value was calculated as the ratio between shoot cross-sectional area and phyllode area.

3.3.3 Phyllode characteristics and analyses

In the field I sampled all distal parts (phyllodes and stems) originating from the same segment used for hydraulic conductivity determinations. Phyllodes and stems were separated, oven-dried for 48 h at 75ºC and weighed. The 20 most distal phyllodes were separated in the field (Fig 3.1) and their area was determined with a Leaf Area Meter (Delta-T Devices LTD). They were also oven-dried for 48 h at 75ºC and weighed. Leaf (phyllode) mass area (LMA) was calculated as:

LMA = phyllode mass/phyllode area

The same sub sample was then ground to a fine powder with a mortar and pestle and analysed for total nitrogen and $\delta^{13}C$. Total nitrogen was determined directly by the combustion technique (Carlo Erba total combustion gas chromatograph). Total nitrogen was used as surrogate for photosynthetic capacity because strong correlations have been found between both parameters for many plant species (Field and Mooney 1986). Carbon isotope analyses were made at the West Australian Biogeochemistry Centre of the University of Western Australia, using PDB as the standard. The precision of the carbon isotope measurements was ±0.07‰.

In addition, I also collected phyllode samples from 15 *Acacia papyrocarpa* trees at another site (Nectar Brook; see Chapter 2, for a site description). From each tree two phyllode samples were taken, one from the top of the highest vertical branch and the other from the longest north-facing horizontal branch. Although for this survey I could not exclusively choose isolated trees, most trees were more than 5m from other canopies. Samples were oven dried at 60ºC for 72 h before being ground to a fine powder with a mortar and pestle. They were analysed for carbon isotope composition ($\delta^{13}C$) in the West Australian Biogeochemistry Centre at the University of Western Australia. These measurements were performed in runs different from the previous one and had a precision of ±0.10‰.

3.4 Results

In a sample of 15 mature, isolated trees with complete crowns, the proxy for horizontal pathway (north crown radius plus branching height) was always longer than the vertical height of the tree (Paired t test $p<0.0001$; Fig 3.2). In young trees, the same proxy for horizontal pathway was of similar length as the tree vertical height (Paired t test $p=0.92$; Fig 3.2).

In the six *A. papyrocarpa* trees used for the more detailed study, both height and pathway length were significantly different between north-facing horizontal branches and vertical branches (Table 3.1). For each tree, all horizontal branches measured had a longer path length than vertical branches from the top of the canopy (Fig. 3.3). The difference between means was 1.07m (Table 3.1). In contrast, height was greater for vertical branches from the top of the canopy than for horizontal branches, the difference between means was 2.06m. There was no significant difference in incident PPFD for horizontal and vertical branches (Table 3.1).

Phyllode $\delta^{13}C$ values were significantly different between the two canopy positions, both among branches within trees and also among trees (Table 3.2). $\delta^{13}C$ values of phyllodes from the ends of horizontal, north-facing branches were on average 0.69‰ lower than those from vertical branches at the top of the canopy, representing a gradient of 0.34‰ m^{-1}. In contrast, there were no significant differences in any of the hydraulic characteristics (K, K_{LS},

K_{SS}, and Huber value) between horizontal and vertical branches (Table 3.2). I also found no differences in either phyllode nitrogen concentration or LMA from the two canopy positions (Table 3.2), or any relationship between phyllode nitrogen concentration and $\delta^{13}C$ (Fig. 3.4). In samples collected from 15 trees at Nectar Brook, I also found that $\delta^{13}C$ was lower in phyllodes from the northern part of the canopy than from the top of the canopy (paired t-test, $p<0.001$, N=15; data not shown). North-facing horizontal branches were high enough (~2.45m) to avoid any possible stratification of CO_2 or humidity in the source air, this is supported by the lack of any relationship between $\delta^{13}C$ and height of the north-facing horizontal branches (Fig. 3.5).

In both orientations there was a positive relationship between branch cross-sectional area and hydraulic conductivity (Fig. 3.6A-B) but top-vertical branches had less variation explained by the linear equation (50%; Fig. 3.6A) than horizontal north-facing branches (90%; Fig. 3.6B). Similarly, phyllode area was correlated with branch cross-sectional area (Fig. 3.6C-D), but less variation was explained in top-vertical branches (25%; Fig. 3.6C) than in horizontal north-facing branches (49%; Fig. 3.6D).

3.5 Discussion

Our results show that mature *A. papyrocarpa* trees growing isolated from neighbouring trees have longer horizontal, north-facing stems than vertical stems. This suggests either that horizontal branches grow more than vertical ones, and/or vertical ones suffer more damage at the top (die-back). The greater variability in branch diameter for vertical branches than for north-facing horizontal branches, when related to hydraulic conductivity and foliage area, is consistent with more damage at the top vertical branches than in north-horizontal ones. *A. papyrocarpa* has a seasonal flush of canopy growth in spring and summer, followed by a period of increased phyllode mortality in winter (Maconochie and Lange 1970), but it is unknown if there is more dieback in upper than in north oriented branches. In other species shoot shedding or shoot-tip abortion seems to occur more frequently in the upper parts of the crown (Davidson and Remphrey 1990, Stromberg et al. 1992). Nevertheless, more research is needed to characterize growth and die-back in top, vertical branches in comparison with equator-oriented, horizontal branches. Whatever the case, in *A. papyrocarpa* gravity is likely to contribute more than pathlength to any hydraulic limitation in height, because water must flow through a longer pathway in the horizontal branches.

Hydraulic mechanisms have been identified as possible factors limiting tree height in broad crowned trees (see section 3.2, Phillips et al. 2003, Chapter 2). It is possible, however, that differences in stem hydraulic conductivity, rather than gravity, could produce the characteristic shape of trees such as *A. papyrocarpa* if hydraulic conductivity in horizontal branches were greater than that in vertical ones. Differences in hydraulic conductivity amongst branch types have been reported for a number of species. For example, in *Quercus robur* it was found that vigorously growing branches had greater hydraulic conductivity than less vigorous ones (Rust and Roloff 2002). Studies on grape vines have shown that hydraulic conductivity was greater in upright than in horizontal stems (Schubert et al. 1999, Schubert et al. 1995), and in *Pinus contorta* it was found that shaded branches had lower hydraulic conductivity than exposed branches (Protz et al. 2000). In contrast, I found no evidence of any compensation in hydraulic conductivity on horizontal north-facing branches that could reduce resistances resulting from longer pathways. However, my comparisons of hydraulic conductivity were made on the last ~50 cm of branches and thus, I cannot rule out completely the possibility that anatomical changes may occur in other portions of the stems. Nevertheless, in oak trees variation in hydraulic conductivity was found in the distal regions of branches (Rust and Roloff 2002).

Phyllode $\delta^{13}C$ can be affected by internal partial pressure of CO_2, light, photosynthetic capacity and $\delta^{13}C$ of the source air (Farquhar et al. 1989). In this study phyllode $\delta^{13}C$ should reflect a long-term internal partial pressure of CO_2 for the following reasons. First, both, top and north-facing parts of the canopy received similar PFDs when measured in winter. The solar path moves to the north during winter in the southern hemisphere, so it is the south-facing branches that will be in shade at this time of the year. In addition, I also selected isolated trees thus eliminating any possibility of shading by neighboring trees. Second, both canopy parts were similar in total nitrogen and LMA, which suggests their photosynthetic capacity is similar because total nitrogen correlates with photosynthetic capacity (Field and Mooney 1986). LMA did not change with height as was found in another study (Koch et al. 2004). Third, I assume minor effects of $\delta^{13}C$ in the source air because significant turbulent mixing is expected in open stands and the $\delta^{13}C$ in the air is expected to remain almost constant throughout the canopy, with some variations only very close to the ground surface (Buchmann et al. 1997, Buchmann et al. 2002). The lower branches were more than 2m in height and stratification in $\delta^{13}C$ in the air has been observed to be in less than 1m close to the ground (Buchmann et al. 1997, Buchmann et al. 2002). This is also supported by the lack of any relationship between north-facing branches and $\delta^{13}C$, which also discard any possible stratification in air humidity. I conclude that phyllode $\delta^{13}C$ values in this study are an indication of internal partial pressure of CO_2, and therefore an indication of long-term water stress as has been assumed in other studies of the hydraulic limitation hypothesis (e.g. Yoder et al. 1994, Phillips et al. 2003, McDowell et al. 2002a). Phyllode $\delta^{13}C$ was lower in north-facing branches than in those from the top of the canopy, suggesting that phyllodes from horizontal north-facing branches were less water stressed than those from vertical branches, despite horizontal north-facing branches having longer pathways. Considering only branch length, my results are contrary to those reported for *Pinus radiata* and *Pinus pinaster*, i.e. where $\delta^{13}C$ was less negative in longer branches than shorter ones (Walcroft et al. 1996, Warren and Adams 2000). The gradient in $\delta^{13}C$ of 0.34‰ m^{-1} found in this study is well into the range found for other species, both in short and large trees. For example, gradients of 0.23 and 0.18‰ m^{-1} have been reported for 6.9 and 25.6m trees of *Eucalyptus saligna* (Barnard and Ryan 2003), 0.5‰ m^{-1} for a 8.5m *Picea sitchensis* tree (Heaton and Crossley 1995), 0.77‰ m^{-1} for a 6.2m *Pinus radiata* tree (Livingston et al. 1998), while for ~110m *Sequoia sempervirens* trees a gradient of ~0.08‰ m^{-1} was reported for most of the tree height but more than 1.5‰ m^{-1} at the top of the tree (the last ~6m; Koch et al. 2004). My findings that $\delta^{13}C$ may respond more to height than to path length resistances in small trees are new. While this idea is well accepted for taller trees and it was explicitly suggested for the tallest trees on Earth (Koch et al. 2004), it is not commonly accepted for small trees (e.g. Hubbard et al. 1999, Pillips et al. 2003). However, as I have already explained (see introduction), some previous studies may have underestimated the effect of gravity by comparing it against midday water potential or soil-plant resistances instead to resistances due to plant length. Others may have confounded the effects of path length and height (i.e. gravity) when analysing $\delta^{13}C$ data. Another problem with some previous studies, as highlighted by Duursama and Marshall (2006), is that the effect of leaf photosynthetic capacity was not considered.

None of the evidence in my study supported the idea that path length resistance is the main source of hydraulic limitation to height in *A. papyrocarpa*. An alternative explanation is that gravity effects on water transport may be important in limiting height even in small trees. If this were true, hydraulic limitation would be greater for vertical branches at the top of trees than for horizontal north-facing branches, because of their differences in height. This may explain the observation that in *A. papyrocarpa* horizontal branches could grow until weight causes them to fall, explaining the presence of procumbent branches in older individuals (Lange and Purdie 1976, Ireland 1997). Gravity could affect long-distance water transport in trees through different pathways. One is the direct effect of the hydrostatic gradient in the water potential of the leaves and more specifically on cell turgor. This mechanism, turgor limitation, relates to the role of turgor pressure in cell expansion and organ growth (Koch et al. 2004, Woodruff et al. 2004). The turgor of cells in buds and leaves decreases in direct proportion with the gravitational component of water potential unless osmotic adjustments occurs (Koch et al. 2004, Woodruff et al. 2004). Because cell turgor is necessary for cell growth, then it would diminish and eventually stop leaf and bud expansion at treetops. In addition, a reduction in leaf area with tree height would reduce photosynthate production. If the osmotic potential does not remain constant, i.e. when osmotic adjustment occurs, then, the cost of producing osmolytes would compete for resources needed for growth (Woodruff et al. 2004). This gravity effect may be related to stomatal conductance (as suggested by Eq. 3.1) and it is consistent with the reduced stomatal conductance at treetops found in many studies of the HLH (see review in Ryan et al. 2006). Another possible gravity effect is on the daily and seasonal refilling of cavitated vessels. Cavitation is a very common phenomenon (Canny 1997b, McCully et al. 1998, Salleo et al. 1996, Tyree et al. 1999) and it has been suggested that its recovery involves parenchyma cells that accompany xylem vessels (Canny 1997b, Zwieniecki et al. 2000, Hacke and Sperry 2003, Pickard 2003). Supporting this, it has been found that parenchyma cells and vessels are hydraulically connected (Schneider et al. 1999, Thurmer et al. 1999, Wistuba et al. 2000). Because these parenchyma cells are interconnected and form a three-dimensional net (Chaffey and Barlow 2001), a gradient in osmotic potential along the vertical axis of a tree would be necessary to overcome the effect of gravity and to refill vessels evenly throughout the plant. This compensatory osmotic adjustment has been observed in lianas and matches the magnitude needed to overcome the gravitational hydrostatic gradient (Wistuba et al. 2000). It could be expected that as plant height increases, the parenchyma cells eventually reach a limit in their ability to adjust osmotically because production of osmolytes, or solute uptake, would compete for resources with plant growth. Eventually, this could make cavitation recovery more costly and difficult, affecting the water relations of the higher plant parts. This gravity effect would also affect stomatal conductance and carbon isotope composition as in the HLH. It would explain crown shape in broad crowned trees because higher branches would be more water-limited than lower but longer lateral branches.

In conclusion, our results with *A. papyrocarpa* are consistent with the suggestion that gravity effects on water transport may be greater than path length resistances in limiting tree height. First, in broad crowned trees horizontal stems can be longer than vertical ones. This indicates less growth and/or more dieback in the taller top branches in comparison with the longer but lower north-oriented branches. Secondly, longer water pathways did not result in higher $\delta^{13}C$ as would be expected if path length were the main source of hydraulic limitation. In contrast, as expected by gravity effects, higher branches had higher $\delta^{13}C$ than lower branches. These differences are probably caused by differences in stomatal conductance and not by differences in photosynthetic capacity between the two canopy parts because total nitrogen was similar in both groups of branches. Finally, there was no apparent hydraulic compensation in the longer horizontal stems. From evidence in this and other papers (e.g. Koch et al. 2004, Woodruff et al. 2004), it seems reasonable to suggest that gravity could be more important than path-length resistances as a source of hydraulic limitation to tree height. Among other impacts, gravity could affect the recovery of cavitated vessels and the cell turgor needed for growth. I have also shown that isolated broad crowned trees represent a good model for studies of the HLH.

3.6 Tables and figures

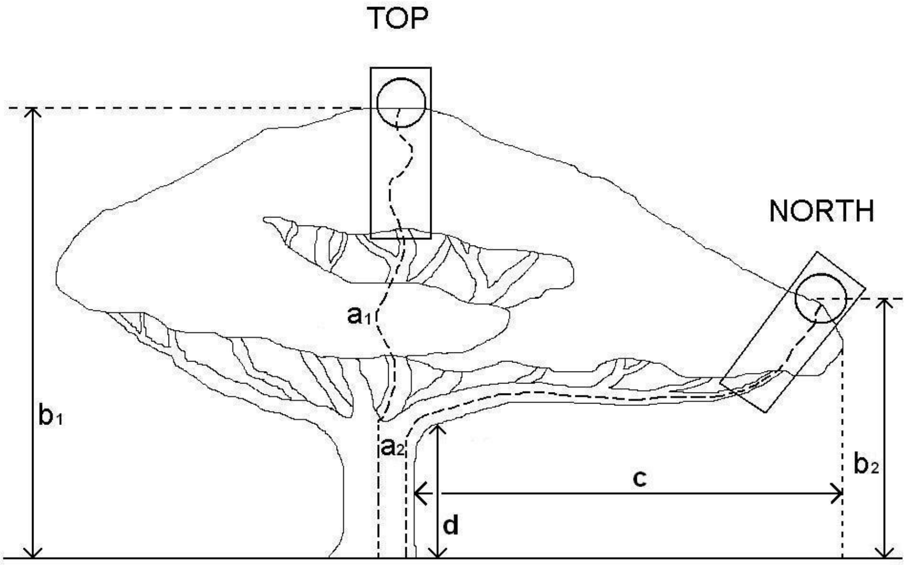

Fig. 3.1. A diagram of Western Myall showing sampling areas and measurements that were performed on vertical and horizontal branches. Broken lines a_1 and a_2 represent sinuous pathway length; continuous lines b_1 and b_2 represent height; the proxy for horizontal pathway length was the sum of lines c and d; circles show areas of phyllode sampling for carbon isotopes ratios, total nitrogen and LMA, and of measurement of incident photon flux density (PFD); finally, rectangles show areas of stem and foliage sampling for hydraulic conductivity.

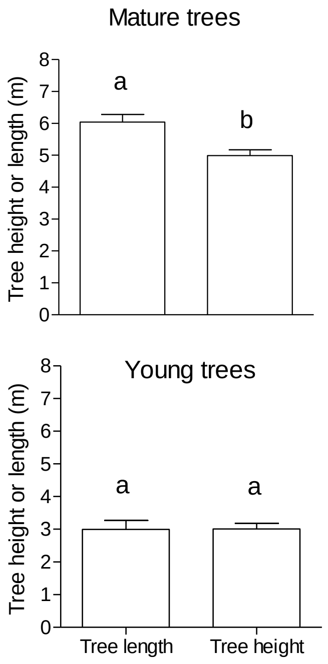

Fig. 3.2. Vertical tree height (b_1 from Fig 1) and the proxy for horizontal pathway length (c + d from Fig 1) of mature (n=15), and young (n=10) *A. papyrocarpa* trees, at Middleback, South Australia.

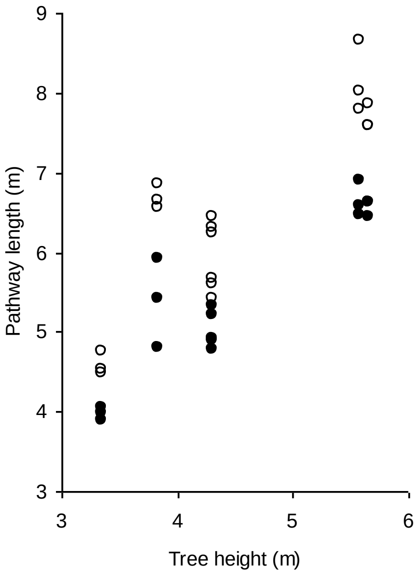

Fig. 3.3. Relationship between sinuous pathway length and tree height in six mature *A. papyrocarpa* trees. Open circles represent horizontal north facing branches and closed circles vertical top branches. Three vertical and three horizontal branches were measured per tree. Two trees had the same height of 4.31m and their data overlap.

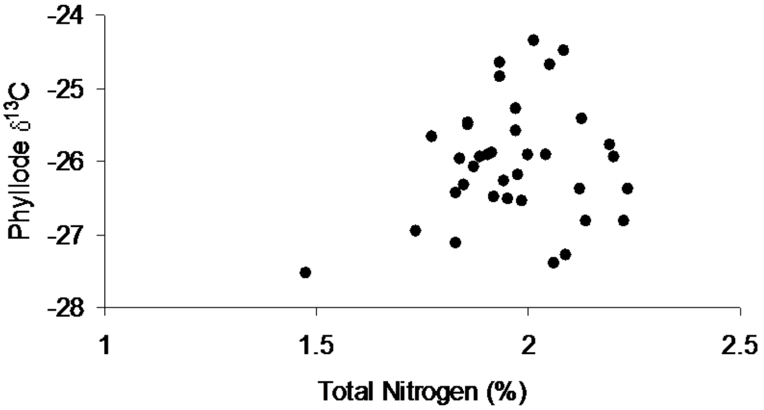

Fig. 3.4. Relationship between phyllode total nitrogen (% dwt) and carbon isotope composition ($\delta^{13}C$) from the six, mature *A. papyrocarpa* trees (r^2=0.02, p=0.33).

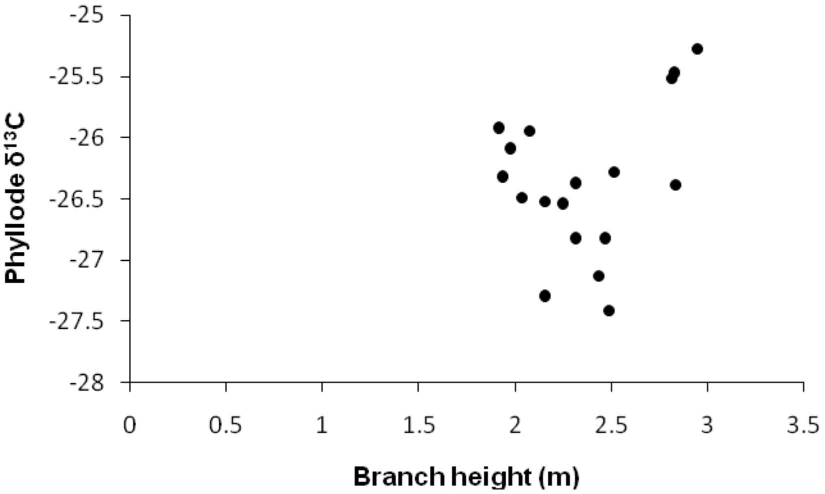

Fig. 3.5. Relationship between branch height and carbon isotope composition ($\delta^{13}C$) from the six, mature *A. papyrocarpa* trees (r^2=0.09).

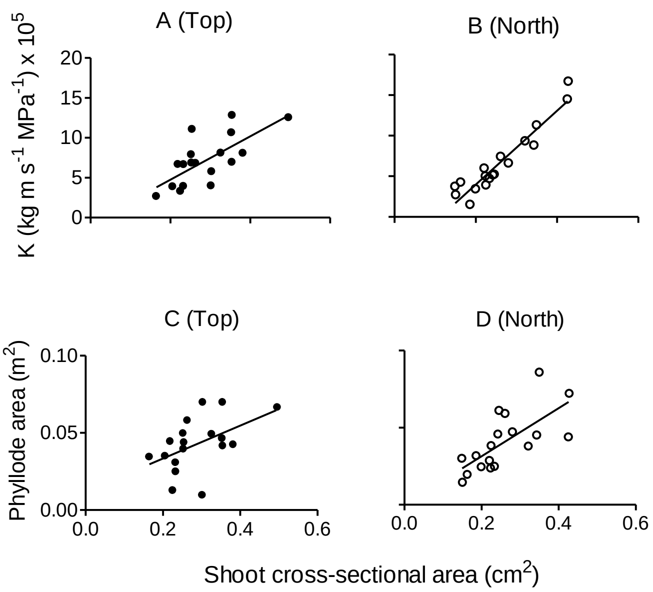

Fig. 3.6. Relationships between stem cross-sectional area and hydraulic conductivity, and phyllode area of vertical (A&C) and horizontal (B&D) branches of mature *A. papyrocarpa*. The r^2 and p values, respectively, for each relationship are: A) 0.50 and 0.001; B) 0.90 and <0.001; C) 0.25 and 0.04; and D) 0.49 and 0.001.

Table 3.1. Height, sinuous pathway length and incident PFD of vertical and horizontal branches of mature, isolated *A. papyrocarpa* trees at Middleback, South Australia. See Fig 1 for an explanation of how measurements were obtained. Paired t tests were made between the top vertical branches and the distal part of north facing horizontal branches. Data are means±SEM.

	Top Means±SEM	North Means±SEM	Paired t test (per tree, n=6) p	
Height (m)	4.51±0.38	2.45±0.28	0.0004	***
Pathway length (m)	5.43±0.23	6.50±0.30	0.001	***
PFD (μmol m^{-2} s^{-1})	1215±35.0	1149±24.5	0.109	

Table 3.2. Stable carbon isotope ratios, branch hydraulic characteristics, phyllode nitrogen concentration and LMA of vertical branches and north facing horizontal branches of isolated *A. papyrocarpa*, from Middleback, South Australia. Data are shown as means±SEM. Results of paired and unpaired t-tests are also shown for each parameter.

	Top Means±SEM	North Means±SEM	Paired t test (per tree, n=6) p	Unpaired t test (per branch, n=18) p
$\delta^{13}C$ (‰)	-25.68±0.20	-26.37±0.14	0.05 *	0.008 **
K (kg m s^{-1} MPa^{-1}) x 10^5	7.15±0.72	6.66±0.96	0.73	0.69
K_{LS} (kg s^{-1} m^{-1} MPa^{-1}) x 10^3	1.88±0.21	1.68±0.16	0.29	0.47
K_{SS} (kg s^{-1} m^{-1} MPa^{-1})	2.46±0.19	2.41±0.17	0.88	0.83
Huber value x 10^4	8.45±1.8	7.04±0.76	0.39	0.39
Total nitrogen (% dwt)	1.93±0.04	2.00±0.03	0.29	0.19
LMA (g cm^{-2}) x 10^3	50.0±1.2	48.9±1.2	0.56	0.5

Chapter 4) Precision, bias and equilibrium assumptions during pressure chamber measurements in non-transpiring leaves placed in free water

4.1 Abstract

I investigated the precision and bias of the pressure chamber technique and tested two water potential equilibrium assumptions underlying accurate measurements of the pressure chamber technique. According to theory, xylem pressure potential (Ψ_{xp}) of non-transpiring leaves placed in pure water should eventually equilibrate to 0 kPa. In addition, non-transpiring leaves should have constant xylem pressure potentials. I measured balance pressures (BP) using the pressure chamber technique on detached leaves of *Viburnum tinus, Eucalyptus pachyphylla, E. incrassata,* and *E. microcorys* that had been covered with packaging tape and placed in water for 15 hours in a dark room. Using a pressure chamber with a digital pressure gauge and a stereo microscope I was able to measure BP with a precision of 2 kPa and a bias of 3 to 12 kPa. Despite this high accuracy, BP in leaves varied from 3 to 250 kPa. In addition, repeated BP measurements of the same leaf over one hour showed a non-constant BP in some leaves. I suggest that BP disequilibrium and non-constant BP were related to processes occurring in living tissues rather than caused by experimental manipulation. I suggest that leaf growth could explain to some extent the disequilibrium found here. These results may require revising some interpretations of pressure chamber data, especially when very accurate measurements of small changes in Ψ_{xp} are required, such as measurements of gravitational potential.

4.2 Introduction

The pressure chamber is the most frequently used technique for measuring xylem pressure potential (Ψ_{xp}) in plants (Scholander et al. 1965, Boyer 1995). Most comparisons between techniques are made against the pressure chamber (e.g. Hardegree 1989, Holbrook et al. 1995a, Pockman et al. 1995, Wei et al. 1999b). In addition, much of the evidence for the Cohesion-Tension theory, central to our understanding of plant water relations in general, has been based on pressure chamber data (Tyree 1997). However, over the last decade there has also been some discussion about the possibility that measurements made with this technique may be unreliable (see Meinzer et al. 2001, Tyree 1997, Zimmermann et al. 2004, Canny and Roderick 2005). Thus, experimentally testing the assumptions and errors associated with the pressure chamber technique remains critical.

The pressure chamber technique relies on four main assumptions (see Scholander et al. 1965, Turner 1988, Tyree 1997, Wei et al. 2000, Tyree and Zimmerman 2002): firstly, when a leaf is cut in the air the water column recedes and is held as a meniscus at the pits of the remaining intact vessels; secondly, there is a hydraulic continuum in the whole leaf; thirdly, the pressure applied -within the pressure chamber- to the living cells in the blade is transmitted directly to the xylem fluid in the petiole; and fourthly, a non-transpiring, detached leaf eventually reaches an internal water potential equilibrium, i.e. any differences in water potential within the leaf should disappear. The first assumption was challenged by data obtained by Canny (1997a), but subsequently supported by the work of Tyree et al. (2003). The second assumption, the hydraulic continuum, has been challenged by Zimmerman and co-workers who argue that there may be embolized vessels, i.e. air-filled spaces in the leaf (Zimmermann et al. 1993, Zimmermann et al. 1994, Zimmermann et al. 2004). This discontinuity would affect the third assumption because some pressure may be attenuated by the presence of air-filled spaces in the leaf and/or pressure may be expended in refilling those air-filled spaces with water to restore hydraulic continuum (Zimmermann et al. 1993, Zimmermann et al. 1994, Zimmermann et al. 2004). In relation with this third assumption, it has also been suggested that pressure is expended when air dissolves into the water of the leaf during pressurization within the pressure chamber (Canny and Roderick 2005). A new interpretation of pressure chamber measurements suggests that the toughness of cell walls may also affect measurements (Roderick and Canny 2005). However, the second and third assumptions have been supported by the strong correlations between pressure chamber measurements of Ψ_{xp} and those made with other techniques (e.g. Holbrook et al. 1995a, Pockman et al. 1995, Wei et al. 1999b). Nevertheless, the discussion has continued because a study found no correlation between pressure chamber and pressure probe measurements when using transpiring leaves (Melcher et al. 1998). There have been no studies testing the equilibrium assumption in relation with the pressure chamber technique.

The equilibrium assumption underpins the interpretation of pressure chamber measurements because this technique is indirect in both space and time (it pressurizes a living tissue in order to balance the former tension in the xylem vessels, i.e. when they were cut). Thus, the equilibrium assumption should have two components that satisfy both the spatial and temporal dimensions. First, there should be a water potential equilibrium across different tissues. Therefore, if non-transpiring leaves are placed in free water, not only their apoplast but also their symplast should eventually reach water potential equilibrium with that water. Secondly, a non-transpiring leaf should have constant water potentials. These two components have not been specifically studied in relation with pressure chamber measurements. However, because the equilibrium assumption is a general principle in plant water relations, it has received some attention, especially at the whole plant level (see Donovan et al. 1999, 2001). This assumption suggest that during the night plants reach water potential equilibrium with soil water (Donovan et al. 1999, 2001). However, such equilibrium has not been found for plants of some species even without transpiration and with soils at field capacity (Donovan et al. 1999, 2001). Furthermore, non-equilibrium has also been observed in potted plants (Passioura and Munns 1984, Rieger and Litvin 1999), in plants with roots placed directly in free water (Koide 1985, Passioura and Munns 1984, Rieger and Motisi 1990), in plants shoots placed in free water (Meron et al. 1989) and even on individual leaves placed in free water (Koide 1985). This departure from equilibrium was suggested to originate in the roots (Koide 1985, Passioura and Munns 1984, Rieger and Litvin 1999) because sometimes removing roots eliminated the disequilibrium (Rieger and Motisi 1990), or because leaving the roots but removing the shoot did not change the disequilibrium (Passioura and Munns 1984). However, the elimination of root systems did not always eliminate the disequilibrium (Stoker and Weatherly 1971, Meron et al. 1989).

It is important to study the accuracy and sources of error during pressure chamber measurements because some studies require very accurate determinations. For example, the study of the gravity head in the static water column of plants requires an accuracy of few kPa because the expected gradient is just 10 kPa per metre. Most studies of the hydrostatic gradient have been made in tall trees (e.g. Scholander et al. 1965, Tobiessen et al. 1971, Connor et al. 1977, Koch et al. 2004, Woodruff et al. 2004), very few in small plants (e.g. Begg and Turner 1970, Turner and Begg 1973). Large trees are favored because a large height difference helps to overcome any measurement errors intrinsic to the pressure chamber technique. In contrast, small plants may be easier to manipulate and would represent less expensive research but they require more accurate measurements. These studies also strongly rely on the assumption of water potential equilibrium within the measured leaf and along the stems during measurements.

The accuracy of an instrument or technique depends on both its precision and bias (Grubbs 1973, Walther and Moore 2005). Precision is the closeness to each other of repeated measurements (Zar 1999); it depends only on the observed value but not on the true value (Walther and Moore 2005). In contrast, bias is the difference between the measurements and the accepted reference or true value, i.e. is the distance to the true value (Walther and Moore 2005). Being a systematic error, bias leads to an under- or overestimate of the true value, while precision is a random error (Walther and Moore 2005). A bias on the technique can be detected by comparing their measurements against a reference instrument (e.g. Holbrook et al. 1995a, Pockman et al. 1995, Melcher et al. 1998, Wei et al. 1999b), a chemical standard or a theoretical value. In this work I am using this last approach.

The purpose of this study was to address precision and bias during pressure chamber measurements under controlled conditions and to test two aspects of the equilibrium assumption as they may affect interpretations when using the technique. First, I tested the assumption that leaves reach water potential equilibrium with free water using non-transpiring, detached leaves left overnight with their petioles immersed in water. Secondly, using repeated measurements of balance pressure (BP) on the same single leaves I tested the prediction of a constant measurable xylem pressure potential in the same leaf.

4.3 Methods

4.3.1 Plant material

I used four species representing a gradient in leaf mass area (LMA): *Eucalyptus pachyphylla* F. Muell., *E. incrassata* Labill., *Viburnum tinus* L. *and E. microcorys* F. Muell. (0.021, 0.016, 0.012, 0.005 gdwt cm^{-2} respectively; see Chapter 5). The first two species are small trees that occur in semiarid woodlands in Australia. *E. microcorys* is a tall forest tree (50 m) that grows in moist forests in eastern Australia. *V. tinus* is an evergreen shrub (1.8 to 3 m) native to the Mediterranean region of Europe. Twenty-one potted plants of each species were obtained from local nurseries and maintained in a naturally illuminated glasshouse with a cooling system. Plants were fertilized with a slow release fertilizer and watered to field capacity 2-3 times a week. I also used leaves from seven adult *V. tinus* (around 2 m height and 1.5 m in crown diameter) growing in close proximity to each other in the gardens of the North Terrace Campus of the University of Adelaide, Adelaide, South Australia.

4.3.2 Leaf sampling and equilibration in water

To test the water potential equilibrium between leaves and free water I re-analysed data from several experiments (those from Chapter 5) performed during 2002 and 2003, including some designed to test the impact of leaf position and age on water potential equilibrium (see Chapter 5). Therefore, my dataset includes leaves of different sizes, sampled from different positions on the plants. For all experiments, however, the method for leaf sampling and preparation was the same. Leaves were cut with a razor blade on the afternoon of the day before the measurements were made. Cut leaves were brought to the laboratory and the blade was covered completely on both surfaces with a box-sealing tape for packaging (Scotch 3M, St. Paul, MN, U.S.A.) to prevent transpiration. Leaves were placed standing up with just the petiole immersed in free water (tap water). All leaves from each sampling day were left together in the same container for at least 15 hours in a photographic dark room. Balance pressure determinations took place the following morning. Under these conditions apoplastic water and the recipient free water would homogenise and the pressure potential in the xylem would be 0 kPa for equilibrated leaves, i.e. those with no differences in water potential between different tissues. This concept can be explained more formally as follows. Because there are no barriers between the free water in the recipient and the apoplast of the leaf, then the water potential (Ψ) of both compartments would equilibrate completely:

$$\Psi_w = \Psi_a \tag{1}$$

where the subscript w refers to the free water in the recipient and a to the apoplast. The water potential of the free water in the recipient depends only on the solute potential (π_w) while the water potential of the apoplast would depend on both the solute (π_a) and the pressure xylem potential (P_a) (Boyer 1995, Tyree and Zimermann 2002). Gravitational potential can be neglected. Therefore, substituting these components of the water potential in Eq. 1 gives:

$$\pi_w = \pi_a + P_a \qquad (2)$$

Because the solute would homogenize between the free water and the apoplast, then the solute potential would be the same for the apoplast and the free water in the recipient. Isolating the pressure xylem potential from Eq. 2, gives:

$$P_a = \pi_w - \pi_a = 0 \qquad (3)$$

Therefore, independently of the original osmotic potentials of the free water and apoplast, the xylem pressure potential would be cero. Thus, xylem pressure potential, as measured by the pressure chamber, would be a useful parameter to identify water potential equilibrium between leaf and free water. Any measured tension would indicate lack of equilibrium and may be originated at the symplast but not at the apoplast.

4.3.3 Balance pressure measurements

Balance pressure was measured using a Scholander-type pressure chamber equipped with a digital gauge with a resolution of 0.1 kPa (DTG-6000 3D Instruments, Huntington Beach CA, U.S.A.). Measurements of BP were made with very high precision because the digital pressure gauge could store pressures in its memory with the press of a button, allowing the observer to concentrate on determining the balance point in the petiole. In this way I could work with a precision of just 2 kPa (see results).

Observations of BP were made under a stereoscopic microscope. Each leaf was taken from the water and the petiole surface was re-cut (less than 1 mm) in order to present a clear surface. The leaf was put into the chamber as quickly as possible (between 1 and 3 minutes). I did not have to put filter paper with water into the pressure chamber as is usually recommended (Boyer 1995) because the leaves were taped to prevent any evaporation into the pressure chamber. I was consistent in leaving 2 mm of petiole to be out of the pressure chamber, and used a small chamber to cover the exposed cut surface of the petiole to avoid any evaporation. The upper part of this „petiole chamber“ had a glass cover slip that permitted observation of the cut surface. I also put humid paper on the walls of the „petiole chamber“. This „petiole chamber“ was used mainly when repeated measurements were taken on the same leaf.

Since previous trials indicated that BP was affected by the pressurization rate (data not shown), I maintained the same pressurization rate (~5 kPa s^{-1}) in most measurements. In some leaves that presented a film of water on the cut surface, ~2 kPa s^{-1} was used to provide more time to identify the balance point precisely. In exceptional cases with very high BP I increased the pressurization rate.

BP was measured as the pressure at very first appearance of water in xylem vessels at the cut surface of the petiole. However, some leaves had a film of water on the cut surface before pressurization started. When the amount of water was very small, BP was measured as the very first movement of the water film. This was repeatable for several minutes or even hours afterwards in leaves with a constant BP. When there was more than a very thin water film on the cut surface I waited until the water was absorbed by the leaf, after which BP was measured. When BP measurements were made with killed leaves (see below), the amount of water expelled was greater than for live lives and no reabsorption occurred. In these cases I blotted the excess water with a small piece of tissue paper until a tiny water film was left on the cut surface.

4.3.4 Hydration kinetics in V. tinus

I analysed hydration kinetics using two leaves of *V. tinus*. Leaves were taken at midday of a cloudy winter day from *V. tinus* plants growing in the glasshouse. Leaves were covered with a packaging tape before being removed from the plant. They were quickly transported to the laboratory where BP was determined using the „petiole chamber" during measurements. In this experiment, BP was determined before placing these leaves in water (tap water) to hydrate. After the initial BP measurement, the leaf was removed from the pressure chamber, weighed and placed in free water for 4 minutes, the leaf was then re-weighed and BP was then re-measured again. I repeated this procedure until a plateau was reached for BP versus time. This curve indicates how much time was needed to reach equilibrium between water and leaf.

4.3.5 Live versus killed leaves

Using *E. microcorys,* I compared BP between recently killed and living leaves after overnight hydration. Killed leaves are not proper control for biological processes during BP determinations with the pressure chamber because they would not have a turgor pressure in the cells, would have the membrane broken and the vacuole and symplastic water mixed with the apoplastic water. However, measurements with the pressure chamber on killed leaves may represent the matric potential of the leaves as suggested by Boyer (1967). Because there would not be any symplast at all, killed leaves would be useful to study specifically the equilibrium between apoplast and free water. In addition, killed leaves represent a system in complete equilibrium and can be useful to compare some characteristics of that equilibrium with live leaves. Comparisons can also be made on the evaporation of water in the petiole and the error associated in detecting the measurement point with the pressure chamber technique. One leaf was taken from each of 20 potted plants of *E. microcorys* growing in a glasshouse. Ten of those leaves were killed by immersing them in boiling water for 4 minutes. Immediately afterwards, all leaves, including the live ones, were covered with packaging tape and their petioles placed in free water overnight to hydrate. BP measurements were performed the following day as described above. I also killed leaves by heating them for 10 s or 1 min on a microwave oven. The results obtained with microwaved leaves were similar to those obtained with the boiled leaves; however, it was easier to remove the tape from the boiled leaves than from microwaved ones.

4.3.6 Repeated BP measurements on the same leaf

To test the assumption that BP remains constant in non-transpiring leaves at equilibrium with free water, several measurements of BP were made on single leaves. The rate of change in BP was also determined. I performed these measurements over 4 days in March and April 2003. On each measurement day leaves were sampled from 6 different plants of *V. tinus* growing in the university gardens. I chose healthy and fully exposed leaves from the top of the canopy. Leaves were taken to the lab, taped and left to rehydrate overnight. BP was determined the following day as described above. After the initial BP measurement the pressure in the chamber was released, and after 5 minutes the BP was measured again. Measurements were repeated several times, over a period of 1 h or longer. The rate of change in BP is then shown by the slope of the relationship between time and BP; slopes no different from zero indicated constant BP. The petiole chamber" was used during this experiment, so that all the water extruded during BP measurements was reabsorbed by the leaf and not lost through evaporation. Leaves were measured sequentially. When a second pressure chamber was available I measured two leaves simultaneously. Thus, several leaves could be measured in a single day. Repeated BP measurements were also made for some recently killed leaves. I also measured evaporation rate from two water reservoirs in the same lab and during the same time that repeated BP measurements were made.

To determine whether frequent pressurization and depressurization affected the rate of change of BP in the living leaves, intervals between repeated BP measurements were varied from 5 to 90 min. In the intervals between BP measurements, the leaf was protected from evaporation with the „petiole chamber".

The area of leaves used for repeated BP measurements was determined with a leaf area meter (Delta-T Devices LTD, Cambridge, U.K.), after removing the packaging tape from the blade.

4.3.7 Precision of the technique

I used three approaches to quantify the precision of my measurements. The first method used the standard deviation of repeated measurements on single leaves. I calculated the standard deviation -of all repeated measurements- for every single leaf of *V. tinus* that showed a constant BP (i.e. regression slopes no different from zero). I also calculated the standard deviations for repeated BP measurements in recently killed leaves. The second approach used the standard deviation in BP for recently killed leaves of *E. microcorys*. The third method involved calculating autocorrelation between BP measurements made on "water-equilibrated" leaves from the same plant but measured on two different days (see Chapter 5). In another study I had shown that mature leaves of *E. pachyphylla* presented characteristics of being fully equilibrated as compared with young and less sclerophyllous leaves (see Chapter 5). For those measurements I took a leaf from the lowest part of 12 potted *E. pachyphylla* (mature leaves, ~10 months old). Leaves were taken to the lab, taped and left to rehydrate overnight. BP was determined the following day as stated before using the „petiole chamber". The experiment was repeated 6 days later using other mature leaves taken from the same plants. In order to quantify the technique precision I analysed plant autocorrelation, i.e. linear regression, and its residuals.

4.4 Results

4.4.1 Precision of the technique

All three methods that I used to calculate my precision gave values consistently less than 2 kPa. The twelve live *V. tinus* leaves that had a constant BP (i.e. slope no different from zero, see below) during repeated BP measurements had standard deviations of around 1.3 kPa (range 0.5 - 2.3 kPa, mean SD=1.3 kPa). A killed leaf from *V. tinus* measured several times (Fig 4.1) showed a similar standard deviation (SD=1.1 kPa, mean BP=9.3 kPa). The standard deviation from 10 killed leaves of *E. microcorys* showed the same magnitude: 1.68 kPa (Fig 4.2). Finally, the autocorrelation found between BP of *E. pachyphylla* leaves measured on separate days (Fig 4.3, and see Chapter 5) was very close to a 1:1 relationship, and explained 89% of the data variability (Fig 4.3). The strong autocorrelation showed that two different leaves from the same plant had similar BP despite being measured on two different days. All the residuals of the linear regression were less than 1.5 kPa (Fig 4.3 inset). Assuming this value to be the magnitude of the measurement random error or precision, it is consistent with the other estimates.

4.4.2 Expected BP values for equilibrated leaves

Killed leaves also permitted me to analyse water evaporation from the petiole. I used a Scholander-type pressure chamber of the rubber tightening type (see Boyer 1995) so when the rubber was tightened during leaf installation into the pressure chamber water was expelled from the petiole prior to BP determinations. I blotted the excess of water until a tiny film was left on the cut surface. This tiny film of water could stay there without being evaporated for several hours. Some live leaves (from the four species) had the same pattern and their BP as low as those of killed leaves. In other leaves the water from the cut surface of the petiole disappeared rapidly. I conclude that water was being re-absorbed by the leaf and there was no evaporation from the petiole because the atmospheric conditions around the petiole were similar for all leaves.

Most old leaves measured for the autocorrelation in *E. pachyphylla* seemed to be at equilibrium with free water, i.e. they had a tiny water film present at the cut petiole surface that was not absorbed (see also Chapter 5). They presented a maximum value of 11.8 kPa. Because equilibrated leaves must have a xylem pressure potential of 0 kPa then BP values as high as 11.8 kPa must represent a small bias in the measurement. This bias and its magnitude are consistent with equivalent BP values obtained for killed leaves, which represent equilibrated systems as well. Recently killed leaves of *E. microcorys* had a mean BP of 6.27 kPa (range 3.2 - 8.7 kPa). In *V. tinus,* repeated measurements had a minimum value of 7.8 kPa and maximum of 11.4 kPa. Therefore, it may have not been possible to measure equilibrated leaves at exactly 0 kPa but at values as high as ~12 kPa due to a small bias in the measurements. It is reasonable to suggest that all leaves with BP below ~15 kPa (i.e. taking into account a bias of 12 kPa plus some random error) were likely to be fully equilibrated with free water.

4.4.3 Testing water potential equilibrium of leaves in free water

According to the hydration kinetics of some *V. tinus* leaves (Fig 4.4), water potential equilibrium could be reached in 70 min. This value is well into the range (~16 to ~100 min) found by Zwieniecki et al. (2007) for leaves of 6 angiosperm species that were cut under water. Nevertheless, many leaves left overnight (around 15 hours) in direct contact with free water did not totally equilibrate their water potential, i.e. their BP measured with the pressure chamber were not close to 0 kPa (Fig 4.5, 4.6). For example, the BP of 244 leaves from seven adult plants of *V. tinus* varied from 5.4 kPa to 251.6 kPa, i.e. some leaves having BP two orders of magnitude greater than the precision of 2 kPa (Fig 4.5). Those 244 leaves, measured on different days, had an average BP of 31.8 kPa (SD = 31.9 kPa) with 69% of the leaves having a BP above 15 kPa. Leaves from potted plants of *V. tinus* showed similar responses, with 70% of the 56 measured leaves having BP values of more than 15 kPa. The mean BP was 31 kPa (SD = 29.7 kPa, Fig 4.6a).

Lack of complete equilibrium with free water was also found in some leaves from the other three species: *E. pachyphylla*, *E. incrassata* and *E. microcorys* (Fig 4.6b-d). The pattern in *E. incrassata* was similar to that of *V. tinus* with around 61.1% of the leaves having BP above 15 kPa (n = 36, mean = 40.5 kPa, SD = 71.6 kPa, Fig 6b). *E. pachyphylla*, had a lower mean BP of 14.2 kPa (SD = 11.8 kPa, n = 124), and had 32.2% of leaves above 15 kPa (Fig 4.6c). In *E. microcorys* the mean BP was further away from 0 kPa than the other 3 species, with a mean BP of 55.5 kPa (SD = 38 kPa, n = 58), and with 91.4% of the leaves above 15 kPa (Fig 4.6d).

In *E. microcorys,* the species with more leaves in disequilibrium, BP of living leaves was significantly different from that of the recently killed leaves (t-test $p<0.001$, Fig 4.2). Mean BP for living leaves was 39.7 kPa (+/-23.9 SD, n = 10, Fig 4.2). In contrast, killed leaves were closer to 0 kPa, with a mean of 6.3 kPa (+/-1.68 SD, n = 10, Fig 4.2). Values in live leaves are influenced by both symplast and apoplast, while in killed leaves are influenced only by the apoplast.

4.4.4 Testing the assumption of a constant water potential

Balance pressures measured in this study were not as constant as is usually assumed for hydrated, non-transpiring leaves (Fig 4.5, 4.7, 4.8). I can rule out that the changes in BP were caused by chamber pressurization and depressurization, since the rate of change in BP was similar when pressurization and depressurization was frequent (i.e. when measurements were taken every 5 min) or when there were periods of more than 90 min without pressurization and depressurization (Fig 4.5). In addition, different leaves had different rate of change in BP or even constant BP, despite the same conditions of frequent pressurization and depressurization (Fig 4.7).

I can also rule out that the change in BP was caused by evaporation in the laboratory because: a) petioles were protected from evaporation with a small chamber, b) evaporation was constant in the laboratory during measurements whereas the rate of change in BP was different among leaves (Fig 4.7), c) two leaves measured simultaneously showed different rates of change in BP (Fig 4.7b), and d) there were some live leaves (and all killed leaves) that had constant BP and could held water at the cut surface of the petiole for around one hour without being evaporated, despite being exposed at the same measurement conditions than leaves with non-constant BP. Evaporation from the leaf blade into the pressure chamber was also highly improbable because all leaves were completely covered with packaging tape. Lack of evaporation into the chamber is also evident because some leaves maintained constant BP despite being exposed to the same conditions than leaves with non-constant BP.

The rate of change in BP varied between leaves in *V. tinus* (Figs 4.7 and 4.8). Of 12 leaves measured on the same day, only 2 had a slope that was not significantly different from zero (Fig. 4.7). From 39 leaves measured in total over 4 days I found only 12 leaves with a constant BP (Fig. 4.8). The rest of the leaves (69.2%) had rates of change in BP that ranged from very close to zero to 40 kPa per hour.

The rate of change in BP was correlated with leaf area on two measurement days (March 31 and April 2, Fig 4.8). On March 20 there were too few data to perform a regression analysis. When all the data were pooled, two distinct responses were observed (Fig 4.8). One group of leaves, of different sizes, had rates of change in BP very close to 0 kPa h^{-1}. When these leaves were included in the analysis, the linear model explained only 13% of the variation ($p=0.02$, Fig 4.8). The analysis of residuals of that linear model showed a bimodal distribution, therefore, indicating that data came from two different populations. After eliminating the data close to 0 kPa h^{-1} and one outlier, the linear model explained 80% of the variation ($p<0.001$, Fig 4.8).

4.5 Discussion

Our results show that the pressure chamber technique can detect accurately the equilibration that do happen between leaf and water; but also show that sometimes leaves do not equilibrate under the conditions here studied. Two basic equilibrium assumptions of plant water relations were not always supported by my results: a) in most cases (32, 61, 70 and 91% of all leaves of *E. pachyphylla*, *E. incrassata*, *V. tinus*, and *E. microcorys*, respectively), the water potential of non-transpiring leaves did not reach equilibrium with free water and b) more than 60% of the leaves had non-constant BP. I have substantial and diverse evidence suggesting that these results were not artefacts of the techniques. First, even when leaves were exposed to the same hydration time in water, laboratory conditions, chamber pressurization and manipulation conditions, only some showed the expected equilibrium and constant BP. Secondly, my random errors, of around 2 kPa, were much smaller than the variability of the BP determinations, from 3 to 100 kPa or more. This high precision in my measurements is supported by strong evidence since estimates using three different approaches in three different species all gave consistent results. Thirdly, some live leaves had similar characteristics to those of recently killed leaves, the model of a complete equilibrium: they had very low and constant BP. They also held a water film at the cut petiole surface, i.e. without it being reabsorbed, as would be expected for leaves at equilibrium. Although killed leaves may not be a proper control for biological influences during pressure chamber measurements, they illustrated the characteristics of a complete equilibrium. Lastly, another possible artefact is the diminished hydraulic conductivity of leaves being cut in the air, because more embolism is present than when petioles are cut under water (Tyree et al. 2003). However, the protocol used here may not have affected BP measurements for the following reasons: a) a comparison between leaves cut in the air and under water did not show a significant difference between either groups of leaves (see Apendix); b) the balancing point is identified as the very first signal of expelled water, therefore, it does not take into account if water was expelled from few vessels or from all of them, thus the method may not be sensitive to a possible partial blockage of vessels; c) it is expected that when a petiole is cut at least some vessel endings, and therefore regions with the water meniscus, remain very close to the cut surface as suggested by the distribution of vessels lengths (e.g. Tyree et al. 2003); d) the hydration kinetics of leaves cut in the air was very similar to those of leaves cut under water; e) hydration time of more than 15h may have allowed some recovery from embolism; and f) the many leaves that had a BP below 15 kPa is also evidence that petioles conducted water and that leaves reached equilibrium.

The small bias in equilibrated leaves, from 0 to around 12 kPa, could be explained by factors producing a threshold pressure. A threshold pressure is the amount of pressure that is consumed before the water column in the petiole is pushed. Zimmermann et al. (2002) proposed that a “threshold pressure” could depend on wood density, elastic forces of the tissue, intercellular spaces, hydraulic coupling between xylem and tissue cells, cellular osmotic pressure, cavitation and the presence of cellular mucopolysaccharides. I assume that cavitation was not a factor in my experiments, since leaves were in water before measurements. The remaining factors cannot be ruled out in live leaves. In killed leaves pressure would only be expended in overcoming mechanical properties of the tissue. Therefore, the magnitude of this “mechanical” threshold may be of only ~11.4 kPa, i.e. the maximum pressure required to move water from killed leaves. Some live leaves that held a tiny water film at the cut, petiole surface without being absorbed had a maximum BP value of 11.8 kPa, i.e. the same magnitude as killed leaves. I suggest that these live leaves had a water potential equilibrium with free water and a threshold pressure would explain the departure from 0 kPa, i.e. those values between 0 to 12 kPa.

4.5.1 Lack of leaf equilibrium with free water

In general, I suggest that most leaves with BP below ~15 kPa (threshold pressure plus random error) were at complete equilibrium with free water while values higher than ~15 kPa represent disequilibrium and must have been caused by variable processes in the symplast. The origin of the disequilibrium for the species and conditions here studied may not be related to solute accumulation in leaf apoplast as has been suggested for other species (Donovan et al. 1999, 2001) because diffusion would dissipate any ion gradients between leaf apoplast and free water. This is consistent with the low values obtained in killed leaves that may represent the apoplast. Thus, the isolated apoplastic influence would not explain values above 12 kPa, the highest pressure value from killed leaves. On the other hand, it is possible that this disequilibrium is originated at the symplast and is related to living processes, specifically to leaf growth as had been suggested by Boyer (1968, see also chapter 5). Wall yielding during leaf growth causes a drop in turgor pressure in the symplast, which reduce xylem pressure potential in the apoplast (Boyer 2001, Boyer and Silk 2004). This growth effect on water potential is called growth-induced water potential (Martre et al. 1999, Tang and Boyer 2002, for a review see Boyer and Silk 2004). Leaf growth is expected when leaves are exposed to high water potentials, like the conditions here studied.

The rate of change in BP (from repeated BP measurements) by itself does not explain completely (although can contribute to) the lack of leaf equilibrium with free water, i.e. the departure from 15 kPa in leaves recently taken from the water reservoir (1 to 3 minutes). One of the highest rates of change in BP found in hydrated leaves was 40 kPa per hour and this rate would only explain a change of 2 kPa during those three minutes before a measurement was made. If I add this 2 kPa to the 12 kPa of the threshold pressure, plus 2 for the random error, the result (~16 kPa) is still not big enough to explain the variability in BP from 15 to 100 kPa or more.

4.5.2 Repeated BP measurements

The non-constant BP during repeated BP measurements is another indication of the lack of water potential equilibrium in some of the leaves I studied. If there were a complete equilibrium within a single leaf then repeated BP measurements would yield constant values. However, my repeated BP measurements on single leaves showed a non-constant BP in more than 60% of the leaves. As explained before, the non-constant BP of the leaves was not caused by evaporation, experimental manipulation, measurements errors or the frequency of pressurization and depressurization to which leaves were exposed. The change in BP in leaves had been observed previously and was suggested to be related to leaf growth (Milburn 1979). In soybean seedlings placed into the pressure chamber it has also been observed that the water film from the recently cut stem is absorbed by the growing tissue (Boyer 2001). In fact, a strong negative relationship was detected between the elongation rate of soybean seedlings under different pressures into a pressure chamber with the pressure required to put back the water film into a recently cut stem (Boyer 2001). With my data it is not yet clear if growth was the cause of the changing BP. If more growth is expected in small rather than big leaves, then, there should be a negative relationship between leaf area and the change in BP. However, I found the opposite: the rate of change in BP seems to be proportional to leaf area. Independently of the biological process that originates the non-steady BP (without discarding even leaf growth), I suggest that water is removed from the xylem in the petiole toward cells in the blade. More volume in bigger leaves would result in more water being taken from the xylem vessels, causing the BP to increase more in large than in small leaves. My data also show that there are two behaviours: in some leaves water is being consumed and this is related to leaf area, whereas in other leaves there is no such water use and it occurs independent of leaf size. It would require more research to determine if those results represent differences between growing and non-growing leaves or contrasting behaviours during other living processes. More research is needed to fully understand the causes of BP change.

4.5.3 Implications for pressure chamber measurements

Evidence presented here does not challenge recent tests supporting the use of the pressure chamber (Wei et al. 1999b, Tyree et al. 2003). However, my results have clear implications for the interpretation of pressure chamber data in studies that require very accurate measurements. For example, studies investigating water potential equilibrium between non-transpiring plants and saturated soil or free water (Rieger and Litvin 1999, Donovan et al. 2001) should also take into account the variability discussed here. The lack of equilibrium may, at least partially, originate at the leaf level and not just in the root system as has previously been suggested (Rieger and Litvin 1999). My results are also relevant to studies testing the presence of a gravity head in the static water column of a plant. These studies require very accurate measurements because of the very small differences in water potentials (of only 10 kPa per metre) between leaves from different heights on the same plant. There is a controversy in this topic because some studies have found gradients close to 10 kPa per metre (Scholander et al. 1965, Connor et al. 1977, Baurle et al. 1999, Koch et al. 2004) whereas others have found smaller values or even absence of gradients (Hellkvist et al. 1974, Zimmermann et al. 1994, 2002). The differences in BP that I found here were of about the same magnitude as the gradient expected for leaves separated by a height of 10 to 25m. My results suggest that this controversy on the gravity head may have been enhanced by leaf disequilibrium rather than real effects of the gravity head itself. In general, interpretations of pressure chamber data could be complicated when very accurate measurements of small water potential changes are required. Certainly more research is needed to completely control all variables affecting measurements with the pressure chamber. More importantly, my results suggest that there may be some biological activity that controls water movement between different parts of the leaf that we do not fully understand.

I conclude that the pressure chamber technique can produce repeatable determinations with high accuracy. The balancing point can be detected with a precision of 2 kPa. In addition, those determinations can be away from the true xylem tension, i.e. biased, by only 3 to 12 kPa due to a small pressure threshold. However, leaves do not always equilibrate under non-transpiring conditions because some living processes may not permit full equilibration. If this leaf disequilibrium is not taken into account when interpreting pressure chamber data then it may become a significative interpretative bias in some studies.

4.6 Tables and figures

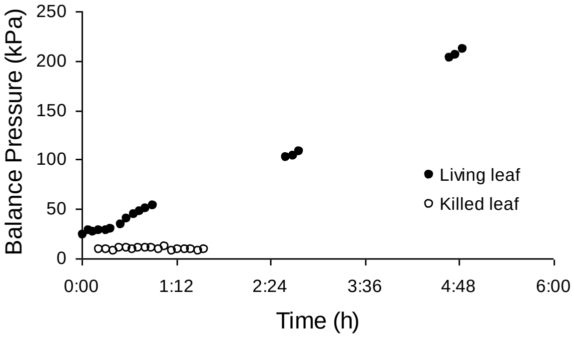

Fig 4.1. Typical repeated-balance pressure measurements on a single killed (empty circles) and a living hydrated leaf (filled circles) of *Viburnum tinus*. Petioles of the taped leaves were left immersed in free water for at least 15 hours before measurements. BP was measured with a pressure chamber provided with a “petiole chamber”. After the initial measurement the pressure was released and after a 5 min period BP was measured again. This procedure was repeated several times for the same leaf. On the living leaf BP-measurements were suspended for long periods (>90 min) to study if the frequency of pressurization and depressurization was affecting BP.

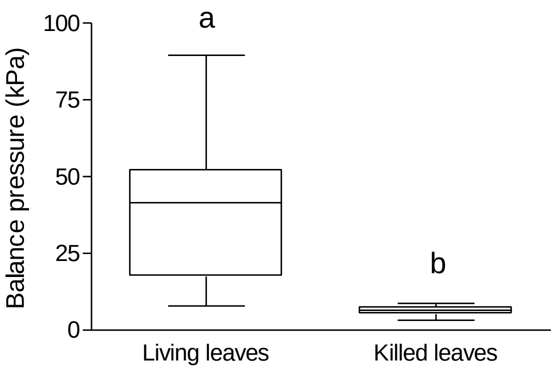

Fig 4.2. Balance pressures of 10 killed and 10 living hydrated leaves from 20 potted plants of *Eucalyptus microcorys*. Killed leaves were immersed in boiling water for 4 min. Petioles of the 20 taped leaves were left immersed in free water for at least 15 hours before measurements. BP was measured with a pressure chamber. The two groups were significantly different ($P<0.001$, student‟s t test).

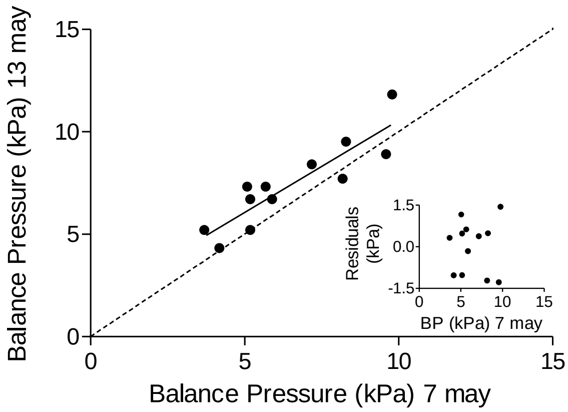

Fig 4.3. Autocorrelation of balance pressures measured in two different days in hydrated leaves from potted plants of *Eucalyptus pachyphylla*. A point represents two different leaves from the same potted plant but from two different dates. Every point is a different plant. The dotted line represents the one to one relationship. Linear regression: $r^2=0.79$, $p<0.001$, $n= 12$ plants. Inset: residuals from the linear model. Data taken from Chapter 5.

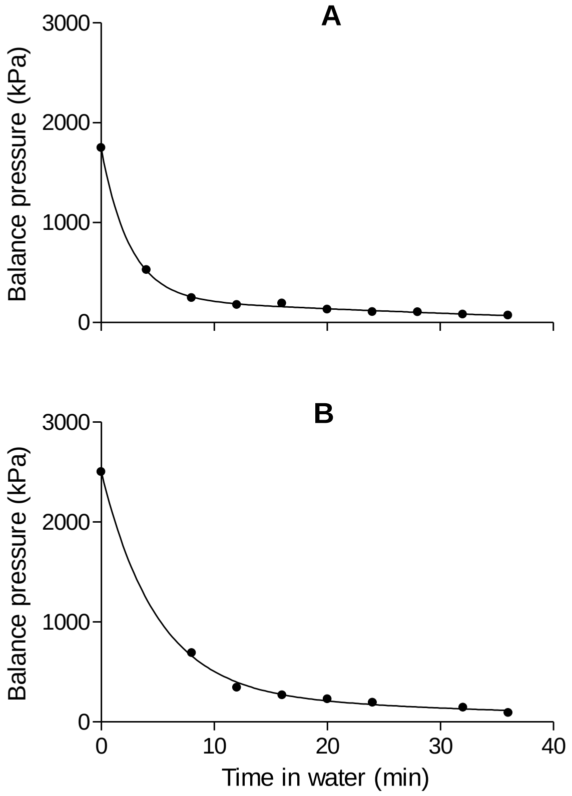

Fig 4.4. Hydration kinetics for two non-transpiring leaves of *Viburnum tinus.* The initial balance pressure measurement was made following leaf sampling from the plant, then the leaf was placed in free water for 4 min and BP was measured again, I repeated this procedure several times for the same leaf until the total time in water was 36 min. The model used for Fig 1a was $y=677.1e^{-0.007873x}+1515e^{-0.3995x}-441.8$ and predicts an x intercept of 54 min, i.e. the time required to reach full equilibrium with free water. The model for Fig 1b was $y=1151e^{-0.003529x}+2255e^{-0.2037x}-900.6$ and predicts 69 min to reach full equilibrium with free water.

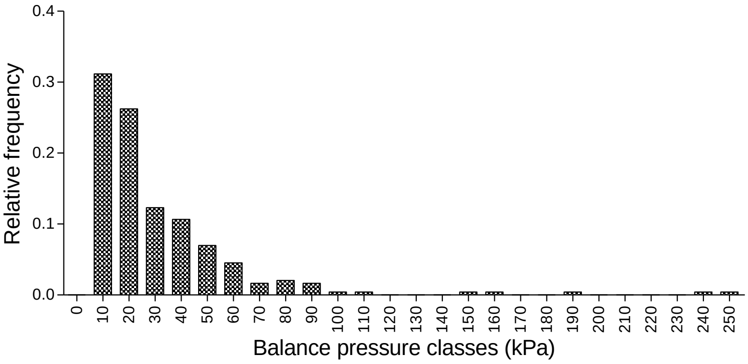

Fig 4.5. Frequency histogram for balance pressures of 244 hydrated leaves from seven mature plants of *Viburnum tinus*. Petioles of the taped leaves were left immersed in free water for at least 15 hours before measurements. BP was measured with a pressure chamber. Values in the horizontal axis represent the center of 10 kPa BP classes. Data were compiled from several measuring days.

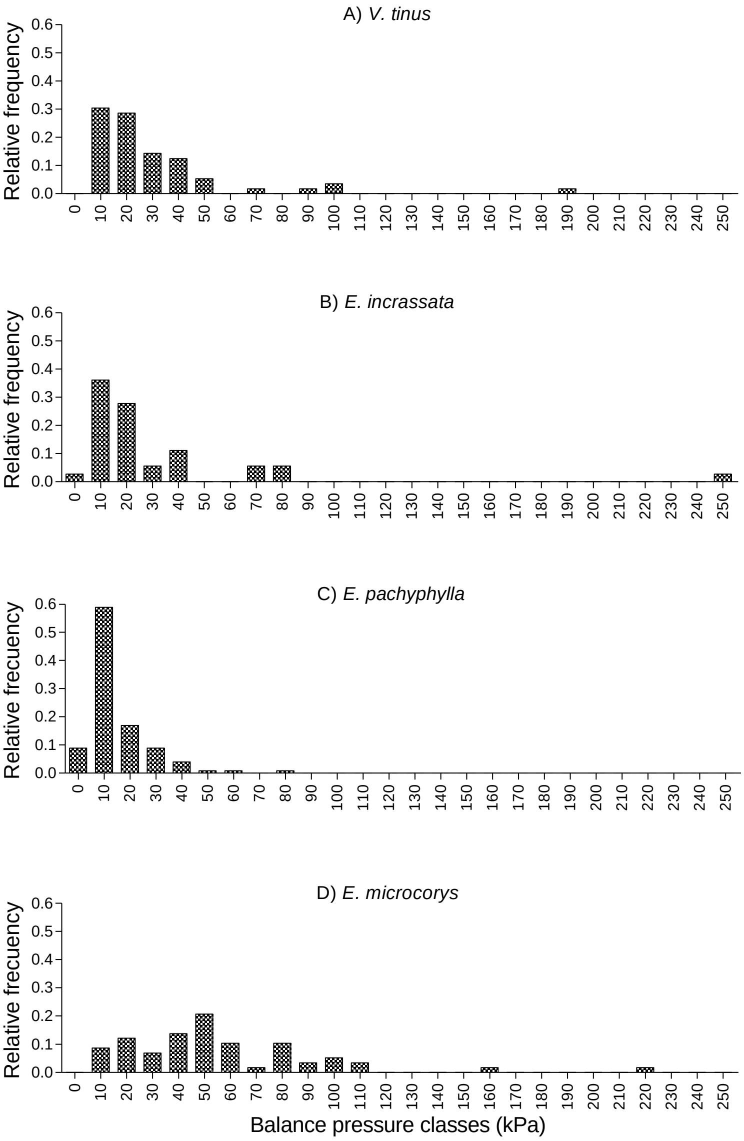

Fig 4.6. Frequency histogram for balance pressures of hydrated leaves from 21 potted plants of the four studied species: A) *Viburnum tinus* n=244 leaves, B) *Eucalyptus incrassata* n=36 leaves, C) *E. pachyphylla* n=124 leaves, and D) *E. microcorys* n=58 leaves. Petioles of the taped leaves were left immersed in free water for at least 15 hours before measurements. BP was measured with a pressure chamber. Values in the horizontal axis represent the center of 10 kPa balance pressure classes. Data were compiled from several measuring days.

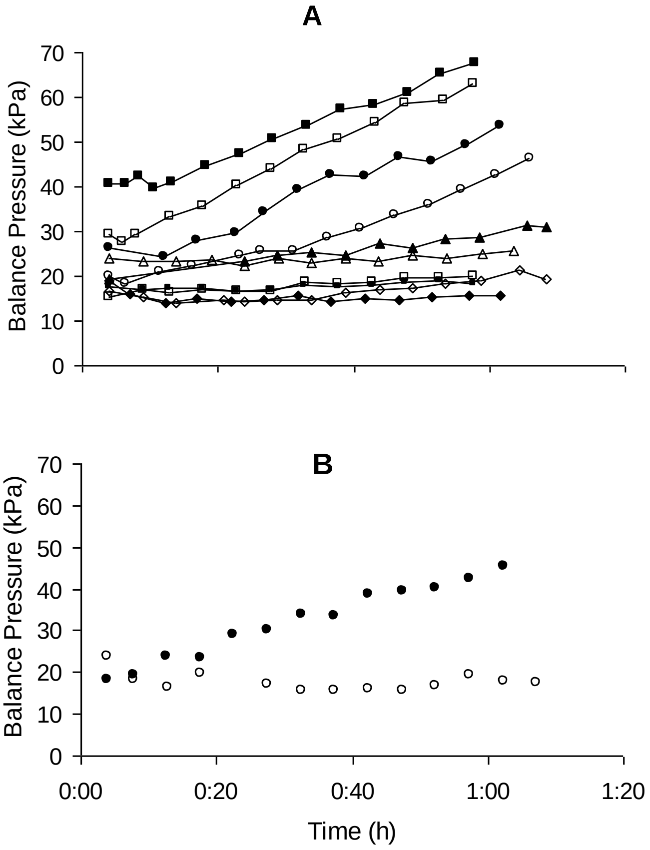

Fig 4.7. Repeated-BP measurements for 12 hydrated leaves of *Viburnum tinus* mature plants measured on April 9 2003. Each different symbol and color represents a different leaf. All measurements were conducted during the same day but using two pressure chambers. Laboratory evaporation, measured on two different flasks, was constant during repeated-BP measurements. A: only one of the 10 regressions analyzed had a slope not different from zero. B: two leaves measured at the same time in two different pressure chambers; the slope of the empty circles was not different from zero.

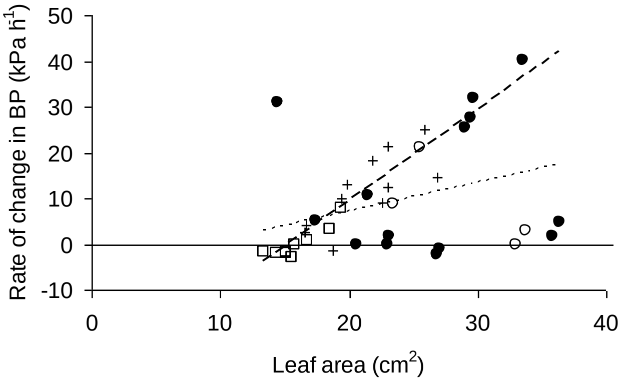

Fig 4.8. Relationship between the rate of change in BP and leaf area in hydrated leaves of *Viburnum tinus*. Data are from different measurement days: March 20 (empty circles, n=4, no linear regression performed due to the few data available), March 31 (empty squares, n=10 leaves, $R^2 = 0.8$ and $p< 0.001$.), April 2 (plus sign, n=11 leaves, $R^2 = 0.6$ and $p< 0.01$) and April 9 (filled circles, n=14 leaves, $R^2 = 0.01$ and $p= 0.69$). Note: two leaves measured on April 9 that always held water at the petiole cut surface were considered to be 0 kPa h^{-1} and were used in this analysis. The dotted line represents the overall linear regression considering all data together (n=39, leaves $R^2 = 0.13$ and $p= 0.023$). Dashed line represents the overall linear regression but eliminating data with a BP change below 5 kPa h^{-1} and one outlier (n=17 leaves, $R^2 = 0.8$ and $p< 0.001$).

Chapter 5) Sources of variability in balance pressure during pressure chamber measurements on hydrated, non-transpiring leaves

5.1 Abstract

I measured balance pressure (BP), as determined by a pressure chamber, on detached, non-transpiring leaves of *Viburnum tinus, Eucalyptus pachyphylla, E. incrassata,* and *E. microcorys*. Prior to measurement, leaves were sealed with water-proof tape and placed in water for 15 hours in a dark room. Leaf position, type of leaf (sun or shade leaves), previous watering regime, leaf mass area or hydration time did not explain the variability in balance pressure (BP) found throughout the four species. Leaf area, age and RGR, however, seem to be related with the variability in BP suggesting the involvement of leaf growth in BP variability. In *V. tinus* leaf area explained 26 to 48 % of the variability in BP with smaller leaves, the expected to have more growth, having more BP. I also compared young and mature leaves in *E. pachyphylla*. BP of young leaves was more variable than that of mature leaves. Comparison among the four species showed that BP was highest and most variable in the taller species with the higher RGR, *E. microcorys*. All these results are consistent with leaf growth as the main cause of BP variability. My hypothesis is that differences in growth-induced water potentials caused part of the variability in BP in the leaves here studied.

5.2 Introduction

The pressure chamber has been the most frequently used technique for measuring xylem pressure potential in plants (see Scholander et al. 1965, Boyer 1995). However, over the last decade there has also been some discussion about the possibility that measurements made with this technique may be unreliable (see Tyree 1997, Meinzer et al. 2001, Zimmermann et al. 2004). This discussion has promoted innovative studies and some of them have lead to more evidence validating the use of the technique (e.g. Holbrook et al. 1995, Pockman et al. 1995, Wei et al. 1999b). Others, however, have reported differences between the results obtained with pressure chamber and pressure probe in transpiring leaves (Melcher, et al. 1998; but see Wei, et al. 1999b). Recently, it was suggested that pressurization of leaves in pressure chamber forces air into solution in leaf-water, and this affects measurements (Canny and Roderick 2005). Furthermore, a new interpretation of pressure chamber measurements suggests that the toughness of cell walls may also affect measurements (Roderick and Canny 2005). The widespread use of the pressure chamber, including its role in providing evidence in support of the Cohesion-Tension theory (Tyree 1997), makes it imperative that all the assumptions on which its operation is based are well tested. Recently, I tested if the pressure chamber technique can measure accurately water potential equilibrium (Chapter 4). I used non-transpiring leaves placed in free water; I expected that under these conditions leaves would eventually equilibrate to a xylem pressure potential of 0 kPa. However, the balance pressures (as measured by the pressure chamber; BP) varied between 3 and 250 kPa despite the technique had a precision of 2 kPa and a bias of 3 to 12 kPa (Chapter 4). I also found that repeated measurements of BP in the same leaf, within an hour, yielded non constant BP in some leaves. It remains to be studied what causes the variability on those BP measurements.

Even small amounts of variability in pressure chamber data can be important. For example, measuring the gravity head in the static water column of plants requires an accuracy of few kPa because the expected gradient is just 10 kPa per metre in height. In fact, variation in the measurement of the gravity head has produced widely differing reports, with some studies have found gradients close to 10 kPa per metre (Scholander et al. 1965, Connor et al. 1977, Baurle et al. 1999, Koch et al. 2004) whereas other have found smaller values or even the absence of a gradient (Hellkvist et al. 1974, Zimmermann et al. 1994, 2002). This not only questions the existence of the hydrostatic gradient itself but also cast doubts on our understanding of the mechanisms of water transport in plants (Zimmermann et al. 1994, 2002, 2004). However, as previously mentioned, my measurements on the technique accuracy (Chapter 4) found that BP varied from 3 to 250 kPa, i.e. the same magnitude as the gradient expected for a 25m tall tree. Thus, the different reports of the magnitude of the gravity head mentioned above may have been caused by other factors creating BP variability rather than real effects of the gravity head itself. Underlying variability in BP may also affect studies of the hydraulic limits to tree height, which are performed by comparing water potentials among leaves into the same plant (Woodruff et al. 2004, Koch et al. 2004). These studies have been performed only in tall trees because a large height difference helps to overcome any measurement errors intrinsic to the pressure chamber technique. Small plants represent an opportunity to make less expensive research, using more controlled conditions and more experimental manipulations than tall trees, but studies on small plants are limited because measurement errors in pressure chamber determinations are usually bigger than the expected differences in water potential for every metre in height.

In this chapter I report results from experiments in which I investigated variability in pressure chamber measurements, and some of the possible causes. Factors such as leaf position, light history (i.e. sun or shade leaves), watering regime, leaf mass area, leaf area and hydration time were all studied.

5.3 Methods

5.3.1 Plant material

I used four species that represented a gradient in leaf mass area: *Eucalyptus pachyphylla*, *E. incrassata*, *Viburnum tinus and E. microcorys* (0.021, 0.016, 0.012, 0.005 g cm^{-1} in LMA respectively; see results). *E. pachyphylla* and *E. incrassata* are small trees (1.5 to 5m and 3 to 7m, respectively) that occur in open mallee woodland of semiarid Australia. *E. microcorys* is a tall forest tree (30 to 50 m) from the moist forests of eastern Australia. *V. tinus* is an evergreen shrub (1.8 to 3 m) from the Mediterranean region of Europe. Potted plants were obtained from local nurseries and maintained in a naturally illuminated glasshouse with a cooling system. The plants were fertilized with a slow release fertilizer and watered to field capacity two or three times a week. I also used seven adult plants of *V. tinus* growing in the gardens of the University of Adelaide, Adelaide, South Australia. They were around 1.8 m tall and 1.5 m in crown diameter.

5.3.2 Leaf sampling and preparation in water

The day before pressure chamber measurements, leaves were cut and transported to the laboratory where the blade was covered on both surfaces with box-sealing tape for packaging (Scotch 3M, St. Paul, MN, U.S.A.) to prevent transpiration. All leaves were left overnight in the same container in a photographic dark room with their petioles immersed in free water for 15 hours or more. Balance pressure determinations were made the following morning.

5.3.3 Balance pressure (BP) measurements

Balance pressures (BP) were measured with a Scholander-type pressure chamber equipped with a digital gauge with a resolution of 0.1 kPa (DTG-6000 3D Instruments, Huntington Beach CA, U.S.A.). Each leaf was taken from water, its petiole was re-cut (less than 1 mm removed) and accommodated into the chamber as quickly as possible (usually 1 to 2 minutes). Around 2 mm of petiole was left out of the chamber and for some experiments that portion was covered with a small chamber with a cover slide (“petiole chamber” or “petiole cover”). This was done to prevent any evaporation from the cut surface of the petiole. Observations of water extrusion from the petiole were made under a stereoscopic microscope.

Pressurization rate was ~5 kPa s^{-1} in most measurements although I also used ~2 kPa s^{-1} when leaves had a water film on the cut surface. BP was measured as the very first appearance of water in xylem vessels or the first movement in a water film at the cut surface of the petiole. It was possible to make very accurate assessment of balancing point in the petiole because the pressure gauge had a button for storing data in its memory, allowing the observer to concentrate totally on the petiole surface. With this method I could work with an accuracy of just 2 kPa (see Chapter 4).

5.3.4 Leaf characteristics

After removing the packaging tape from the blade I measured: leaf area, length, width, thickness, density, leaf mass per area (LMA), and petiole length and diameter. Leaf length, maximum blade width and petiole length and diameter were measured with a calliper with a resolution of 0.05 mm. Leaf area was measured with a leaf area meter (Delta-T Devices LTD, Cambridge, U.K.). Leaf thickness was measured with a micrometer with resolution of 0.01 mm. Several measurements were made along the blade avoiding the midrib and major nerves, and mean leaf thickness was calculated. Leaf dry mass was determined on a digital balance after drying the leaves in an oven at 60 degrees for 72 h.

LMA was used as a measure of sclerophylly. This index is considered the best currently available measure of sclerophylly (Groom and Lamont 1999). The interpretation of LMA requires knowledge of the two other components of mass: thickness and density. LMA and density were calculated according to Witkowski and Lamont (1991):

Density = mass/[(area)(thickness)]

LMA = (thickness)(density) = mass/area

5.3.5 Between species variability

Comparisons between species were performed during several experiments using 21 potted plants per species. Plants were maintained as explained above: in a glasshouse and watered to field capacity three times a week. In the first experiment I sampled only 15 potted plants per species but measured all four species during a single day (November 18). Measurements were made on the youngest fully expanded leaf, which was usually the third to fifth youngest leaf, from the upper, most exposed parts of the plants. One leaf per potted plant was taken to the lab, taped and left to hydrate overnight. BP was determined the following day as described above.

I performed three more comparisons between species but this time in pairs. *E. pachyphylla* and *V. tinus* were compared on November 29, 2002, *E. microcorys* and *V. tinus* on December 13, 2002, and *E. microcorys* and *E. pachyphylla* on January 23, 2003. Fifteen to 21 potted plants per species were sampled during each experiment. The measurement procedure was the same as explained above.

5.3.6 Within species variability

I was able to analyse developmental changes and their influence on BP measurements because some of the measurements described above (i.e. on November 18 and 29, December 13, 2002, and January 23, 2003) were made on the same potted plants in three species. During this analysis I selected only the measurements that were repeated in the same potted plant along the three days. In *E. pachyphylla* there were 15 repeated measurements, 14 in *V. tinus* and 9 in *E. microcorys*.

In order to analyse the variability into a single plant I sampled 17 to 32 leaves in each of four *V. tinus* adult plants from the campus garden. In each plant I selected leaves with different sizes from the upper most exposed part of the canopy. Each plant was sampled in a different day but all selected leaves from a single plant were sampled the same day. They were taken to the lab, taped and left to hydrate overnight, however, in some of the leaves I extended the hydration time to 43 hours. BP was determined the following day as described above.

5.3.7 Effect of leaf position in V. tinus

I compared BP after overnight hydration on leaves taken from different heights of plants from the campus garden. Leaves exposed to full sunlight were taken from different heights [the upper (~1.7m), middle (~1.2m) and lower (~0.8m) part of the canopy] of seven individuals of *V. tinus.* They were taped and left to hydrate the whole night and BP was measured the following day as stated before.

I also compared BP on fully expanded sun leaves versus fully expanded shade leaves from six individuals of *V. tinus*. Sun leaves were taken from the upper, most exposed part of the canopy, and shade leaves from the lower, internal, least exposed part of the canopy. I took two leaves from every position from six individuals growing in the university gardens. Leaves were taped, hydrated and BP measured as described above.

5.3.8 Soil water regime

Three different water regime treatments were imposed on the experimental plants. Twenty-one plants of each of the four species were maintained in a glasshouse and watered to field capacity three times a week for a month. Then, groups of 7 plants per species were exposed to three treatments of 1, 3 and 4 days without watering. I determined the volumetric soil water content of every pot with a theta-probe (ML2x Delta-T devices, Cambridge, England). Measurement were made on the youngest fully expanded leaf; this was usually the third to fifth youngest leaf, usually from the upper, most exposed parts of the plants. Leaves were taken to the lab, taped and left to hydrate overnight. BP was determined the following day as described above. The experiment was repeated in *E. pachyphylla*, *V. tinus and E. microcorys* but with 1, 3 and 6 days without watering.

5.3.9 Leaf age and BP autocorrelation

I compared BP after overnight hydration in mature versus young leaves of *E. incrassata* and *E. pachyphylla* previously exposed to two water regime treatments. A 7 day drought treatment was imposed on 8 plants of each species while the other 8 plants remained well watered. Both groups were sampled the same day and the volumetric soil water content of all pots was measured with a theta-probe (ML2x Delta-T devices, Cambridge, England). I took the third to fifth youngest leaf of each of the 32 plants (young leaves, ~1 month old) and a leaf from the lowest part of the plants (old mature leaves, ~10 months old). Leaves were then taken to the lab, taped and left to hydrate overnight. BP was determined the following day as stated before but using a “petiole cover”. I watered all plants to field capacity and then imposed a second drought regime on the same plants the following week. Thus, the experiment was repeated with leaves taken from the same plants, enabling me to analyse autocorrelation in BP of individual plants.

5.4 Results

5.4.1 Between species variability

During some days there were differences in BP between some species, which may be related with differences in LMA (Fig 5.1, 5.2). In the first experiment there was a clear gradient in

LMA among the four species [*E. pachyphylla* (0.021±0.0012 g/cm^2), *E. incrassata* (0.016±0.0009 g/cm^2), *V. tinus* (0.012±0.0004 g/cm^2), *and E. microcorys* (0.005±0.0003 g/cm^2)] and only *E. microcorys* had more variation and higher balance pressure than the other species (Fig 5.1). However, in following days there were differences in BP between *E. pachyphylla* and *V. tinus,* but not between *E. microcorys* and *V. tinus* (Fig 5.2). Differences between *E. microcorys* and *E. pachyphylla*, as well as differences in LMA between all species, resembled results of the first experiment (Fig 5.2). Every time that BP was different between species, the species with the higher BP had a lower LMA (Fig 5.1, 5.2). This suggests that differences in BP between species may be related to differences in LMA. All these results also suggest a temporal variation in BP in some species.

5.4.2 Within species variability

Developmental changes and their influence on BP measurements could be analysed because the experiments were repeated on the same potted plants in three species. In *E. pachyphylla* a trend to a lower LMA coincided with a higher BP on January 23 (Fig 5.3) while leaf area remained constant during three different measurement days. This may indicate that differences in LMA contribute to temporal differences in BP for plants of the same species. This is consistent with the above results of comparisons between species (see above, Fig 5.2). In *V. tinus* an increasing in BP coincided with a diminishing in leaf area, while LMA remained constant (Fig 5.3). This may indicate that leaf area also contributes to temporal differences in BP for plants of the same species. Results in *E. microcorys* were consistent with the two previous conclusions, because BP remained constant when there was a lower LMA and a higher leaf area in November 18. A higher leaf area may be related with a reduction in BP while a lower LMA with an increase in BP, thus balancing theirs effects, and no affecting BP, during November 18.

In order to study the origin of the intra-specific variability in BP I also performed regression analyses between BP and different leaf characteristics. None of the leaf characteristics measured, including leaf area (Fig 5.4), explained BP variability across leaves from different potted plants in each of the four species. However, as will be shown below, LMA in *E. pachyphylla* did explain part of the BP variability. When measurements were made on leaves from a single adult plant of *V. tinus* leaf area explained 26-57 % of BP variability (Fig 5.5a-b). These measurements were repeated in four adult plants and the results were similar (only two individuals are shown in Fig 5.5). In all these cases, leaf area accounted for more of the variability than any other leaf characteristics (leaf thickness, density, length, width, LMA, petiole length and diameter). Smaller leaves (<15 cm^2) were the main cause of the negative non-linear relationship between leaf area and BP. Leaf area could not explain the BP variability in leaves larger than 15 cm^2, and across different plants (Fig 5.5c-d). The negative relationship between leaf area and BP is consistent with the results previously described for temporal differences in BP for plants of the same species (see above, Fig 5.3).

5.4.3 Hydration time and leaf position in V. tinus

Extending the hydration time to 40 hours did not reduce the magnitude of BP in *V. tinus* (Fig 5.5a-b). Neither the difference in leaf position into the plant affected the magnitude of BP (Fig 5.5c-d). A similar result was found when comparing sun and shade leaves. Despite differences in LMA in sun (0.013±0.0003 g/cm^2) and shade leaves (0.009±0.0003 g/cm^2), and also differences in position on the plant (upper and lower canopy), there were no differences in the balance pressures (Fig 5.6). It should be noted that in all these experiments leaf area was similar between the selected groups of leaves (Fig 5.5 and 5.6).

There were some days where BP for *V. tinus* was less variable and also much closer to equilibrium with water (Fig 5.5d). In contrast, there were other days where leaves of similar size and sampled from the same plants had higher BP and more variability (Fig 5.5c). This was observed in both adult (Fig 5.5c-d) and potted plants (pers. obs.).

5.4.4 Soil water regime

Because the magnitude in BP sometimes changed with measurement day I tested whether this could have been caused by the previous water regime of the plants. However, no relationship was found between BP and watering regime before detaching leaves (Fig 5.7). Results were similar across the four species, and also when I repeated the experiments.

5.4.5 Leaf age and BP autocorrelation

Young leaves of *E. pachyphylla* were more variable in BP and, on average, less sclerophyllous than old leaves (Fig 5.8a-b). However, it was sclerophylly, rather than age, the factor more closely related to BP variability. LMA explained 61% of BP variability ($y=0.011x^{-1.7035}$) during May 7 but only 12% during May 13. However, rather than a gradual change in BP with respect to LMA, there was a clear sudden change in BP variability at LMA around 0.014 g/cm^2, which was consistent when the experiment was repeated (Fig 5.8a-b). BP in these more sclerophyllous leaves did not rise above 12 kPa during both experiments. In contrast, BP variability was similar in all *E. incrassata* leaves regardless of age or LMA (Fig 5.8c).

I was able to analyse the autocorrelation of individual plants because the experiment was repeated on the same potted plants. There was autocorrelation in both, young and old leaves in *E. pachyphylla*, and young leaves of *E. incrassata* (Fig 5.9). The autocorrelation in *E. pachyphylla* old leaves was very close to a one to one relationship explaining 79% of the data variability (Fig 5.9a) and their residuals were less than 1.5 kPa (see Chapter 4). In young leaves of both species only explained around 40 % of data variability (Fig 5.9c-d). The autocorrelation was independent of the previous water regime.

5.5 Discussion

5.5.1 BP variability and leaf growth

Several leaf characteristics by themselves were not able to explain the deviations from equilibrium with free water in the four species investigated. Nor were position within the plant, hydration time or previous watering regime related to variability in BP. Nevertheless, I found that leaf area in *V. tinus* and LMA in *E. pachyphylla* explained part of the variability in BP. LMA may also be related to some of the differences in BP between species and together with leaf area to some of the temporal differences in BP within the same species. All these results, together with other pieces of evidence, suggest that departure from equilibrium may be related, at least partially, to leaf growth. First, leaf area explained part of the variability in *V. tinus* with very small leaves, the ones expected to have more growth activity, being further away from equilibrium. Secondly, when I compared BP from the four species, the least sclerophyllous, *E. microcorys*, had the most BP variability. This species had the higher relative growth rate (RGR) than the other four (pers. obs.). Differences in BP and LMA between pairs of species also showed what seems to be a pattern: the species with the lower LMA had the higher BP. This may be related to differences in RGR between species because LMA has been shown to be negatively correlated with RGR in many species including *Eucalyptus* (Wright and Westoby 1999, 2000). Therefore, the observed BP variability may be related to differences in leaf growth not only within species but also between species. Finally, sclerophylly (LMA) rather than leaf size was more closely related to BP variability in *E. pachyphylla*, with more sclerophyllous (and mainly mature) leaves achieving equilibrium with free water. In this case, higher LMA could be a consequence of reduced leaf expansion rate (Tardieu et al. 1999) and/or be related to a reduction in elasticity of the cell wall (e.g. Salleo and Lo Gullo 1990, Groom and Lamont 1997, Galmes et al.

2007). Cell wall stiffening is a major cause of leaf growth reduction (see Cosgrove 1997a, Cosgrove 1997b, Schopfer 2006). Cell growth depends on the balance between wall-loosening and wall-stiffening (Schopfer 2006). The irreversible cessation of growth in mature leaves is related with the continued addition of secondary wall material that causes wall stiffening in aged leaves (Cosgrove 1997a, Cosgrove 1997b, Schopfer 2006). The observed LMA value where BP variability abruptly changes (around 0.014 g/cm^2) may indicate a LMA limit to leaf expansion for *E. pachyphylla* under the conditions here studied.

Regarding LMA two other conclusions can be made. Firstly, LMA was related to BP variability but only when LMA was related to leaf aging or development, not when differences in LMA were caused by sun exposition (sun versus shade leaves). This contrasting response is consistent with the idea that part of the BP variability is related to leaf growth. Secondly, Roderick and Canny (2005) in their "plastic bottle analogy" suggested that measurements with the pressure chamber would be directly related to leaf toughness and here I did not found such relationship between LMA and BP when comparing sun vs shade leaves. In addition, in *E. pachyphylla*, and when comparing differences in BP between species, I found an opposite relationship to the one suggested by Roderick and Canny (2005).

Leaf growth could be affecting BP measurements through growth-induced water potential. Growth-induced water potential (for a review see Boyer and Silk 2004) has been suggested to originate from wall yielding (Boyer 2001). In growing tissues cell walls yield and create a water potential below that of water supply in the xylem vessels, this gradient cause water to move into the growing cells (Boyer 2001, Boyer and Silk 2004). Growth-induced water potentials have been measured in growing hypocotyls (Boyer 2001, for a review see Boyer and Silk 2004) and leaves (Boyer 1968, Martre et al. 1999, Tang and Boyer 2002), and by using different techniques: pressure probe (e.g. Martre et al. 1999), psychrometer (e.g. Boyer 1968, Tang and Boyer 2002) and pressure chamber (e.g. Boyer 2001). By using this last technique a strong negative relationship was detected between the elongation rate of soybean seedlings under different pressures into a pressure chamber with the pressure required to return the water film into a recently cut stem (Boyer 2001). The magnitude of growth-induced water potentials in leaves have been found to be between 150 and 500 kPa (Boyer 1968, Martre et al. 1999, Tang and Boyer 2002). Because growth-induced water potentials generate tension on water in the apoplast it can be measured as xylem pressure potential with the pressure chamber (Boyer 2001). My measurements with the pressure chamber represent a weighted average of both growing and non-growing tissue in the same leaf; this may explain the lower values for most of my BP determinations (12 to 100 kPa) relative to those reported for growing leaves. In contrast, very small leaves in *V. tinus* were within the range found by previous authors; I assume that these leaves were comprised mostly of growing tissue. I suggest that the growth-induced water potential could partially explain the BP variability found in this study. This possibility needs further experimentation with more direct tests. More studies are also needed to completely explain and control all sources of variability during very precise pressure chamber determinations. For example, in my study there were fully expanded leaves which did not equilibrate with free water. On the other hand, there were young or small leaves that were at equilibrium. In addition, it seems that the frequency of leaves that had equilibrium varied with measurement day (Fig 5.5c-f). Part of this temporal variation may be explained by developmental differences in the sampled leaves but also by the temporal variation in growth rates (e.g. Schurr et al. 2006). The autocorrelation of the measurements suggest that there are genetic characteristics or particular plant conditions during leaf development that are affecting BP measurements; this also needs further research. Among other possible mechanisms that may explain partially the BP variability found here and that need further research are: variable osmotic potential due to differences in starch-to-sugar conversion, differences in hydraulic resistances due to maturation of xylem vessels, variable hydraulic coupling due to plasmodesmata and aquaporins.

5.5.2 Implications for pressure chamber measurements

Our results have important implications for the interpretation of pressure chamber data in some particular studies. For example, the disequilibrium that is sometimes found between non-transpiring plants and saturated soil (Donovan, et al. 2001) or free water (Rieger and Litvin 1999) has been explained in terms of solute accumulation in leaf apoplast (Donovan et al. 1999, 2001) or apoplastic barriers in the root (Rieger and Litvin 1999) but it may be partially explained by growth-induced water potential as well. My results are also relevant for studies testing the hydraulic limits to tree height (e.g. Woodruff et al. 2004, Koch et al. 2004) or the existence of the hydrostatic gradient (e.g. Scholander et al. 1965, Baurle et al. 1999, Zimmermann et al. 1994, 2002) because small differences in water potentials (of only 10 kPa per metre in height) are compared between non-transpiring leaves from the same plant. Those studies usually assume conditions of water potential equilibrium along the plant and also between the plant and the soil. Although, in very tall trees, the distances involved, can usually overcome some of these errors and variability in the measurements (e.g. Woodruff et al. 2004, Koch et al. 2004) growth-induced water potential could also affect measurements and interpretations, and studies with small plants may be problematic. Contrasting results relating to existence of the gravity head (Zimmermann et al. 1994, 2002) may also be related to the BP variability arising from leaf growth. This variability needs to be taken into account before interpreting gradients in water potentials. As was shown here, however, it is difficult to avoid BP variability when selecting leaves on the basis of area alone. For some species, like *E. pachyphylla*, I could recommend using age or LMA to avoid leaves with variable BP.

Finally, it is interesting to note that the correlations found by Melcher et al. (1998) and Wei et al. (1999b) between pressure chamber and the pressure probe measurements in non-transpiring leaves, were made in monocot plants. Leaf growth in monocots is restricted to the first few centimetres of the leaf base (Tang and Boyer 2002, Tardieu et al. 2000) and it is there where the growth induced water potential is manifested (Martre et al. 1999, Tang and Boyer 2002). Above the growth zone a mature tissue with homogenous and constant water potential is expected when leaves are not transpiring (Tang and Boyer 2002). It is likely that when the non-growing leaf tips of monocots are used in such studies, stable measurements will be obtained (e.g. Melcher et al. 1998, Wei et al. 1999b). In contrast, early evidence against the pressure chamber was based mainly on work with dicot plants (e.g. Balling and Zimmermann 1990, Benkert et al. 1995, Zimmermann et al. 1993, Zimmermann et al. 1994) where measurements with the pressure chamber are made at the petioles, thus including all the growing parts of the blade and adding more variability to their measurements. Comparison between the two techniques with dicots would clarify whether the discrepancies were related to BP variability arising from leaf growth.

5.5.3 Concluding remarks

Evidence found here suggests that the non-equilibrium between leaves and free water may be caused by leaf growth and more specifically by growth-induced water potential. However, this possibility and the involvement of other mechanisms need further research. Nevertheless, the BP variability found in some leaves, if not controlled or taken into account, would affect interpretations of pressure chamber data. More research is needed to fully control BP variation and sources of error in very accurate measurement with the pressure chamber.

5.6 Tables and figures

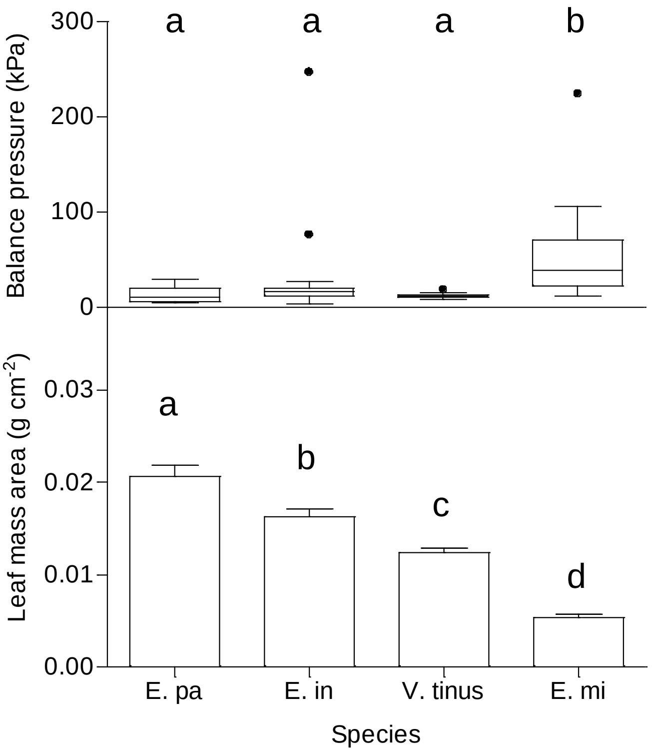

Fig 5.1. A single day experiment (November 18) showing BP and LMA in rehydrated leaves from the four species. E. pa = *E. pachyphylla,* E. in = *E. incrassata,* V. ti = *V. tinus*, E. mi = *E. microcorys.* n=15 leaves, each one from a different potted plant. Different letters indicate significant differences from one-way ANOVA and Tukey test. For the statistical analysis BP data were log-transformed. The upper box plots show the median, the 25th and 75th percentiles (borders of the box), the outermost data points within the 1.5 interquartile range (bars) and outliers.

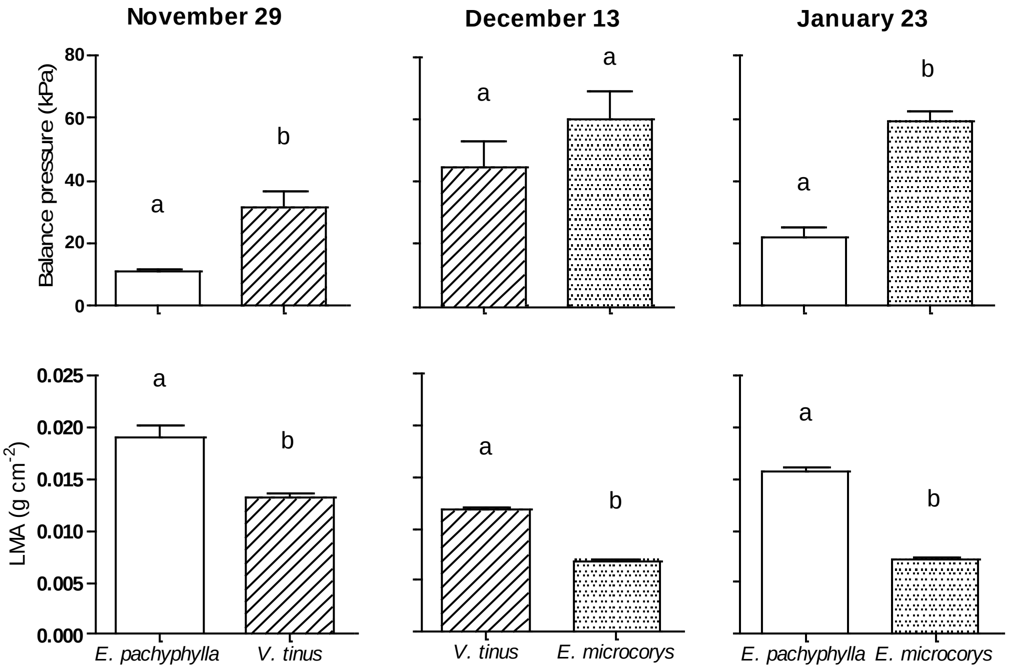

Fig 5.2. Balance pressure and LMA in hydrated leaves from three different species that were measured in pairs during three different days. Every measured leaf during the same single day was from a different potted plant. On November 29 n=21 for *E. pachyphylla* and *V. tinus*, on December 13 n=19 for *E. microcorys* while n=20 for *V. tinus*, on January 23 n=15 for *E. microcorys* while n=20 for *E. pachyphylla*. Different letters indicate significant differences from a student"s t test. For the statistical analysis BP data were log-transformed.

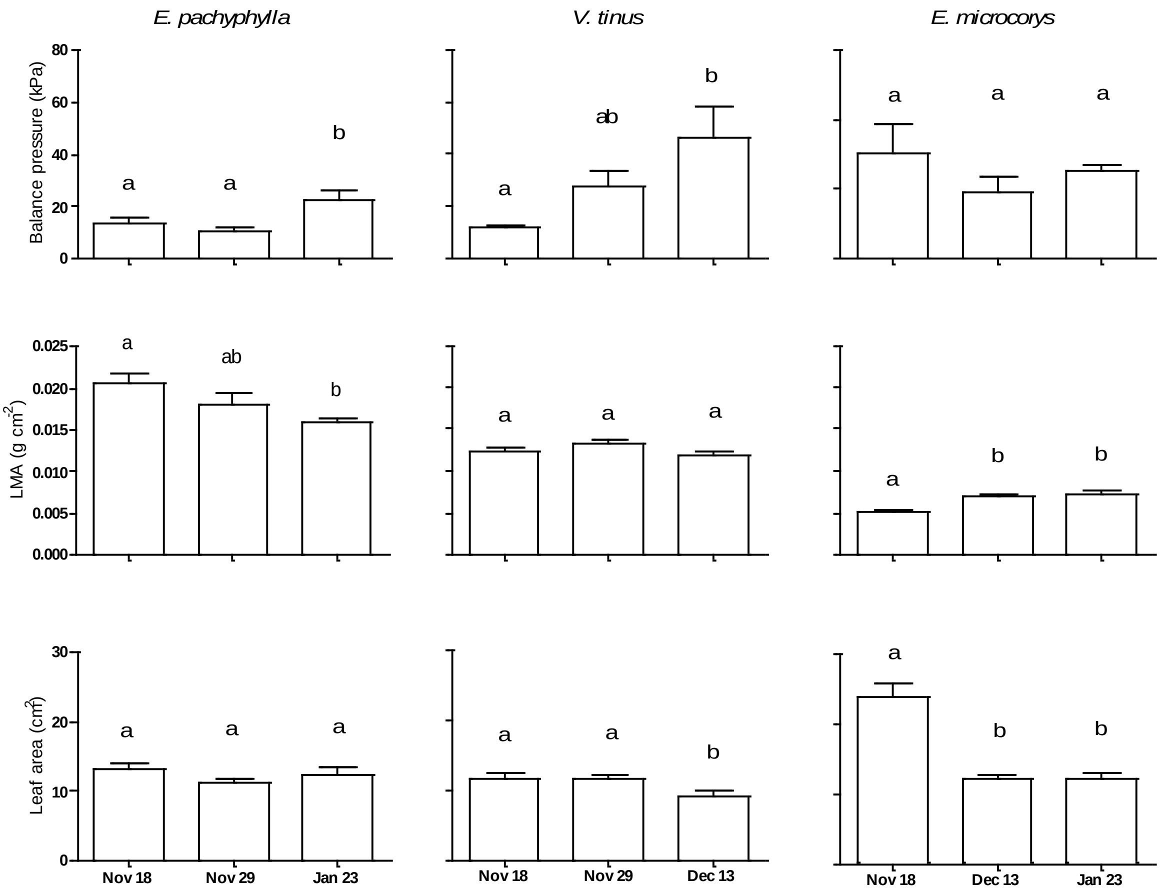

Fig 5.3. Balance pressure, LMA and leaf area on different days from the same potted plants of three species. Data used for this analysis were obtained from the experiments in Fig 1 and 2. However, I only used measurements from those potted plants that were sampled during the three days (repeated measurements). *E. pachyphylla* n=15, *V. tinus* n=14 *E. microcorys* n=9. Different letters indicate significant differences from one-way ANOVA of repeated measures and Tukey test.

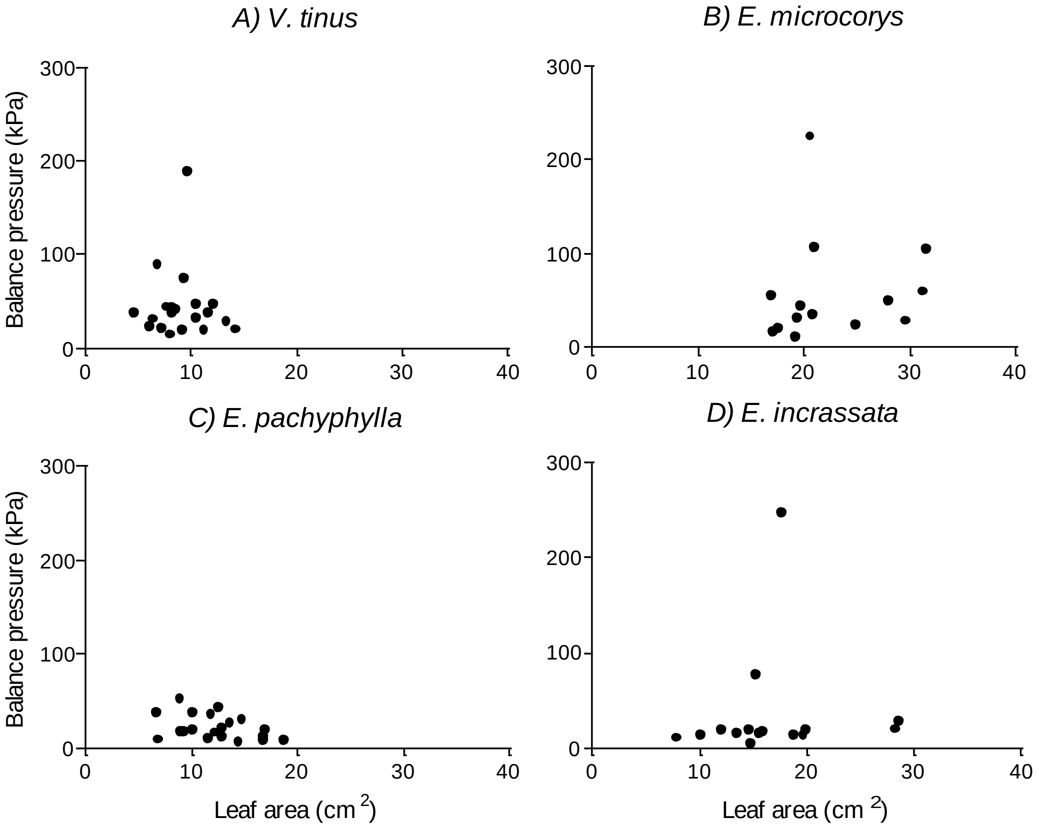

Fig 5.4. Balance pressure and leaf area in hydrated leaves from potted plants from the four species. Every point represents a different leaf from a different potted plant growing in a glasshouse. Measurements were performed on November 18 (B and D), December 13 (A), 2002 and January 23, 2003 (C).

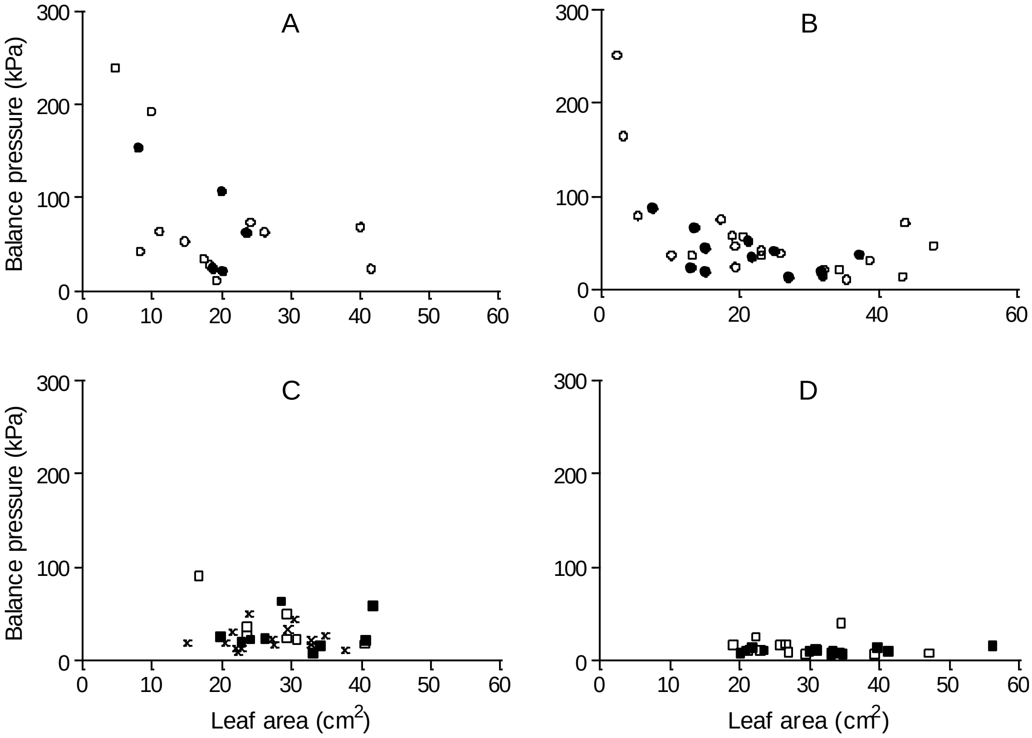

Fig 5.5. Leaf area and balance pressure in hydrated leaves from *V. tinus* adult plants growing on the gardens of the University of Adelaide. Each point represents a different leaf. A-B: each graph represents a different adult plant; leaves were left on water for 15 to 21 h (empty circles) or 40 to 43 h (filled circles). C-D: leaves sampled from three different positions on each of seven adult plants: the upper part of the canopy (filled circles), lower part of the canopy (empty circles) and an intermediate position (stars); measurements were performed on August 2 (C) and 5, 2002 (D).

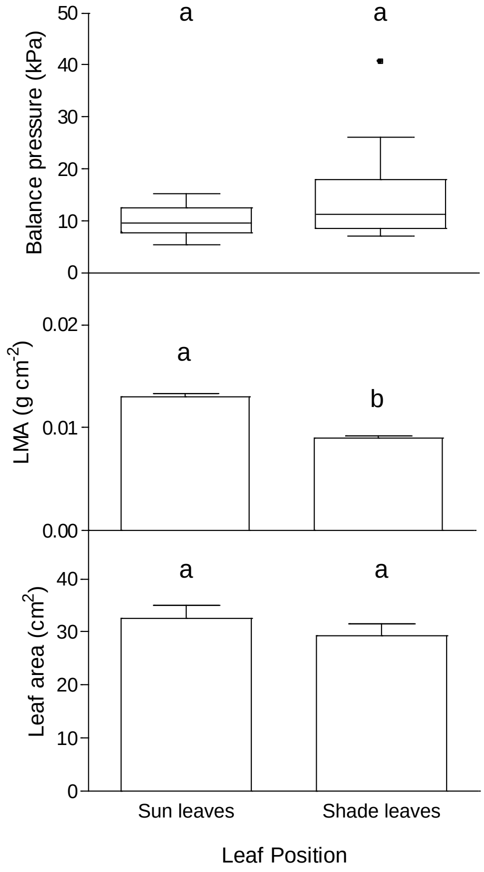

Fig 5.6. Balance pressure (BP), LMA and leaf area on hydrated leaves from the upper most exposed part of the canopy (sun leaves) and from the lower less exposed part of the canopy (shade leaves). n=14 leaves, 2 from each of seven *V. tinus* adult plants growing on the gardens of the University of Adelaide. Different letter indicates significant differences from a student‟s t test. For the statistical analysis BP data were log-transformed. The upper box plots show the median, the 25th and 75th percentiles (borders of the box), the outermost data points within the 1.5 interquartile range (bars) and outliers.

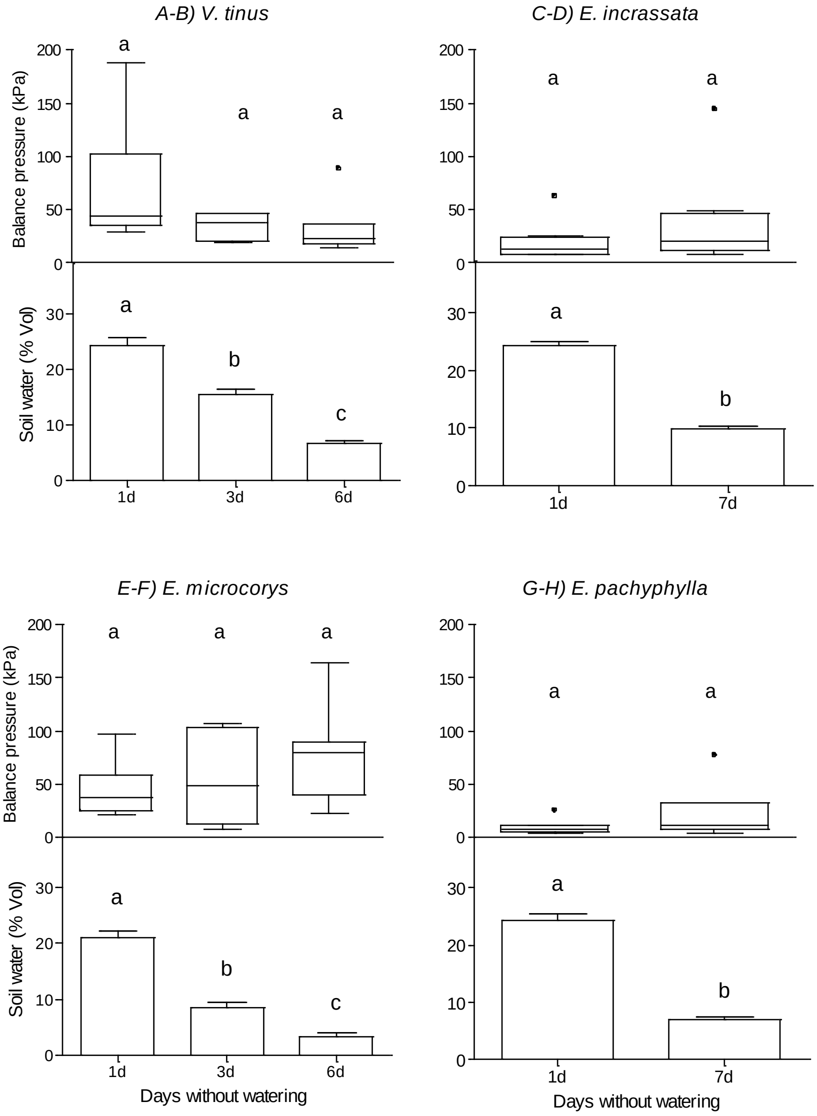

Fig 5.7. Experiments showing the effect of previous watering regime on the Balance Pressure of hydrated leaves from the four species. The watering regime was imposed on the potted plants before detaching the leaves. n=6 for *E. microcorys,* n=7 for *V. tinus* and n=8 for *E. pachyphylla* and *E. incrassata*. Each leaf was from a different potted plant. Different letters indicate significant differences from student"s t test or one-way ANOVA and Tukey test. For the statistical analysis BP data were log-transformed. The upper box plots show the median, the 25th and 75th percentiles (borders of the box), the outermost data points within the 1.5 interquartile range (bars) and outliers.

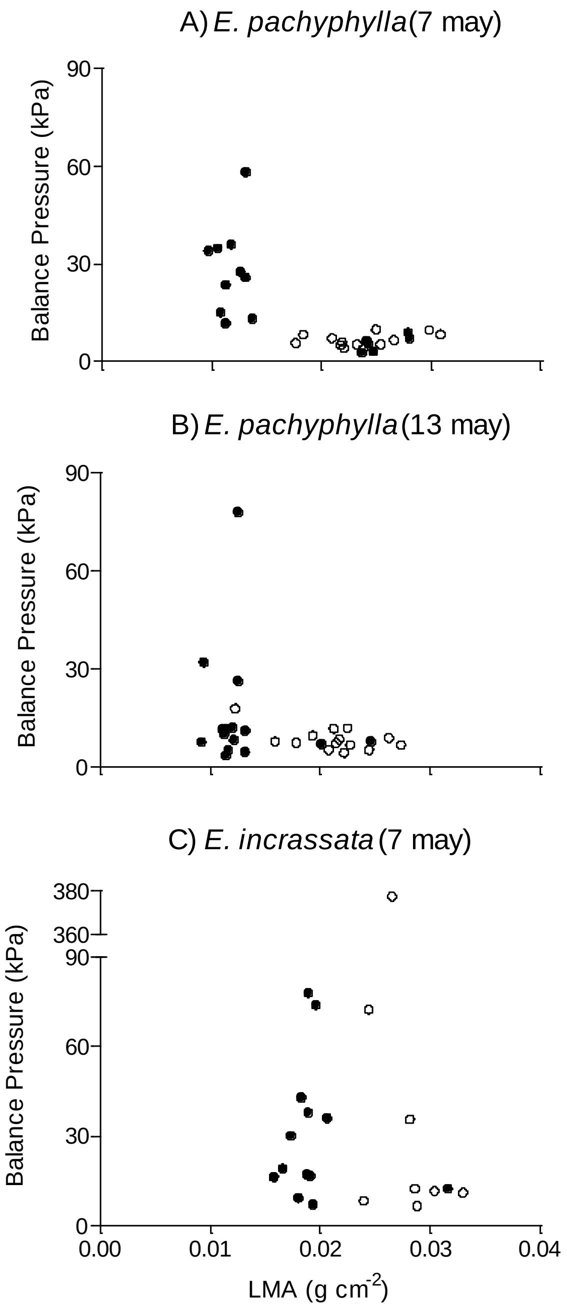

Fig 5.8. Balance pressures and LMA for old (~10 months; empty circles) and young hydrated leaves (~1 month; filled circles) from potted plants of *E. pachyphylla* and *E. incrassata*.

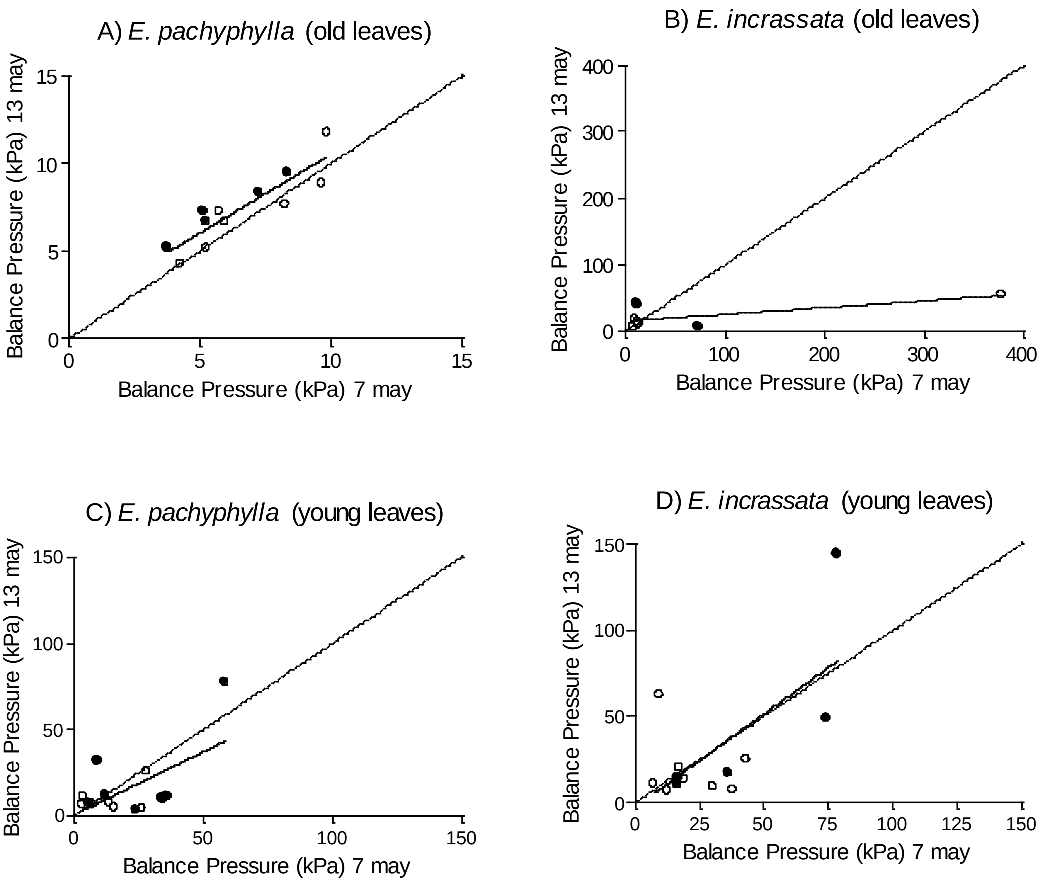

Fig 5.9. Autocorrelation in hydrated leaves from of *E. pachyphylla* and *E. incrassata*. A point represents two different leaves from the same potted plant but from two different dates. Every point is a different plant. Those plants had been subjected to different water regime, watered (filled circles) and drought (empty circles). The dotted line represents the one to one relationship. Overall results for each graph are: A: r^2=0.79, p=0.0001; B: r^2=0.54, p=0.058; C: r^2=0.37, p=0.016; D: r^2=0.43, p=0.015.

Chapter 6) General conclusions

During the last decade the Hydraulic Limitation Hypothesis (HLH) has received much attention from researchers and there is a good body of data supporting its role in explaining the mechanisms that limit tree height. However, in a recent review it was concluded that the HLH is not universal and that there are inconsistencies, particularly with respect to water relations, that require further study. In my literature review I noted that most studies testing the HLH have confounded the path length of water transport with tree height when using tall narrow trees. In addition, studying tall trees can be logistically difficult and expensive. For these reasons different approaches and model trees are required to identify the specific mechanisms limiting tree height. During my research I used broad-crowned trees as models to study the HLH (Chapters 2 and 3). I also investigated variability in pressure chamber measurements to determine whether difficulties in measuring water potentials accurately could be hampering investigations into the role of the HLH (Chapters 4 and 5).

Many factors contribute concurrently to determine tree shape and height (Westoby et al. 2002). However, in certain species and/or under particular conditions, one or two of these factors become dominant. It is well documented that hydraulic mechanisms limit tree height in many species under a range of conditions, but this factor is not always the most limiting. For example, wind is the most important factor limiting tree height in many exposed locations. Thus, in my study I investigated a range of factors that could be limiting tree height in *Acacia papyrocarpa* so as to properly identify the role of hydraulic limitations (Chapter 2). Once I had determined that hydraulic limitations were an important factor in limiting the height of *Acacia papyrocarpa*, I then investigated the mechanisms contributing to hydraulic limitations in this species, as its broad crown enabled me to disentangle the effects of gravity and water pathway length on hydraulic limitations (Chapter 3).

My results excluded other height-limiting factor such as plant-herbivore interactions, escape from fire, light competition or wind damage (Chapter 2). Wind was ruled out as a critical factor limiting tree height in this system because if wind affected tree height then it would also affect crown orientation in isolated trees. Crown measurements were consistent with crown orientation being related to sun exposition but not to wind effects. This research was the first to take into account most of the factors proposed to explain height and crown shape in broad crowned trees. They had been studied before but only independently and their predictions had never been tested against a water transport limitation. If water transport is involved in limiting tree height then trees growing in sites with greater water availability should be taller on average, as I found for *A. papyrocarpa*. In addition, the carbon isotope composition of the upper branches of trees across a water availability gradient was similar as would be expected if the magnitude of the hydraulic limitation at the top of the trees was the same across the gradient; despite the trees differing in height (see also Koch et al. 2004).

I also used the crown shape to compare the contribution of pathway length and height to the carbon isotope composition of the foliage (Chapter 3). Previous studies used tall narrow trees to test the HLH and its mechanisms. However, this confounds height and water pathway length, especially when studying the carbon isotope composition of the foliage. One additional problem frequently present when studying the HLH has been the use of crowded trees that add the confounding factor of differential light penetration into the canopy. I overcame these two problems with a novel approach: I used isolated broad crowned trees as model trees. With those trees I was able to compare carbon isotopes ratios from two contrasting parts of the canopy of the same tree, one with shorter path length but greater height (i.e. the top middle vertical stems) and the other with longer path length but less height (i.e. the horizontal north oriented branches). The north part of the crown in *A. papyrocarpa* is as exposed to the sun as the treetop and would be subjected to similar water vapour deficit. Both branches had similar hydraulic conductivity and photosynthetic capacity in their foliage. However, they were different in the carbon isotope composition of the foliage: $\delta^{13}C$ was lower in foliage from the longer but lower horizontal stems. Thus, longer water pathways did not result in higher $\delta^{13}C$ as would be expected if path length were the main source of hydraulic limitation. The difference in height between canopy parts seems to be the only factor that could explain the difference in $\delta^{13}C$. This suggests that the effects of gravity on water transport can be more important than path-length resistances as the source of hydraulic limitation to tree height. Among other effects, gravity could affect recovery of cavitated vessels and/or the maintenance of cell turgor needed for growth. I proposed that refilling of cavitated vessels may be more important than the actual rupture of the water column. Thus, gravity effects could explain both the difference in length and $\delta^{13}C$ between horizontal branches and branches from the treetop.

Testing the HLH in small trees may be less expensive and logistically easier in comparison with tall trees. However, one of the limitations for using small trees may be the high intra-canopy variability (of around 100 to 200 kPa for leaves at the same position) during xylem pressure potential determinations with the pressure chamber at hydrostatic conditions (e.g. Brooks et al. 2003). This variability is considerable in comparison with the magnitude of the hydrostatic gradient, which is only 10 kPa per meter of height. Using tall trees has been the common approach to overcome this problem because substantial differences in xylem pressure potential usually compensate for such variability. However, it is important to understand and control the source of this variability because this technique is the main tool used for measuring water potential in plants and much of the research depends on it. I investigated precision and bias (Chapter 4), as well as sources of variability during pressure chamber measurements under controlled conditions (Chapter 5). My approach was to study detached and non-transpiring leaves left overnight to equilibrate with free water. I found a precision of 2 kPa and a small bias of 4 to 12 kPa when using the pressure chamber with stereomicroscope and a pressure gauge with a digital display (Chapter 4). The resulting accuracy (6 to 14 kPa) would be good enough for reliable studies on the hydrostatic gradient in small plants. However, I also found deviations from the expected equilibrium with free water (from 15 to more than 100 kPa) that were not caused by measurement errors but were seemingly related to the biological processes of the leaf. If this disequilibrium is not taken into account when interpreting results it would become a bias of the technique. I studied possible causes of this disequilibrium and my results suggest that it may be related specifically to leaf growth, and consistent with this hypothesis, it was of similar magnitude as the growth-induced water potential (Chapter 5). This magnitude is also similar to the intra-canopy variability found in field studies. Therefore, differences in leaf growth may explain, at least partially, the intra-canopy variability in xylem water potentials often found in field studies. This may also explain some conflicting results in previous water relations studies, for example, differences between measurements conducted with pressure chambers and with pressure probes (e.g. Balling and Zimmermann 1990), the controversy about the continuity of the water column (e.g. Zimmermann et al. 2002), and the water potential disequilibrium between soil and plant (e.g. Donovan et al. 2001). My results may have implications for interpreting pressure chamber data especially when trying to measure under hydrostatic conditions. For example, the contribution of the gravity head in limiting tree height is usually determined at predawn when hydrostatic conditions are expected; however, morning conditions (low VPD, low transpiration and high water potential) are also the most suitable for leaf growth and this may cause the mentioned variability. My study also opens the possibility of having very accurate measurements with the pressure chamber technique. However, more experimental work is needed to fully identify and control all sources of variability during pressure chamber measurements.

In summary, I have made some novel contributions that can be grouped into three areas. Firstly, my thesis represents an effort to identify and control previously confounding factors that obscure our search of more precise understanding of the mechanisms that limits tree height. I have shown that trees with broad canopies offer a more appropriate architecture for these studies, making it possible to separate pathway and height effects, which are confounded in tall, narrow trees. Light penetration into the canopy is a confounding factor that can be controlled by studying isolated trees. I have also investigated the accuracy of one of the main techniques used to measure xylem pressure potentials in studies of the role of the HLH in tall trees. From this study I have identified a potential extra confounding factor that needs to be taken into account: leaf growth. Secondly, my thesis also contributes to making studies testing the HLH more accessible. I have shown that using small trees is a reliable and economic alternative to the studies using tall trees. This would facilitate studies testing limits in tree height. Thirdly, my thesis takes into account a new, alternative, paradigm in water relations: the involvement of the living tissue in water transport. The discovery of aquaporins as well as the frequent refilling of the cavitated vessels contributed to the establishment of this new paradigm. I have proposed that the water relations of the living tissue through the refilling of cavitated vessels may be an important factor limiting tree height. I have also documented that pressure chamber determinations may also be affected by some living process like leaf growth.

By testing basic assumptions in one of the most popular techniques for measuring xylem pressure potential, by using different model trees and approaches when testing the HLH, and by being perceptive to the new discoveries in water relations my thesis provides original information not only with respect to the HLH but also to water relations in general. Although the issues presented here are far from being solved I have shown some directions for further studies.

7) References

Amodeo G, Dorr R, Vallejo A, Sutka M, Parisi M (1999) Radial and axial water transport in the sugar beet storage root. *Journal of Experimental Botany* **50**, 509-516.

Anfodillo T, Carraro V, Carrer M, Fior C, Rossi S (2006) Convergent tapering of xylem conduits in different woody species. *New Phytologist* **169**, 279-290.

Anisimov AV (1993) The symplast radial-axial water transport in plants: a NMR approach. Pages 141–146 *in* M. Borghetti, J. Grace, and A. Raschi, editors. Water transport in plants under climatic stress. Cambridge University Press, Cambridge, U. K.

Archibald S, Bond WJ (2003) Growing tall vs growing wide: Tree architecture and allometry of *Acacia karroo* in forest, savanna, and arid environments. *Oikos* **102**, 3-14.

Arora R, Rowland LJ, Tanino K (2003) Induction and release of bud dormancy in woody perennials: a science comes of age. *Hortscience* **38**, 911-921.

Backhouse SL, Pegg RK (1984) The effects of the prevailing wind on trees in a small area of south-west Hampshire. *Journal of Biogeography* **11**, 401-411.

Balfour DA, Midgley JJ (2006) Fire induced stem death in an African acacia is not caused by canopy scorching. *Austral Ecology* **31**, 892-896.

Balling A, Zimmermann U (1990) Comparative measurements of the xylem pressure of *Nicotiana* plants by means of the pressure bomb and pressure probe. *Planta* **182**, 325-338.

Barrieu F, Chaumont F, Chrispeels MJ (1998) High expression of the tonoplast aquaporin ZmTIP1 in epidermal and conducting tissues of maize. *Plant Physiology* **117**, 1153-1163.

Barnard HR, Ryan MG (2003) A test of the hydraulic limitation hypothesis in fast-growing *Eucalyptus saligna*. *Plant Cell and Environment* **26**, 1235-1245.

Batschelet E (1981) Circular statistics in biology. Academic Press Inc., London, U. K.

Baurle WL, Hinckley TM, Cermak J, Kucera J (1999) The canopy water relations of old-growth Douglas-fir trees. *Trees* **13**, 211-217.

Becker P, Gribben RJ, Lim CM (2000a) Tapered conduits can buffer hydraulic conductance from path- length effects. *Tree Physiology* **20**, 965-967.

Becker P, Meinzer FC, Wullschleger SD (2000b) Hydraulic limitation of tree height: a critique. *Functional Ecology* **14**, 4-11.

Begg JE, Turner NC (1970) Water potential gradients in field tobacco. *Plant Physiology* **46**, 343-346.

Benkert R, Zhu JJ, Zimmermann G, Tuerk R, Bentrup FW, Zimmermann U (1995) Long-term xylem pressure measurements in the liana *Tetrastigma voinierianum* by means of the xylem pressure probe. *Planta* **196**, 804-813.

Bhaskar R, Ackerly DD (2006) Ecological relevance of minimum seasonal water potentials. *Physiologia Plantarum* **127**, 353-359.

Bond BJ, Czarnomski NM, Cooper C, Day ME, Greenwood MS (2007) Developmental decline in height growth in Douglas-fir. *Tree Physiology* **27**, 441-53.

Bond BJ, Ryan MG (2000) Comment on 'Hydraulic limitation of tree height: a critique' by Becker, Meinzer and Wullschleger. *Functional Ecology* **14**, 137-140.

Boyce SG (1954) The Salt Spray Community. *Ecological Monographs* **24**, 29-67.

Boyer JS (1967) Matric potentials of leaves. *Plant Physiology* **42**, 213-217.

Boyer JS (1968) Relationship of water potential to growth of leaves. *Plant Physiology* **43**, 1056-1062.

Boyer JS (1995) Measuring the water status of plants and soils. Academic Press, San Diego, California, USA.

Boyer JS (2001) Growth-induced water potentials originate from wall yielding during growth. *Journal of Experimental Botany* **52**, 1483-1488.

Boyer JS, Silk WK (2004) Hydraulics of plant growth. *Functional Plant Biology* **31**, 761-773.

Brodribb TJ, Feild TS (2000) Stem hydraulic supply is linked to leaf photosynthetic capacity: evidence from New Caledonian and Tasmanian rainforests. *Plant Cell and Environment* **23**, 1381-1388.

Brodribb TJ, Holbrook NM (2003) Stomatal closure during leaf dehydration, correlation with other leaf physiological traits. *Plant Physiology* **132**, 2166-2173.

Brodribb TJ, Holbrook NM (2004) Diurnal depression of leaf hydraulic conductance in a tropical tree species. *Plant Cell and Environment* **27**, 820-827.

Brodribb TJ, Holbrook NM, Edwards EJ, Gutierrez MV (2003) Relations between stomatal closure, leaf turgor and xylem vulnerability in eight tropical dry forest trees. *Plant Cell and Environment* **26**, 443-450.

Brooks R, Schulte P, Bond B, Coulombe R, Domec J, Hinckley T, McDowell N, Pillips N (2003) Does foliage on the same branch compete for the same water? Experiments on Douglas-fir trees. *Trees - Structure and Function* **17**, 101-108.

Brough DW, Jones HG, Grace J (1986) Diurnal changes in water content of the stems of apple trees, as influenced by irrigation. *Plant, Cell and Environment* **9**, 1-7.

Brown CL, McAlpine RG, Kormanik PP (1967) Apical dominance and form in woody plants: a reappraisal. *American Journal of Botany* **54**: 153-162.

Brown W (1960) Ants, acacias and browsing animals. *Ecology* **41**, 587-592.

Buchmann N, Kao WY, Ehleringer J (1997) Influence of stand structure on carbon-13 of vegetation, soils, and canopy air within deciduous and evergreen forests in Utah, United States. *Oecologia* **110**, 109-119.

Buchmann N, Brooks JR, Ehleringer JR (2002) Predicting daytime carbon isotope ratios of atmospheric CO_2 within forest canopies. *Functional Ecology* **16**, 49-57.

Bullock SH (2000) Developmental patterns of tree dimensions in a neotropical deciduous forest. *Biotropica* **32**, 42-52.

Canny MJ (1997a) Vessel contents of leaves after excision: A test of Scholander's assumption. *American Journal of Botany* **84**, 1217-1222.

Canny MJ (1997b) Vessel contents during transpiration: Embolisms and refilling. *American Journal of Botany* **84**, 1223-1230.

Canny MJ, Roderick ML (2005) A second pathway for gas out of the pressure chamber-what is being squeezed? *Plant Physiology and Biochemistry* **43**, 315-321.

Chaffey N, Barlow P (2001) The cytoskeleton facilitates a three-dimensional symplasmic continuum in the long-lived ray and axial parenchyma cells of angiosperm trees. *Planta* **213**, 811-823.

Connor DJ, Legge NJ, Turner NC (1977) Water relations of mountain ash (*Eucalyptus regnans* F. Muell.) forests. *Australian Journal of Plant Physiology* **4**, 753-762.

Cordero RA, Fetcher N, Voltzow J (2007) Effects of wind on the allometry of two species of plants in an Elfin cloud forest. *Biotropica* **39**, 177-185.

Cosgrove DJ (1986) Biophysical control of plant cell growth. *Annual Review of Plant Physiology* **37**, 377-405.

Cosgrove DJ (1997a) Assembly and enlargement of the primary cell wall in plants. *Annual Review of Cell and Developmental Biology* **13**, 171-201.

Cosgrove DJ (1997b) Relaxation in a high-stress environment: the molecular bases of extensible cell walls and cell enlargement. *The Plant Cell* **9**, 1031-1041.

Cruiziat P, Cochard H, Améglio T (2002) Hydraulic architecture of trees: main concepts and results. *Annals of Forest Science* **59**, 723-752.

Cullen S (2002) Trees and wind: Wind scales and speeds. *Journal of Arboriculture* **28**, 237-242

Davidson CG, Remphrey WR (1990) An analysis of architectural parameters of male and female *Fraxinus pennsylvanica* in relation to crown shape and crown location. *Canadian Journal of Botany* **68**, 2035-2043.

Delzon S, Sartore M, Burlett R, Dewar R, Loustau D (2004) Hydraulic responses to height growth in maritime pine trees. *Plant, Cell and Environment* **27**, 1077–1087.

Dixon HH, Jolly J. (1895) On the ascent of sap. *Philosophical Transactions of the Royal Society of London* **186**:563-576.

Donovan LA, Grisé DJ, West JB, Papert RA, Alder NN, Richards JH (1999) Predawn disequilibrium between plant and soil water potentials in two cold-desert shrubs. *Oecologia* **120**, 209-217.

Donovan LA, Linton MJ, Richards JH (2001) Predawn plant water potential does not necessarily equilibrate with soil water potential under well-watered conditions. *Oecologia* **129**, 328-335.

Duursama RA, Marshall JD (2006) Vertical canopy gradients in δ^{13}C correspond with leaf nitrogen content in a mixed-species conifer forest. *Trees* **20**, 496-506.

Ehleringer J R, Schulze ED, Ziegler H, Lange OL, Farquhar GD, Cowar IR (1985) Xylem-tapping mistletoes: water or nutrient parasites? *Science* **227**, 1479-1481.

Ehleringer JR, Werk KS (1986) Modifications of solar-radiation absorption patterns and implications for carbon gain at the leaf level. Pages 57-82 *in* T. J. Givnish, editor. On the economy of plant form and function. Cambridge University Press, New York, New York, USA.

Falster DS, Westoby M (2003) Plant height and evolutionary games. *Trends in Ecology and Evolution* **18**, 337-343.

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* **40**, 503-537.

Field C, Mooney H (1986) The photosynthesis-nitrogen relationship in wild plants. Pages 25-55 *in* T. J. Givnish, editor. On the economy of plant form and function. Cambridge University Presss, Cambridge, U. K.

Franks P, Brodribb TJ (2005) Stomatal control and water transport in the xylem. Pages 69-89 *in* N. M. Holbrook, and M. A. Zwieniecki, editors. Vascular transport in plants. Elsevier Academic Press, Boston, Massachusetts, USA.

Friend AD (1993) The prediction and physiological significance of tree height. Pages 101–115 *in* A. M. Solomon and H. H. Shugart, editors. Vegetation dynamics and global change. Chapman and Hall, New York, New York, USA.

Galmes J, Flexas J, Save R, Medrano H (2007) Water relations and stomatal characteristics of mediterranean plants with different growth forms and leaf habits: Responses to water stress and recovery. *Plant and Soil* **290**, 139-155.

Goldstein G, Andrade JL, Meinzer FC, Holbrook NM, Cavelier J, Jackson P, Celis A (1998) Stem water storage and diurnal patterns of water use in tropical forest canopy trees. *Plant Cell and Environment* **21**, 397-406.

Groom PK, Lamont BB (1997) Xerophytic implications of increased sclerophylly: Interactions with water and ligth in *Hakea psilorrhyncha* seedlings. *New Phytologist* **136**, 231-237.

Groom PK, Lamont BB (1999) Which common indices of sclerophylly best reflect differences in leaf structure? *Ecoscience* **6**, 471-474.

Grubbs FE (1973) Errors of measurement, precision, accuracy and the statistical comparison of measuring instruments. *Technometrics* **15**, 53-66.

Hacke UG, Sperry JS (2003) Limits to xylem refilling under negative pressure in *Laurus nobilis* and *Acer negundo*. *Plant Cell and Environment* **26**, 303-311.

Hallé F, Oldeman RAA, Tomlinson PB (1978) Tropical trees and forest: an architectural analysis. Springer-Verlag, Berlin, Germany.

Hardegree SP (1989) Xylem water holding capacity as a source of error in water potential estimates made with the pressure chamber and thermocouple psychrometer. *American Journal of Botany* **76**, 356-360.

Heaton THE, Crossley A (1995) Carbon isotope variations in a plantation of sitka spruce, and the effect of acid mist. *Oecologia* **103**, 109-117.

Hellkvist J, Richards GP, Jarvis PG (1974) Vertical gradients of water potential and tissue water relations in Sitka spruce trees measured with the pressure chamber. *Journal of Applied Ecology* **11**, 637-667.

Holbrook NM, Burns MJ, Field CB (1995a). Negative xylem pressures in plants: A test of the balancing pressure technique. *Science* **270**, 1193-1194.

Holbrook NM, Whitbeck JL, Mooney HA (1995b) Drought responses of neotropical dry forest trees. Pages 243-276 *in* S. H. Bullock, H. A. Mooney, and E. Medina, editors. Seasonally dry tropical forests. Cambridge University Press, Cambridge, U. K.

Holbrook NM, Zwieniecki MA (1999) Embolism repair and xylem tension: Do we need a miracle? *Plant Physiology* **120**, 7-10.

Horn HS (1971) The adaptive geometry of trees. Princenton University Press, Princenton, USA.

Hubbard RM, Bond BJ, Ryan MG (1999) Evidence that hydraulic conductance limits photosynthesis in old Pinus ponderosa trees. *Tree Physiology* **19**, 165-172.

Hubbard RM, Ryan MG, Stiller V, Sperry JS (2001) Stomatal conductance and photosynthesis vary linearly with plant hydraulic conductance in ponderosa pine. *Plant Cell and Environment* **24**, 113-121.

Ireland C (1997) Sustaining the western myall woodlands: ecology and management. PhD Thesis, Department of Environmental Science and Rangeland Management, The University of Adelaide, Adelaide, Australia.

Iwasa Y, Cohen D, Leon JA (1984) Tree height and crown shape, as results of competitive games. *Journal of Theoretical Biology* **112**, 279-297.

Jessop JP, Toelken HR (1986) Flora of South Australia. Part II. Leguminosae-Rubiaceae. State of South Australia, Adelaide, Australia.

King DA (1990) The adaptive significance of tree height. *American Naturalist* **135**, 809-828.

Koch GW, Sillett SC, Jennings GM, Davis SD (2004) The limits to tree height. *Nature* **428**, 851-854.

Koch GW, Fredeen AL (2005) Transport challenges in tall trees. Pages 437-456 *in* N. M. Holbrook, and M. A. Zwieniecki, editors. Vascular transport in plants. Elsevier Academic Press, Boston, Massachusetts, USA.

Koide R (1985) The nature and location of variable hydraulic resistance in *Helianthus annus* L. (Sunflower). *Journal of Experimental Botany* **36**, 1430-1440.

Kolb KJ, Davis SD (1994) Drought tolerance and xylem embolism in co-occurring species of coastal sage and chaparral. *Ecology* **75**, 648-659.

Kolb T, Stone J (2000) Differences in leaf gas exchange and water relations among species and tree sizes in an Arizona pine-oak forest. *Tree Physiology* **20**, 1-12.

Ladiges PY, Ashton DH (1974) Variation in some central Victorian population of *Eucalyptus viminalis* Labill. *Australian Journal of Botany* **22**, 81-102.

Lambers H, Stuart Chapin III F, Pons TL (1998) Plant physiological ecology. Springer-Verlag, New York, New York, USA.

Langan SJ, Ewers FW, Davis SD (1997) Xylem dysfunction caused by water stress and freezing in two species of co-occurring chaparral shrubs. *Plant Cell and Environment* **20**, 425-437.

Lange R, Purdie R (1976) Western myall (*Acacia sowdenii*), its survival prospects and management needs. *Australian Rangelands Journal* **1**, 64-69.

Lange RT, Sparrow AD (1992) Growth rates of western myall (*Acacia papyrocarpa* Benth.) during its main phase of canopy spreading. *Australian Journal of Ecology* **17**, 315-320.

Lawton R (1982) Wind stress and elfin stature in a montane rain forest tree: an adaptive explanation [Didymopanax pittieri]. *American Journal of Botany* **69**, 1224-1230.

Legge N (1985) Anatomical aspects of water movement through stems of Mountain Ash (*Eucalyptus regnans* F. Muell.). *Australian Journal of Botany* **33**, 287-298.

Leyser O (2005) The fall and rise of apical dominance. *Current Opinion in Genetics and Development* **15**, 468-471.

Livingston NJ, Whitehead D, Kelliher FM, Wang YP, Grace JC, Walcroft AS, Byers JN, McSeveny TM, Millard P (1998) Nitrogen allocation and carbon isotope fractionation in relation to intercepted radiation and position in a young *Pinus radiata* D. Don tree. *Plant Cell and Environment* **21**, 795-803.

Maconochie JR, Lange R (1970) Canopy dynamics of trees and shrubs with a particular reference to arid-zone topfeed species. *Transactions of the royal society of South Australia* **94**, 243-248.

Magnani F, Mencuccini M, Grace J (2000) Age-related decline in stand productivity: the role of structural acclimation under hydraulic constraints. *Plant Cell and Environment* **23**, 251-263.

Marshall JD, Monserud RA (2003) Foliage height influences specific leaf area of three conifer species. *Canadian Journal of Forest Research* **33**, 164-170.

Martre P, Bogeat-Triboulot MB, Durand JL (1999) Measurement of a growth-induced water potential gradient in tall fescue leaves. *New Phytologist* **142**, 435-439.

McCully ME, Huang CX, Ling LEC (1998) Daily embolism and refilling of xylem vessels in the roots of field-grown maize. *New Phytologist* **138**, 327-342.

McCully ME (1999) Root xylem embolisms and refilling. Relation to water potentials of soil, roots, and leaves, and osmotic potentials of root xylem sap. *Plant Physiology* **119**, 1001-1008.

McDowell NG, Phillips N, Lunch C, Bond BJ, Ryan MG (2002a) An investigation of hydraulic limitation and compensation in large, old Douglas-fir trees. *Tree Physiology* **22**, 763-774.

McDowell N, Barnard H, Bond BJ, Hinckley T, Hubbard RM, Ishii H, Kostner B, Magnani F, Marshall JD, Meinzer FC, Phillips N, Ryan MG, Whitehead D (2002b) The relationship between tree height and leaf area: Sapwood area ratio. *Oecologia* **132**, 12-20.

McMahon T (1973) Size and shape in biology: Elastic criteria impose limits on biological proportions, and consequently on metabolic rates. *Science* **179**, 1201-1204.

Meinzer FC, Clearwater MJ, Goldstein G (2001) Water transport in trees: Current perspectives, new insights and some controversies. *Environmental and Experimental Botany* **45**, 239-262.

Melcher PJ, Meinzer FC, Yount DE, Goldstein G, Zimmermann U (1998) Comparative measurements of xylem pressure in transpiring and non-transpiring leaves by means of the pressure chamber and the xylem pressure probe. *Journal of Experimental Botany* **49**, 1757-1760.

Mencuccini M, Grace J (1996) Hydraulic conductance, light interception and needle nutrient concentration in Scots pine stands and their relations with net primary productivity. *Tree Physiology* **16**, 459-68.

Mencuccini M, Magnani F (2000) Comment on 'Hydraulic limitation of tree height: a critique' by Becker, Meinzer and Wullschleger. *Functional Ecology* **14**, 135-137.

Mencuccini M, Martínez-Vilalta J, Hamid HA, Korakaki E, Vanderklein D (2007). Evidence for age- and size-mediated controls of tree growth from grafting studies. *Tree Physiology* **27**, 463-73.

Meron M, Grimes DW, Phene CJ, Hutmacher RB (1989) Shoot resistance to water flow in cotton. *Journal of Experimental Botany* **40**, 919-923.

Midgley JJ (2003) Is bigger better in plants? The hydraulic costs of increasing size in trees. *Trends in Ecology and Evolution* **18**, 5-6.

Midgley JJ, McLean P, Botha M, and Balfour D (2001) Why do some African thorn trees (*Acacia spp*.) have a flat-top: a grazer-plant mutualism hypothesis? *African Journal of Ecology* **39**, 226-228.

Milburn JA (1979) Water flow in plants. Longman, London, U. K.

Neufeld HS, Meinzer FC, Wisdom CS, Sharifi MR, Rundel PW, Neufeld MS, Goldring Y, Cunningham GL (1988) Canopy architecture of *Larrea tridentata* (DC.) Cov., a desert shrub: Foliage orientation and direct beam radiation interception. *Oecologia* **75**, 54-60.

Nicolotti G, Rettori A, Paoletti E, Gullino ML (2005) Morphological and physiological damage by surfactant-polluted seaspray on *Pinus pinea* and *Pinus halepensis*. *Environmental Monitoring and Assessment* **105**, 175-191.

Niklas KJ (1993) The scaling of plant height: A comparison among major plant clades and anatomical grades. *Annals of Botany* **72**, 165-172.

Niklas KJ (1994) Size-dependent variations in plant growth rates and the "3/4 power rule". *American Journal of Botany* **81**, 134–144.

Niklas KJ (2007) Maximum plant height and the biophysical factors that limit it. *Tree Physiology* **27**, 433-40.

Niklas KJ, Spatz H (2004) Growth and hydraulic (not mechanical) constraints govern the scaling of tree height and mass. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 15661–15663.

Nobel PS (1986) Form and orientation in relation to PAR interception by cacti and agaves. Pages 83-103 *in* T. J. Givnish, editor. On the economy of plant form and function. Cambridge University Press, New York, New York, USA.

Nobel PS (1991) Physicochemical and environmental plant physiology. Academic Press, San Diego, California, USA.

Noguchi Y (1979) Deformation of trees in Hawaii and its relation to wind. *Journal of Ecology* **67**, 611-628.

Ogden GL (1980) Sea-salt aerosol damage to *Quercus agrifolia* and *Quercus lobata* in the Santa Ynez Valley, California. Pages 230–237 *in* T. R. Plumb, editor. Proceedings of the symposium on the ecology, management, and utilization of California oaks. Volume General Technical Report PSW-44. USDA Forest Service, Pacific Southwest Forest and Range Experiment Station, Berkeley, California, USA.

Otto B, Kaldenhoff R (2000) Cell-specific expression of the mercury-insensitive plasma-membrane aquaporin NtAQP1 from *Nicotiana tabacum*. *Planta* **211**, 167-172.

Passioura JB (1982) Water in the soil-plant-atmosphere continuum. Pages 5–33 *in* O. L. Lange, P. S. Nobel, C. B. Osmond, and H. Ziegler, editors. Water relations and carbon assimilation. Encyclopedia in plant physiology, NS, vol 12B, Springer-Verlag, New York, New York, USA.

Passioura JB, Munns R (1984) Hydraulic resistance of plants. II. Effects of rooting medium, and time of day, in barley and lupin. *Australian Journal of Plant Physiology* **11**, 341-350.

Phillips N, Bond BJ, McDowell NG, Ryan MG, Schauer A (2003) Leaf area compounds height-related hydraulic costs of water transport in Oregon White Oak trees. *Functional Ecology* **17**, 832-840.

Pickard WF (2003) The role of cytoplasmic streaming in symplastic transport. *Plant Cell and Environment* **26**, 1-15.

Pockman WT, Sperry JS, O Leary JW (1995) Sustained and significant negative water pressure in xylem. *Nature* **378**, 715-716.

Pockman WT, Sperry JS (2000) Vulnerability to xylem cavitation and the distribution of Sonoran desert vegetation. *American Journal of Botany* **87**, 1287-1299.

Protz CG, Silins U, Lieffers VJ (2000) Reduction in branch sapwood hydraulic permeability as a factor limiting survival of lower branches of lodgepole pine. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere* **30**, 1088-1095.

Putz FE, Milton K (1982) Tree mortality rates on Barro Colorado Island. Pages 95–100 *in* E. G. Leigh, S. Rand, and D. M. Windsor, editors. The ecology of a tropical forest: Seasonal rhythms and long-term changes. Smithsonian Institution Press, Washington, D.C., USA.

Rambal S, Leterme J (1987) Changes in aboveground structure and resistances to water uptake in *Quercus coccifera* along a rainfall gradient. Pages 191-200 *in* J. D. Tenhunen, F. M. Catarino, O. L. Lange, and W. C. Oechel, editors. Plant responses to stress: functional analysis in Mediterranean ecosystems. NATO ASI ser. Vol. G15, Springer-Verlag, Berlin, Germany.

Rasmuson, KE, Anderson JE, Huntly N (1994) Coordination of branch orientation and photosynthetic physiology in the Joshua tree (*Yucca brevifolia*). *Great Basin Naturalist* **54**, 204-211.

Raventós J, De Luís M, Gras MJ, Cufar K, González-Hidalgo JC, Bonet A, Sanchez JR (2001) Growth of *Pinus pinea* and *Pinus halepensis* as affected by dryness, marine spray and land use changes in a Mediterranean semiarid ecosystem. *Dendrochronologia* **19**, 211-220.

Rich PM (1987) Mechanical structure of the stem of arborescent palms. *Botanical Gazette* **148**, 42-50.

Rieger M, Litvin P (1999) Root system hydraulic conductivity in species with contrasting root anatomy. *Journal of Experimental Botany* **50**, 201-209.

Rieger M, Motisi A (1990) Estimation of root hydraulic conductivity on intact peach and citrus rootstocks. *Hortscience* **25**, 1631-1634.

Roderick ML, Canny MJ (2005) A mechanical interpretation of pressure chamber measurements-what does the strength of the squeeze tell us? *Plant Physiology and Biochemistry* **43**, 323-336.

Rundel PW, Cowling RM, Essler KJ, Mustart PM, Van Jaarsveld E, Bezuidenhout H (1995) Winter growth phenology and leaf orientation in *Pachypodium namaquanum* (Apocynaceae) in the succulent karoo of the Richtersveld, South Africa. *Oecologia* **101**, 472-477.

Rust S, Roloff A (2002) Reduced photosynthesis in old oak (*Quercus robus*): the impact of crown and hydraulic architecture. *Tree Physiology* **22**, 597-601.

Ryan MG, Bond BJ, Law BE, Hubbard RM, Woodruff D, Cienciala E, Kucera J (2000) Transpiration and whole-tree conductance in ponderosa pine trees of different heights. *Oecologia* **124**, 553-560.

Ryan MG, Phillips N, Bond BJ (2006) The hydraulic limitation hypothesis revisited. *Plant Cell and Environment* **29**, 367-381.

Ryan MG, Waring RH (1992) Maintenance respiration and stand development in a subalpine lodgepole pine forest. *Ecology* **73**, 2100-2108.

Ryan MG, Yoder BJ (1997) Hydraulic limits to tree height and tree growth: What keeps trees from growing beyond a certain height? *Bioscience* **47**, 235-242.

Salleo S, Lo Gullo MA (1990) Sclerophylly and plant water relations in three Mediterranean *Quercus* species. *Annals of Botany* **65**, 259-270.

Salleo S, Lo Gullo MA, De Paoli D, Zippo M (1996) Xylem recovery from cavitation-induced embolism in young plants of *Laurus nobilis*: A possible mechanism. *New Phytologist* **132**, 47-56.

Schneider H, Thurmer F, Zhu JJ, Wistuba N, Gessner P, Linder K, Herrmann B, Zimmermann G, Hartung W, Bentrup FW, Zimmermann U (1999) Diurnal changes in xylem pressure of the hydrated resurrection plant *Myrthamnus flabellifolia*: evidence for lipid bodies in conducting xylem vessels. *New Phytologist* **143**, 471-484.

Scholander PF, Hammel HT, Bradstreet ED, Hemmingsen EA (1965) Sap pressure in vascular plants. *Science* **148**, 339-346.

Schopfer P (2006) Biomechanics of plant growth. *American Journal of Botany* **93**, 1415-1425.

Schubert A, Lovisolo C, Peterlunger E (1999) Shoot orientation affects vessel size, shoot hydraulic conductivity and shoot growth rate in *Vitis vinifera* L. *Plant Cell and Environment* **22**, 197-204.

Schubert A, Restagno M, Novello V, Peterlunger E (1995) Effects of shoot orientation on growth, net photosynthesis, and hydraulic conductivity of *Vitis vinifera* L. cv. Cortese. *American Journal of Enology and Viticulture* **46**, 324-328.

Schulze ED, KÜppers M, Matyssek R (1986) The roles of carbon balance and branching pattern in the growth of woody species. Pages 585-602 *in* T. J. Givnish, editor. On the economy of plant forma and function. Cambridge University Press, New York, New York, USA.

Schurr U, Walter A, Rascher U. (2006) Functional dynamics of plant growth and photosynthesis - from steady-state to dynamics - from homogeneity to heterogeneity. *Plant Cell and Environment* **29**, 340-352.

Simpson JE (1994) Sea breeze and local winds. Cambridge University Press, Cambridge, U. K.

Sperry JS (2000) Hydraulic constraints on plant gas exchange. Agricultural and Forest *Meteorology* **104**, 13-23.

Sperry JS, Donnelly JR, Tyree MT (1988) A method for measuring hydraulic conductivity and embolism in xylem. *Plant Cell and Environment* **11**, 35-40.

Sperry JS, Meinzer FC, McCulloh KA (2008) Safety and efficiency conflicts in hydraulic architecture: scaling from tissues to trees. *Plant Cell and Environment* **31**, 632-45.

Sperry JS, Tyree MT (1988) Mechanism of water stress-induced xylem embolism. *Plant Physiology* **88**, 581-587.

Steudle E (2001) The cohesion-tension mechanism and the acquisition of water by plant roots. *Annual Review of Plant Physiology and Plant Molecular Biology* **52**, 847-875.

Stoker R, Weatherley PE (1971) The influence of the root system on the relationship between the rate of transpiration and depression of leaf water potential. *New Phytologist* **70**, 547-554.

Stromberg JC, Tress JA, Wilkins SD, Clark SD (1992) Response of velvet mesquite to groundwater decline. *Journal of Arid Environments* **23**, 45-58.

Tang AC, Boyer JS (2002) Growth-induced water potentials and the growth of maize leaves. *Journal of Experimental Botany* **53**, 489-503.

Tardieu F, Granier C, Muller B (1999) Modelling leaf expansion in a fluctuating environment: are changes in specific leaf area a consequence of changes in expansion rate? *New Phytologist* **143**, 33-44.

Tardieu F, Reymond M, Hamard P, Granier C, Muller B (2000) Spatial distributions of expansion rate, cell division rate and cell size in maize leaves: a synthesis of the effects of soil water status, evaporative demand and temperature. *Journal of Experimental Botany* **51**, 1505-1514.

Telewski FW (1995) Wind-induced physiological and developmental responses in trees. Pages 237-263 *in* M. P. Coutts, and J. Grace, editors. Wind and trees. Cambridge University Press, Cambridge, U. K.

Tennakoon KU, Pate JS (1996) Effects of parasitism by a mistletoe on the structure and functioning of branches of its host. *Plant Cell and Environment* **19**, 517-528.

Thomas P (2000) Trees: their natural history. Cambridge University Press, Cambridge, U. K.

Thurmer F, Zhu JJ, Gierlinger N, Schneider H, Benkert R, Gessner P, Herrmann B, Bentrup FW, Zimmermann U (1999) Diurnal changes in xylem pressure and mesophyll cell turgor pressure of the liana *Tetrastigma voinierianum*: The role of cell turgor in long-distance water transport. *Protoplasma* **206**, 152-162.

Tobiessen P, Rundel PW, Stecker RE (1971) Water potential gradient in a tall *Sequoiadendron*. *Plant Physiology* **48**, 303-304.

Turner NC (1988) Measurement of plant water status by the pressure chamber technique. *Irrigation Science* **9**, 289-308.

Turner NC, Begg JE (1973) Stomatal behaviour and water status of maize, sorghum, and tobacco under field conditions. *Plant physiology* **51**, 31-36.

Tyerman SD, Niemietz CM, Bramley H (2002) Plant aquaporins: Multifunctional water and solute channels with expanding roles. *Plant Cell and Environment* **25**, 173-194.

Tyree MT (1997) The cohesion-tension theory of sap ascent: Current controversies. *Journal of Experimental Botany* **48**, 1753-1765.

Tyree MT (2003) Hydraulic limits on tree performance: Transpiration, carbon gain and growth of trees. *Trees-Structure and Function* **17**, 95-100.

Tyree MT, Salleo S, Nardini A, Lo Gullo MA, Mosca R (1999) Refilling of embolized vessels in young stems of laurel. Do we need a new paradigm? *Plant Physiology* **120**, 11-21.

Tyree MT, Sperry JS (1989) Vulnerability of xylem to cavitation and embolism. *Annual Review of Plant Physiology and Plant Molecular Biology* **40**, 19-36.

Tyree MT, Cochard H, Cruiziat P (2003) The water-filled versus air-filled status of vessels cut open in air: the 'Scholander assumption' revisited. *Plant Cell and Environment* **26**, 613-621.

Tyree MT, Zimmermann MH (2002) Xylem structure and the ascent of sap. Springer-Verlag, Berlin, Germany.

Vandeleur RK, Mayo G, Shelden MC, Gilliham M, Kaiser BN, Tyerman SD (2009) The role of plasma membrane intrinsic protein aquaporins in water transport through roots: diurnal and drought stress responses reveal different strategies between isohydric and anisohydric cultivars of grapevine. *Plant Physiology* **149**, 445-460.

Wade JE, Hewson EW (1979) Trees as local climatic wind indicator. *Journal of Applied Meteorology* **18**, 1182-1187.

Walcroft AS, Silvester WB, Grace JC, Carson SD, Waring RH (1996) Effects of branch legth on carbon isotope discrimination in *Pinus radiata*. *Tree Physiology* **16**, 281-286.

Walter BA, Moore JL (2005) The concepts of bias, precision and accuracy, and their use in testing the performance of species richness estimators, with a literature review of estimator performance. *Ecography* **28**, 815-829.

Waring RH, Silvester WB (1994) Variation in foliar delta-13C values within the crowns of *Pinus radiata* trees. *Tree Physiology* **14**, 1203-1213.

Warren CR, Adams MA (2000) Water availability and branch length determine $\delta^{13}C$ in foliage of *Pinus pinaster*. *Tree Physiology* **20**, 637-643.

Wei CF, Steudle E, Tyree MT (1999a) "Water ascent in plants: Do ongoing controversies have a sound basis?" *Trends in Plant Science* **4**, 372-375.

Wei CF, Tyree MT, Steudle E (1999b) Direct measurement of xylem pressure in leaves of intact maize plants. A test of the cohesion-tension theory taking hydraulic architecture into consideration. *Plant Physiology* **121**, 1191-1205.

Wei CF, Tyree MT, Bennink JP (2000) The transmission of gas pressure to xylem fluid pressure when plants are inside a pressure bomb. *Journal of Experimental Botany* **51**, 309-316.

West GB, Brown JH, Enquist BJ (1999) A general model for the structure and allometry of plant vascular systems. *Nature* **400**, 664-667.

Westoby M, Falster DS, Moles AT, Vesk PA, Wright IJ (2002) Plant ecological strategies: Some leading dimensions of variation between species. *Annual Review of Ecology and Systematics* **33**, 125-159.

Whibley DJE, Symon DE (1992) Acacias of South Australia. Government Printer, Adelaide, Australia.

Williams JE, Davis SD, Portwood K (1997) Xylem embolism in seedlings and resprouts of *Adenostoma fasciculatum* after fire. *Australian Journal of Botany* **45**, 291-300.

Wilson BF (2000) Apical control of branch growth and angle in woody plants. *American Journal of Botany* **87**, 601-607.

Wistuba N, Reich R, Wagner HJ, Zhu JJ, Schneider H, Bentrup FW, Haase A, Zimmermann U (2000) Xylem flow and its driving forces in a tropical liana: Concomitant flow-sensitive NMR imaging and pressure probe measurements. *Plant Biology* **2**, 579-582.

Witkowski ETF, Lamont BB (1991) Leaf specific mass confounds leaf density and thickness. *Oecologia* **88**, 486-493.

Woodruff DR, Bond BJ, Meinzer FC (2004) Does turgor limit growth in tall trees? *Plant Cell and Environment* **27**, 229-236.

Wright IJ, Westoby M (1999) Differences in seedlings growth behaviour among species: Trait correlations across species, and trait shifts along nutrient compared to rainfall gradients. *Journal of Ecology* **87**, 85-97.

Wright IJ, Westoby M (2000) Cross-species relationships between seedling relative growth rate, nitrogen productiviy and root vs leaf function in 28 Australian woody species. *Functional Ecology* **14**, 97-107.

Yoda K, Shinozaki K, Ogawa H, Hozumi K, Kira T (1965) Estimation of the total amount of respiration in woody organs of trees and forest communities. *Journal of Biology-Osaka City University* **16**, 15–26.

Yoder BJ, Ryan MG, Waring RH, Schoettle AW, Kaufmann MR (1994) Evidence of reduced photosynthetic rates in old trees. *Forest Science* **40**, 513-527.

Zar JH (1999) Biostatistical analysis. 4th ed., Prentice Hall, Upper Saddle River, New Jersey, USA.

Zimmermann MH (1983) Xylem structure and the ascent of sap. Springer-Verlag, Berlin Germany.

Zimmermann U, Haase A, Langbein D, Meinzer F (1993). Mechanisms of long-distance water transport in plants: A re-examination of some paradigms in the light of new evidence. *Philosophical Transactions of the Royal Society of London B Biological Sciences* **341**, 19-31.

Zimmermann U, Meinzer FC, Benkert R, Zhu JJ, Schneider H, Goldstein G, Kuchenbrod E, Haase A (1994) Xylem water transport: Is the available evidence consistent with the cohesion theory? *Plant Cell and Environment* **17**, 1169-1181.

Zimmermann U, Schneider H, Wegner LH, Haase A (2004) Water ascent in tall trees: Does evolution of lands plants rely on a highly metastable state? *New Phytologist* **162**, 575-615.

Zimmermann U, Wagner HJ, Schneider H, Rokitta M, Haase A, Bentrup FW (2000) Water ascent in plants: the ongoing debate. *Trends in Plant Science* **5**, 145-146.

Zimmermann U, Wagner HJ, Heidecker M, Mimietz S, Schneider H, Szimtenings M, Haase A, Mitlohner R, Kruck W, Hoffmann R, Konig W (2002) Implications of mucilage on pressure bomb measurements and water lifting in trees rooting in high-salinity water. *Trees-Structure and Function* **16**, 100-111.

Zwieniecki MA, Brodribb TJ, Holbrook NM (2007) Hydraulic design of leaves: insights from rehydration kinetics. *Plant Cell and Environment* **30**, 910-921.

Zwieniecki MA, Holbrook NM (2000) Bordered pit structure and vessel wall surface properties. Implications for embolism repair. *Plant Physiology* **123**, 1015-1020.

Zwieniecki MA, Hutyra L, Thompson MV, Holbrook NM (2000) Dynamic changes in petiole specific conductivity in red maple (*Acer rubrum* L.), tulip tree (*Liriodendron tulipifera* L.) and northern fox grape (*Vitis labrusca* L.). *Plant Cell and Environment* **23**, 407-414.

8) Appendix

Comparing balance pressures of leaves cut in air versus leaves cut under water during pressure chamber measurements on hydrated, non-transpiring leaves

During the development of the experiments in Chapter 4 and 5 leaves were cut in air and transported to the laboratory where they were covered with packaging tape and placed in water. Here, further measurements were conducted to test if the “cut-in-air” protocol used in chapters 4 and 5 may have significantly affected some of the results.

The sources of leaves were *Malosma laurina* and *Nicotiana glauca* plants growing in patches of natural vegetation in the Ensenada Centre for Scientific Research and Higher Education (CICESE) in Ensenada, Baja California, México. Leaves were cut during the afternoon of the day before pressure chamber measurements. Seven leaves with a gradient in size were cut under water (distilled) from a *Malosma laurina* plant. They were transported to the laboratory where the blade was covered on both surfaces with the same type of box-sealing tape used in chapters 4 and 5 (Scotch 3M, St. Paul, MN, U.S.A.). Leaves were left with their petioles immersed in distilled water (Sparkletts, water purified by steam distillation, filtered and ozonated) for more than 15 hours. Pressure chamber measurements were made the following day. The experiment was repeated using a different *Malosma laurina* plant. Eight leaves were cut under water while nine leaves were cut in air. All leaves were taped before they were cut from the plant. They were left in water for more than 15h and BP was measured the following day. A third run of measurements was performed but using leaves from a *Nicotiana glauca* plant. Eleven leaves were cut in air and 11 under water. The rest of the protocol was similar. When leaves were cut under water (during the three experiments) their petioles remained under water until the following day.

Balance pressures (BP) were measured with a pressure chamber (PMS Instrument Company, Albany OR, U.S.A.) fitted with an adjustable compression gland sealing system. The original needle gauge of the pressure chamber was replaced by a digital gauge with a resolution of 0.1 kPa (Digital Test Gauge XP2i, Crystal Engineering Corporation, San Luis Obispo CA, U.S.A.). The measurement protocol was similar to the one described in chapter 4 and 5. Observations of BP were made under a stereoscopic microscope. Each leaf was taken from the water reservoir and the petiole surface was re-cut (less than 1mm) in order to have a clear surface, and immediately placed in the pressure chamber. The pressure was increased slowly. The balance point was registered as the very first appearance of water in the xylem vessels.

Leaves were scanned and their digitized areas were measured with the Scion Image software (a version of the NIH Image, written by the National Institutes of Health, USA).

There was a non-linear relationship between leaf area and BP in leaves cut under water from *Malosma laurina* (Figs. 8.1 and 8.2). The same result was recorded on the two different days with two different plants (Figs. 8.1 and 8.2), and the results were similar for leaves cut under water or in air (Figs. 8.2 and 8.3). This relationship between BP and leaf area resembles the one found for *V. tinus* in Chapter 5 (Fig. 5.5b; Fig. 8.4). All leaves from *Nicotiana glauca* presented a BP below 12 kPa and there was no difference between leaves cut in air and those cut under water (t-test p=0.24, Fig. 8.5).

It seems that the "cut-in-air" protocol did not affect BP determinations. Leaves cut in air always matched the patterns presented by leaves cut under water. It seems that leaves from *Nicotiana glauca* were all equilibrated with water. The BP measurement protocol detects the very first signal of expelled water, but does not distinguish whether water was expelled from a few vessels or from all of them. Thus, this protocol may not be sensitive to a possible partial blockage of vessels. The evidence found here is in full agreement with all conclusions from chapter 4 and 5.

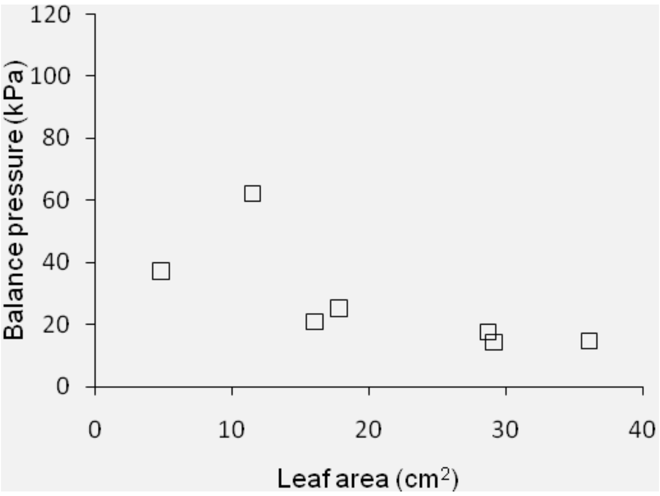

Fig. 8.1. Leaf area and balance pressure of hydrated leaves from *Malosma laurina* adult plants growing in patches of natural vegetation in the CICESE campus. Leaves were taped and cut under water on July 22 2010 and measurements were performed the following day.

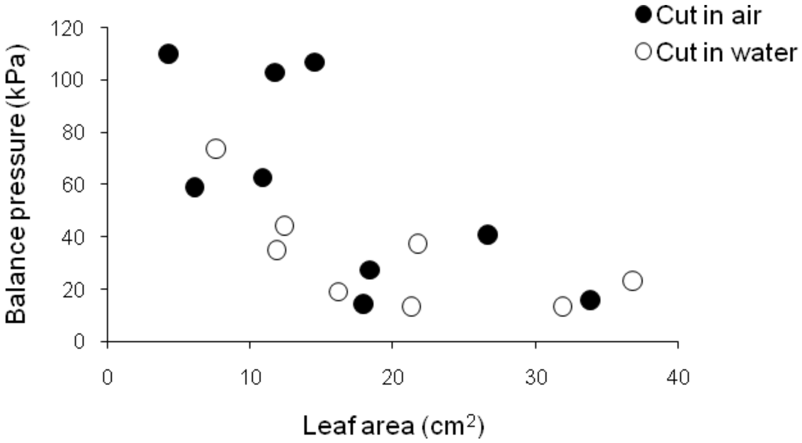

Fig. 8.2. Leaf area and balance pressure of hydrated leaves from *Malosma laurina* adult plants growing in patches of natural vegetation in the CICESE campus. All leaves were taped before they were cut from the plant. Some leaves were cut in the air (filled circles) and some were cut under water (empty circles). They were cut on july 28 2010 and measurements were performed the following day.

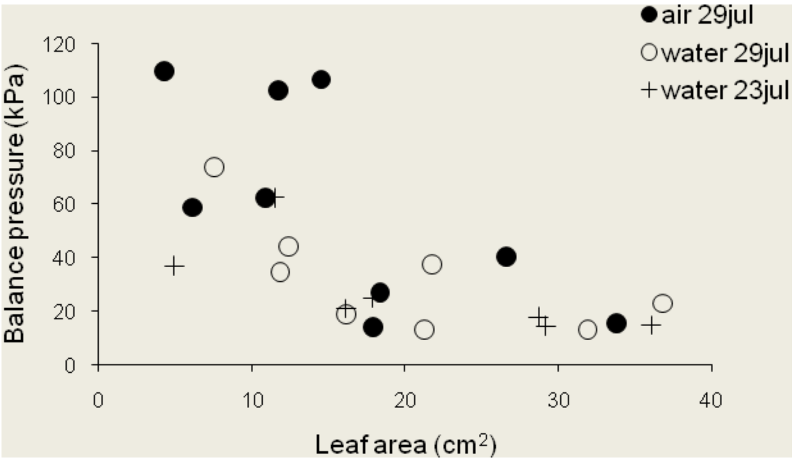

Fig. 8.3. Data from Figs 8.1 and 8.2 are plotted together.

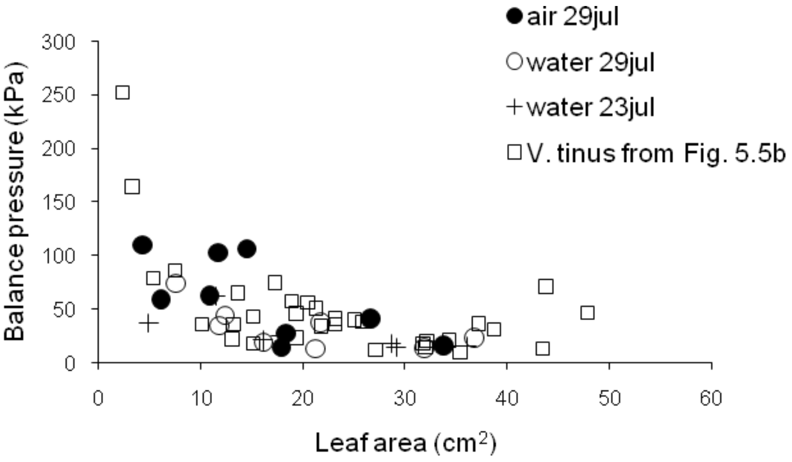

Fig. 8.4. Same as Fig. 8.3 but including data from Fig. 5.5b.

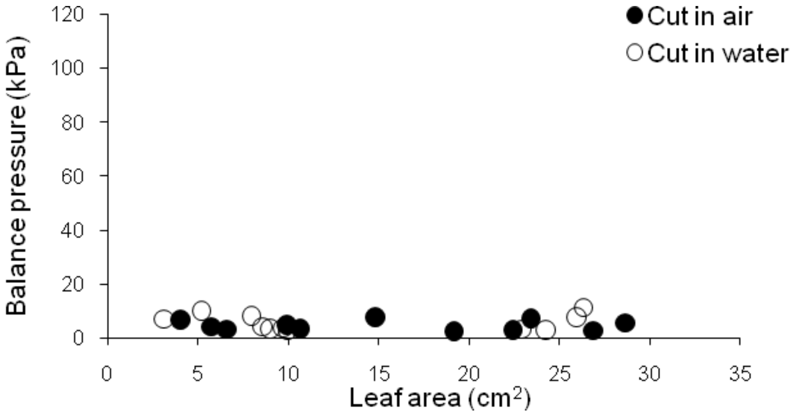

Fig. 8.5. Leaf area and balance pressure of hydrated leaves from *Nicotiana glauca* adult plant growing in the CICESE campus. All leaves were taped before they were cut at the petiole. Some leaves were cut in the air (filled circles) and some were cut under water (empty circles). They were cut on august 11 2010 and measurements were performed the following day.