Leaf hydraulics: implications for understanding leaf structure and function, drought resistance and community assembly

By

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The research associated with this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University.
Abstract

The leaf hydraulic system in plants is charged with supplying water to the sites of evaporation in order to facilitate photosynthesis and growth, while simultaneously resisting negative pressure generated under tension induced by water stress. These processes contribute substantially to the enormous variation in leaf structure and function found across vascular plants and may contribute in fundamental ways to plant function and differences in species ecological strategy.

In this dissertation I examined the leaf hydraulic properties of a phylogenetically, morphologically and ecologically diverse group of woody angiosperm species in order to better understand how leaf hydraulics defines plant function under drought, is integrated with leaf structure and function, and drives differences in species ecological strategy and drought resistance. My results strongly indicate that leaf hydraulics underlie many important aspects of plant function and leaf structure. They also enhance our understanding of the function and assembly of ecological communities, as well as the evolution of plant drought resistance. Furthermore, they provide a potentially crucial tool for predicting the potential impacts of climate change and increasing aridity on plant function and community dynamics.

In drought-stressed seedlings, the recovery of gas exchange following re-watering was strongly correlated with the relatively slow recovery of leaf hydraulic conductance ($K_{\text{leaf}}$) in three ecologically disparate species. This hydraulic-stomatal limitation model of gas exchange recovery observed in these species indicates that leaf hydraulics is a key driver of plant functional recovery from drought. Variation in the hydraulic vulnerability of leaves to water-stress-induced tension ($P50_{\text{leaf}}$) was intimately linked to drought survival in the experimental species. Furthermore, variation in $P50_{\text{leaf}}$ across a larger group of species was significantly correlated with a suite of leaf structural and functional traits that confer increased drought resistance.

As expected, $K_{\text{leaf}}$ was positively correlated with both maximum assimilation and vein density across species. Thus, the water transport capacity of leaves may constrain plant gas exchange and reflect leaf hydraulic design. In addition, insights into the water transport pathway in leaves were generated by different measures of leaf capacitance ($C_{\text{leaf}}$) related to short and long-term fluctuations in transpiration.
Variation in leaf hydraulic vulnerability was strongly correlated with the xylem dimensions in the leaf minor veins that predict the vulnerability of conduits to collapse under negative pressure \((t/b)^3\). While this result does not necessarily indicate a direct link between hydraulic dysfunction and conduit collapse, the relationship between \(P50_{\text{leaf}}\) and \((t/b)^3\) suggests evolved coordination in leaves between xylem structural strength and hydraulic vulnerability that will have major implications for understanding leaf-carbon economy.

Leaf hydraulic vulnerability was also shown to define the bioclimatic limits of species. Species with low \(P50_{\text{leaf}}\) extended into drier regions, while species with high \(P50_{\text{leaf}}\) were restricted to areas of high rainfall. Furthermore, the adaptive significance of \(P50_{\text{leaf}}\) was demonstrated using phylogenetically independent comparisons of species pairs from wet and dry forests. Across these pairings, wet forest species were consistently more vulnerable to water-stress-induced hydraulic dysfunction, despite their generic ecological affinity in both wet and dry forests. This indicates that the evolution of leaf hydraulic vulnerability is bi-directional and adaptive across the rainfall spectrum.

Despite the adaptive significance of leaf hydraulic vulnerability, within-site variability in \(P50_{\text{leaf}}\) differed between two high-rainfall communities that contrast in species diversity and historical ecology. This suggests that the functional composition of modern-day plant communities are not only influenced by current climate but by processes related to long-term climate variability and/or parochial historical constraints.

This detailed examination of leaf hydraulics in woody angiosperms provides key insights into the nature of leaf structure and function and enhances our understanding of the processes that drive plant responses to environmental stress and determine differences in species ecological strategy. Greater understanding of the hydraulic constraints in leaves across different plant groups will therefore lead to better management practices in natural and agricultural systems.
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Chapter 1

General introduction

Leaf hydraulics

Because leaves represent the interface between liquid and vapour-phase processes in plants, they are crucial to understanding whole-plant function. Hydraulically, leaves are designed to supply water to the sites of evaporation in order to facilitate photosynthesis and plant growth. However, until recently, the importance of the hydraulic properties of leaves in defining plant function and driving differences in species ecological strategy has remained largely unrecognised. Recent studies indicate that the pathways for water movement in the leaf can represent more than 30% of the whole-plant hydraulic resistance (Sack & Holbrook, 2006). This hydraulic bottleneck places a significant constraint on plant functional processes, which is reflected by a good correlation between maximum leaf hydraulic conductance ($K_{\text{leaf}}$) and both stomatal conductance (Aasamaa et al., 2001) and maximum photosynthetic capacity (Brodribb et al. 2005). Across species, the hydraulic properties of leaves contribute substantially to the enormous variation in leaf structure, venation architecture and hydraulic design across different plant groups. Furthermore, $K_{\text{leaf}}$ varies 65-fold across species (Sack & Holbrook, 2006), indicating that the leaf water transport capacity in plants has potentially strong ecological importance.

While water transport capacity strongly influences plant performance, the hydraulic pathway in plants is vulnerable to dysfunction under tension. Hydraulic tension is generated by the process of transpiration, which effectively pulls water from the roots through the xylem to the sites of evaporation in the leaf mesophyll (Zimmermann, 1983). As leaf water potentials decline under drought conditions, this tension increases, and can reach levels that compromise the transport system and reduce the hydraulic conductance. The mechanism for this reduced conductance is widely thought to be xylem cavitation caused by air-bubbles entering the water column via pit membranes (Tyree & Sperry, 1989, Zimmermann, 1983), although it may also be a consequence of xylem wall implosion and cell collapse (Brodribb & Holbrook, 2005, Cochard et al., 2004), or increased extra-xylary resistance (Brodribb & Holbrook, 2004b). Regardless, plants have been shown to respond to this water-stress-induced...
hydraulic dysfunction by closing their stomata, thus recovering water potential and avoiding potentially catastrophic hydraulic failure (Brodribb & Holbrook, 2003, Cochard et al., 2002, Nardini & Salleo, 2000). However, it is now accepted that some plants in the field regularly operate at water potentials associated with hydraulic dysfunction (Brodribb & Holbrook, 2004a, Johnson et al., 2009, Lo Gullo et al., 2003) and furthermore, are capable of rapid and complete hydraulic recovery (Brodribb & Holbrook, 2004a, Nardini et al., 2008, Trifilo et al., 2003).

The ability of plants to maintain hydraulic conductance under water-tension influences the range of water potentials within which plants remain functional (Brodribb & Holbrook, 2007, Hubbard et al., 2001) and shapes species distribution patterns along environmental gradients of moisture availability (Engelbrecht et al., 2007). The vulnerability of the water transport pathway to water-stress-induced tension is typically assessed as $P_{50}$, or the water potential causing a 50% reduction in hydraulic conductance. The functional and ecological significance of hydraulic vulnerability has been widely demonstrated in stems (Hacke et al., 2001, Tyree et al., 1998), where it has been shown to vary across broad taxonomic groups in relation to gradients in rainfall (Brodribb & Hill, 1999, Maherali et al., 2004, Pockman & Sperry, 2000). However, there is growing evidence that the hydraulic constraints in leaves are equally if not more important in defining plant function and driving differences in species ecological strategy. The hydraulic properties in leaves are distinct from those in stems and, due to their relatively high hydraulic resistance (Sack & Holbrook, 2006), impose significant constraints on plant function. Compared to stems, leaves are often more vulnerable to hydraulic dysfunction (Brodribb et al., 2003, Choat et al., 2005, Hao et al., 2008, Salleo et al., 2000). Based on a close correspondence between reduced $K_{\text{leaf}}$ and stomatal closure, leaf hydraulic vulnerability may also underlie plant functional responses to short-term water stress (Brodribb & Holbrook, 2003).

Therefore, the hydraulic properties of leaves are potentially fundamental to plant function and ecological strategy. However, further work is required to improve our understanding of the functional and ecological importance of leaf hydraulics, especially in woody angiosperm species. Gaps in our knowledge that require further attention include how leaf hydraulics might be influential in driving plant functional responses during drought as well as in subsequent recovery following re-watering. Further work is also required to elucidate how different leaf hydraulic traits are integrated with leaf structure and function. Finally, because leaf hydraulic vulnerability may potentially represent a fundamental indicator of drought resistance, further work is required to
understand the adaptive significance of leaf hydraulics across the rainfall spectrum and determine how leaf hydraulics relates to processes of community assembly.

Leaf hydraulics and drought

Drought is a major environmental stress that adversely affects plant growth (Kramer & Boyer, 1995) and strongly influences plant distribution patterns (Engelbrecht et al., 2007, Kursar et al., 2009). Given that climate change models predict drought to increase in frequency and severity in many parts of the world (IPCC, 2007), there is an increasingly urgent need to better understand the potential impacts of drought on plant function. An essential step in this process will be to identify plant attributes that drive plant functional responses in relation to drought and directly reflect adaptation to increasing aridity. Because varying degrees of drought-stress effectively reduce the ability of plants to maintain hydraulic conductance, which in turn influences plant function, these attributes are likely to be linked to the water transport properties in plants. Indeed, increased resistance to water-stress-induced hydraulic dysfunction in stems has already been shown to influence species' drought tolerance (Jacobsen et al., 2007, Pockman & Sperry, 2000). However, this functional correlation has yet to be comprehensively tested in leaves. Despite this, recent studies have not only shown that leaf hydraulics influence plant functional responses during drought, but have also identified leaf hydraulics as important drivers of plant functional recovery from drought following re-watering (Brodribb & Cochard, 2009, Resco et al., 2009). Furthermore, reports that leaf hydraulic dysfunction is intimately linked with drought survival in a number of conifer species (Brodribb & Cochard, 2009) indicates that leaf hydraulic vulnerability may be adaptive across different species in determining their drought resistance.

Leaf hydraulics and plant functional traits

Plant functional traits are attributes that positively influence establishment, survival and/or reproduction, and are usually considered adaptive if the expression of trait-states correlates with variation in natural environments (Ackerly, 2003). While many morphological and physiological traits change systematically along global and regional environmental gradients (Cavender-Bares et al., 2004, Wright et al., 2004), many of the most widely studied traits appear to be secondary correlative indicators of function; they are broadly adaptive but do not necessarily reflect direct adaptation to specific, identifiable, environmental stresses. Leaf mass per unit area (LMA), for example, is often cited in studies examining land-plant ecology (Poorter et al., 2009, Reich et al.,
Chapter 1 – General introduction

1999). However, the mechanism that underlies variation in LMA across environments is not necessarily specific and may relate to increasing aridity (Niinemets, 2001) and/or the protection of long-lived leaves from a diverse array of damage types, especially under resource poor conditions (Jordan et al., 2005, Turner, 1994, Wright et al., 2002). Therefore, identifying key functional traits that reflect direct adaptation to specific environmental stress will be highly informative to studies of land-plant ecology and enhance our understanding of plant ecological strategy (McGill et al., 2006).

Because leaf hydraulic vulnerability relates to the ability of plants to maintain function under conditions of water-stress, variation in $P50_{leaf}$ is likely to be a strong candidate of a functional trait that directly reflects plant adaptation to drought. As a result, greater understanding of the hydraulic constraints in leaves might allow us to develop a predictive tool for assessing the likely impacts of increasing aridity on species distribution patterns and community dynamics. Furthermore, such a trait that has a known mechanistic association with an environmental gradient will be highly valuable in understanding the assembly and function of ecological communities.

Objectives and thesis outline

In this dissertation I examine and characterise the leaf hydraulics of a phylogenetically, morphologically and ecologically diverse group of woody angiosperm species. Particular emphasis is placed on examining how key leaf hydraulic traits in these species determine plant functional responses in relation to drought, and relate to leaf structure and function. I also test the adaptive significance of leaf hydraulic vulnerability in defining species bioclimatic limits in terms of minimum rainfall and explore the idea that variation in $P50_{leaf}$ can be used to enhance our understanding of the potential impacts of climate change and increasing aridity on plant function and community dynamics, as well as the processes that shape community assembly and influence community functional composition. The following research questions are addressed:

1) What is the functional significance of leaf hydraulics?

2) What is the adaptive significance and evolutionary basis of variation in leaf hydraulic vulnerability?

3) How does leaf hydraulics relate to the functional composition of plant communities under current and future climates?
4) Are leaf hydraulic traits useful indicators of drought resistance?

In Chapter 2, I assess the importance of leaf hydraulics in influencing plant functional responses during drought and in subsequent recovery following re-watering. I also examine whether variation in leaf hydraulic vulnerability ($P50_{leaf}$) corresponds to species' absolute drought tolerance in terms of drought survivorship.

In Chapter 3, I examine how inter-specific variation in $P50_{leaf}$ is coordinated with leaf structure and function. Specifically, I test whether the structural properties of the leaf xylem that predicts the capacity of xylem conduits to resist implosion under water-stress is related to leaf hydraulic vulnerability. I also test for relationships between $P50_{leaf}$ and other functional traits related to drought tolerance.

In Chapter 4, I test the adaptive significance of variation in $P50_{leaf}$ in defining species bioclimatic limits in terms of minimum rainfall. Here, I also compare the within-site variability in $P50_{leaf}$ of two high-rainfall communities that contrast in species diversity and historical ecology in order to test whether plant functional traits can enhance our understanding of the assembly and function of ecological communities.

In Chapter 5, I examine how different measures of leaf capacitance ($C_{leaf}$) relate to the water transport pathway and influence the determination of absolute hydraulic conductance in leaves using the rehydration kinetics of water potential relaxation.

Finally, in Chapter 6 I combine the major findings of the different chapters in a synthesis aimed at addressing the four research questions outlined above.

Preface

This PhD thesis is composed of two published papers in peer-reviewed international journals (Chapters 2 and 3), and two chapters which are formatted as papers (Chapters 4 and 5). To enhance the structure of this thesis, I have removed the author addresses, keywords and acknowledgements from each paper, but have maintained the reference list for each.

REFERENCES


Chapter 4

The adaptive significance of leaf hydraulic vulnerability and its implications for community assembly

Blackman CJ, Brodribb TJ, Jordan, GJ

ABSTRACT

The ability of plants to maintain water flow through leaves under increasing water-stress-induced tension (defined as the leaf hydraulic vulnerability; $P50_{\text{leaf}}$) is intimately linked with survival. However, our knowledge of the extent to which inter-specific variation in $P50_{\text{leaf}}$ influences species’ ecological tolerances and defines their distributional climatic limits remains limited. Here, we tested the adaptive significance of leaf hydraulic vulnerability in woody angiosperms using paired species from montane rainforest and dry-sclerophyll forest communities in Tasmania, Australia, and exploring the relationship between $P50_{\text{leaf}}$ and each species’ climatic limits in terms of minimum water availability. We also compared the within-site variability in $P50_{\text{leaf}}$ between two ever-wet montane rainforest communities in Western Tasmania and the Central Peruvian Cordillera that contrast in species diversity and historical constraints in order to investigate the role of physiology in community assembly. $P50_{\text{leaf}}$ was strongly and significantly correlated with the minimum rainfall availability for species pairs in wet and dry communities in Tasmania, thus pointing to the adaptive significance of this key functional trait. However, within-site variability in $P50_{\text{leaf}}$ was found to be significantly different between the two montane rainforest communities. Because $P50_{\text{leaf}}$ was shown to be functionally adaptive across a broad range of environments, this divergence in trait variability between two high-rainfall communities suggests that within-site trait distributions may be influenced by some combination of spatial and temporal environmental heterogeneity and parochial historical ecology.
INTRODUCTION

The habitat preferences of species are often explained by invoking functional characteristics or traits that provide competitive advantages under certain environmental conditions. Functional traits are attributes that positively influence establishment, survival and/or reproduction, and are usually considered adaptive if the expression of trait-states correlates with variation in natural environments (Ackerly 2003). While many morphological and physiological traits change systematically along global and regional environmental gradients (Cavender-Bares et al., 2004; Wright et al., 2004), these traits are often secondary correlative indicators of function; they are broadly adaptive but do not necessarily reflect direct adaptation to specific environmental stress. As a result, identifying key functional traits that reflect direct adaptation to specific, identifiable environmental stress will be highly informative to studies of land-plant ecology and enhance our ability to both understand plant ecological strategy and predict the likely impact of changes in environmental conditions on plant function and community dynamics (McGill et al., 2006). In particular, traits that have a known mechanistic association with environmental gradients will be of more value in understanding both the relationships of individual species to their environment and the assembly and function of ecological communities than traits with solely correlative associations.

Recent advances in plant hydraulic research suggest that inter-specific variation in the vulnerability of the hydraulic pathway to drought-induced dysfunction is directly linked to the ability of different species to withstand drought-stress. Hydraulic dysfunction commonly arises once negative water potentials associated with increased tension in the xylem transgress species-specific thresholds, leading to cavitation (Tyree and Sperry 1989) or xylem cell-wall collapse {Blackman, 2010 #1634; Brodribb, 2005 #582; Cochard, 2004 #94}. This process is associated with reduced plant photosynthesis and growth (Brodribb & Holbrook 2007; Meinzer et al., 2001) and, without relief, ultimately leads to plant death. Therefore, it is not surprising that strong evidence points to the adaptive significance of hydraulic vulnerability to dysfunction in numerous plant groups across broad geographic ranges and habitats (Brodribb & Hill 1999; Choat et al., 2007; Maherali et al., 2004; Pockman & Sperry 2000).

Despite consistency in the patterns of association between hydraulic vulnerability and environment, significant variation among species in functional traits related to drought-resistance also occurs within communities. Stem hydraulic vulnerability to
drought-induced dysfunction, for example, has been shown to be highly variable among species co-occurring in Mediterranean-type environments (Jacobsen et al., 2007; Pockman & Sperry 2000). This within-site trait variability has been explained by differences in rooting depth, supporting the idea that within-site functional trait variability is enhanced by differences in ecological strategy that reflect spatial and/or temporal environmental heterogeneity and allow species co-existence (Tilman 1988). However, within-site trait variability is also influenced by the phylogenetic structure of the regional species pool (Webb et al., 2002), which in turn is highly contingent on historical and biogeographic processes (Donoghue 2008). Throughout history, different regions have been open to colonisation from different lineages, while regional variation in rates of speciation and extinction has caused divergence in community structure (McGlone 1996), even among areas sharing similar contemporary environmental conditions (Qian & Ricklefs 2000). As a result, functional trait variability within plant communities that have similar climates but contrasting histories might also be expected to diverge.

In this paper we assess the adaptive significance of a key functional trait related to drought resistance in a phylogenetically and ecologically diverse group of woody cool-temperate angiosperm species. The core functional trait used here is the vulnerability of the leaf hydraulic pathway to water stress-induced dysfunction (defined as the water potential responsible for a 50% loss in maximum leaf hydraulic conductance; \( P50_{\text{leaf}} \)). We measure \( P50_{\text{leaf}} \) on the basis that early evidence points to the functional significance of this trait in terms of plant survival under drought conditions in a number of angiosperm and conifer species (Blackman et al., 2009; Brodribb & Cochard 2009). Further to this, the hydraulic vulnerability of leaves is often higher than that of other plant organs (Brodribb et al., 2003; Hao et al., 2008) and thus may define the climatic limits of different species in terms of minimum water availability. We also compare the within-site variability of \( P50_{\text{leaf}} \) in a Tasmanian montane rainforest community with a recent history of large-scale extinction with that of a species-rich and historically more stable montane rainforest community in the Peruvian Andes. Despite their contrasting histories, these communities both occur in cool wet climates that can be expected to impose minimal water stress on plants and thus, according to processes of local ecological determinism, would be expected to exhibit similar within-site \( P50_{\text{leaf}} \) variability. However, if significant differences in within-site \( P50_{\text{leaf}} \) variability between the two communities are observed, we ask whether functional trait diversity in natural systems can be shaped by processes related to long-term climatic variability and/or historical climate perturbation.
MATERIALS AND METHODS

Community characteristics and species

In Tasmania, we sampled foliage from a phylogenetically diverse range of species co-occurring in montane rainforest below Lake Fenton (42°40'S, 146°37'E) at 900 m within the Mount Field National Park, Tasmania, Australia. The montane rainforest community at Mount Field is characteristic of moist, low fire-frequency sites in subalpine regions on relatively fertile soil throughout much of the central and western half of the state (Crowden 1999). These communities are typically cool and wet receiving a range of annual rainfall from ~3700 mm on the western mountains to ~1500 mm on drier mountains where montane rainforest is often restricted to relatively cool and wet micro-sites such as south facing slopes (Crowden 1999). Fifteen woody angiosperm species from Tasmania were chosen, based on their high abundance and structural dominance within the montane rainforest community (Table 4.1). All species show geographic distributions extending into very wet mountainous regions towards the west coast (e.g. Mount Read), as reflected by the 95th percentile of mean annual rainfall across each species' distribution (see Table A4.1 in Appendix 4). Monthly average temperatures for a representative high-rainfall montane rainforest site range from a minimum of 2.03°C to a maximum of 16.31°C (data for Mount Read sourced from the Australian Bureau of Meteorology and adjusted to allow for a 300 m altitude difference between the climate station and montane rainforest, assuming a local lapse rate of 6.3°C km⁻¹).

To perform phylogenetic independent comparisons between leaf vulnerability and species' climatic limits (see adaptive significance of $P_{50\text{leaf}}$ below), data was collected from five dry sclerophyll forest species with close relatives among the sample of Tasmanian montane rainforest species (Table 4.1). Compared to montane rainforest, these dry forest species occur at lower elevations and in much drier climatic conditions (Table A4.1).

In Peru, we sampled plant material from 14 tree species that phylogenetically overlapped with the Tasmanian montane rainforest sample (Table 4.1; Figure A4.1 in Appendix 4), selected for their high relative abundance and canopy dominance within one-hectare plots (D Catchpole, unpublished). These species grow in tropical montane rainforest between 2400 (lower montane) and 2800 (upper montane) meters above sea level within the Yanachaga-Chemillén National Park, Pasco, Peru (10°31'S, 75°21'W). This small park (122,000 ha) has been identified as one of the most
floristically diverse within Peru, with estimates of around 5000 species, mostly in montane forests that contain a high proportion of epiphytes (Vasquez et al., 2005). The forest is characterised by dominant tree species ranging in height from 12 to 35 m depending on altitude. The upper montane rainforest is typical of forest stands on mountains passes or steep ridges between 2000 and 3200 m throughout the Eastern Peruvian Andes. This forest often develops on young and poorly developed soils that are often waterlogged throughout the year. The lower montane rainforest is typical of forest stands in narrow mountain valleys between 1800 and 2800 m, often forming on highly variable, yet relatively fertile and better drained soils. Annual rainfall within the upper montane rainforest is approximately 3170 mm, with a moderate dry season during the austral winter, while monthly average temperatures range from a minimum of 7.92°C to a maximum of 14.35°C (data based on seven years of measurements, D Catchpole unpublished).

Although the two communities sampled differ in some aspects of climate, they show particular similarities in climatic parameters related to the functional trait studied here, particularly in being more-or-less ever-wet. Both communities are also characterised by cool temperatures, high rainfall all year round and frequent mist (especially in Peru). The montane rainforest in Tasmania is generally exposed to slightly lower winter minimum temperature as well as higher summer maximum temperatures than the Peruvian forest, but the lower minimum temperatures recorded in the Tasmanian community are insufficient to cause freeze-induced drought by soil freezing or xylem embolism (Feild & Brodribb 2001).

The most striking difference between the communities is that the Tasmanian forest has much lower species richness than the Peruvian forest. For example, the Lake Johnston Nature Reserve at Mount Read is one of the most diverse Tasmanian montane rainforest sites, but has only 174 vascular plant species (including 16 tree species) in 138 ha (Parks and Wildlife Service 1999). In contrast, 649 species with 137 species of canopy trees have been recorded in a 1 ha plot within the Yanachaga-Chemillén National Park, Peru (D Catchpole unpublished).

Although these differences in diversity are broadly consistent with well known global latitudinal trends that are often explained in terms of broad-scale climate gradients (Currie & Francis 2004), there is clear evidence for contrasting historical constraints on the diversity of these systems. Palaeobotanical evidence shows that Tasmania’s montane rainforest flora was highly diverse in the late Paleogene, with taxonomic and
physiognomic similarity to modern subtropical-tropical lower montane rainforest (Carpenter et al., 1994). However, increasingly cool and dry conditions during the Neogene (Hill 2004), reduced the diversity of mesic species significantly (Carpenter et al., 1994; Jordan 1997), culminating in extensive extinction without replacement of rainforest species during the early climate cycles of the Pleistocene (Jordan 1997) which involved repeated episodes of cold and dry climates (Hope 1994). The Neogene and Pleistocene also saw a proliferation of sclerophyllous taxa with adaptations for dealing with drier climates (Jordan et al., 2008). This is in marked contrast with Peru, where continuous cloud forest cover within the Andean foothills suggests that moist montane rainforest vegetation remained intact and diverse throughout the last glacial cycle (Urrego et al., 2005).

**Leaf hydraulic vulnerability**

For each species, leaf vulnerability curves were constructed by measuring leaf hydraulic conductivity ($K_{\text{leaf}}$) in leaves rehydrated from a range of initial leaf water potentials ($\Psi_{\text{leaf}}$). $K_{\text{leaf}}$ was measured by assessing the kinetics of $\Psi_{\text{leaf}}$ relaxation upon leaf rehydration, as described by Brodribb and Holbrook (2003). Leaf vulnerability curves for the Tasmanian species were drawn from previous work (Blackman et al., In press). Branches from three individuals of each species were cut early in the morning while $\Psi_{\text{leaf}}$ was high, and immediately double-bagged to arrest water loss. Branches in Peru were sampled the previous evening due to the remoteness of the field sites. Using data for the vessel length of each species (CJ Blackman, unpublished), branches were cut to a length sufficient to prevent emboli from extending into the sample leaves. Branches were transferred to the laboratory and allowed to desiccate slowly for up to 48 hrs, before being carefully bagged to arrest water loss and ensure water potential equilibrium throughout the branch. For each species, this resulted in a set of branches covering a range of water potentials of approximately $-0.4$ to $-3.0$ MPa for drought sensitive species and $-0.4$ to $-5.0$ MPa for drought resistant species. Initial $\Psi_{\text{leaf}}$ was determined by measuring leaves neighbouring the sample leaf in a Scholander pressure chamber (PMS, Albany, OR, USA). The sample leaf was then cut under water and allowed to rehydrate for a period of between 30 and 300 s depending on the initial $\Psi_{\text{leaf}}$, after which their petioles were immediately dabbed dry, and the leaf wrapped in moist paper towel and double bagged ready for final $\Psi_{\text{leaf}}$ determination. During rehydration, the submerged cut end of both petioles and branchlets was regularly recut to ensure the xylem remained unblocked. Final $\Psi_{\text{leaf}}$ was measured with the pressure chamber.
and $K_{\text{leaf}}$ calculated from the ratio of the initial to final $\Psi_{\text{leaf}}$ and the capacitance of the leaf:

$$K_{\text{leaf}} = C_{\text{leaf}} \ln \left[ \frac{\Psi_o}{\Psi_f} \right] / T$$  \hspace{1cm} (4.1)

where $\Psi_o =$ initial leaf water potential (MPa); $\Psi_f =$ final leaf water potential (MPa); $T =$ duration of rehydration (s); $C_{\text{leaf}} =$ leaf capacitance (mmol m$^{-2}$ MPa$^{-1}$).

Mean leaf capacitance ($C_{\text{leaf}}$) was measured from six leaves of each species using the slope of the pressure-volume relationship for each species (Tyree & Hammel 1972). Branches were cut underwater in the morning and rehydrated until $\Psi_{\text{leaf}} > -0.05$ MPa, after which leaves were detached for pressure-volume analysis. Leaf weight and water potential were measured periodically during slow desiccation of sample leaves in the laboratory. Because cell walls in the leaf are elastic, leaf capacitance pre- and post-turgor loss are often quite different. The capacitance function was defined by measuring the turgor loss point from the inflection point of the graph of $1/\Psi_{\text{leaf}}$ verses relative water content (RWC), and then using this value as the intersection of linear regressions fitted through data either side of the turgor loss point. These slopes yielded pre- and post turgor leaf capacitance in terms of RWC.

Determination of $K_{\text{leaf}}$ (mmol m$^{-2}$ s$^{-1}$ MPa$^{-1}$) requires that leaf capacitance, as determined by the pressure-volume curve, be calculated in absolute terms and normalised by leaf area. To do this, leaf capacitance was multiplied by the saturated mass of water in the leaf and then divided by leaf area (Brodribb & Holbrook 2003). Leaf areas were measured as projected areas with a flatbed scanner and image analysis software (Image J, National Institutes of Health, Bethesda, MD, USA).

The vulnerability of leaf hydraulic conductance to decreasing water potential for individual species was defined as the negative $\Psi_{\text{leaf}}$ at which point $K_{\text{leaf}}$ had declined by 50% from maximum ($P50_{\text{leaf}}$). $P50_{\text{leaf}}$ was calculated by fitting a three-parameter sigmoidal regression function of the form $y = 100/(1+e^{a(\Psi_{\text{leaf}} - b)})$, where $y =$ % loss of $K_{\text{max}}$ to the $K_{\text{leaf}}$ versus $\Psi_{\text{leaf}}$ data from each species. Because each species showed a sigmoidal response of $K_{\text{leaf}}$ to increasing xylem tension, $K_{\text{max}}$ was defined for each species independently as the mean $K_{\text{leaf}}$ at water potentials before any significant decline in hydraulic conductance.
Chapter 4 – Adaptive significance of $P50_{\text{leaf}}$

**Adaptive significance of $P50_{\text{leaf}}$**

We tested the adaptive significance of leaf vulnerability to cavitation using five phylogenetically independent species pairs in Tasmania (Table 4.1) from across a taxonomically diverse sample range. Each pairing contrasted a species from the dry sclerophyll forest with a closely related species from the Tasmanian montane rainforest. To allow for difference in ecological tolerances of the species, we estimated the climatic limit for each species by calculating the 5\textsuperscript{th} percentile of mean annual rainfall across each species distribution. Species observation data within Tasmania and southeast Australia was compiled using the Natural Values Atlas database (Department of Primary Industries and Water, Tasmania, http://www.naturalvaluesatlas.tas.gov.au) and Australia’s Virtual Herbarium database (Centre of Plant Biodiversity Research, http://www.anbg.gov.au/avh), respectively. Because of more intensive sampling in the Natural Values Atlas, the data from this source was randomly downsampled to give the same sampling intensity (as determined by comparison of data for the same areas from the two sources). Climate data for each observation was calculated using a fine-scale climate model BIOCLIM (Houlder et al., 2003), which uses a three dimensional spline to estimate climate for latitude, longitude and altitude. The relationship between rainfall and vulnerability was expected to be non-linear because the tensile strength of water constrains minimum $P50_{\text{leaf}}$ to $-22\text{ MPa}$ (Cochard et al., 2007) at the dry end of the spectrum whereas maximum $P50_{\text{leaf}}$ will not exceed the turgor loss point of ever-wet cloud forest species at around $-0.5\text{ MPa}$ (Feild et al., 2009). Hence we fitted a sigmoidal curve of the form $y = a*x/(b+x)+c*x/(d+x)$ through the vulnerability verses climate data for all Tasmanian species having defined the water potential at zero rainfall as $-22\text{ MPa}$. To linearise the vulnerability data, we predicted the expected rainfall from the observed vulnerability according to the fitted curve for each of the species in dry sclerophyll/montane rainforest pairs. We then tested for a phylogenetically independent relationship between vulnerability and climate by estimating the ratio of the change in vulnerability (linearised) to the change in rainfall for each species, and then used a t-test to assess whether the average value of this ratio was greater than zero.

$P50_{\text{leaf}}$ variability

Differences in the variability of $P50_{\text{leaf}}$ values between species from montane rainforest in Tasmania and Peru were tested using Bartlett’s test of homogeneity of variances. The means of $P50_{\text{leaf}}$ for Tasmanian montane rainforest species with true rainforest...
ancestry (Table A4.1) and the Peruvian species were compared using a type 2 Student’s t-Test.

RESULTS

For all Tasmanian and Peruvian species, the response of leaf hydraulic conductivity to decreasing water potential was sigmoidal, with an initial plateau followed by a decline in $K_{leaf}$ to a minimum value close to zero as $\Psi_{leaf}$ declined (Fig. 4.1 shows four typical species’ vulnerability curves).

**Figure 4.1** Responses of leaf hydraulic conductance ($K_{leaf}$) to decreasing leaf water potential for a subset of species sampled from Peru and Tasmania. *Licaria subsessilis* and *Eugenia pubescens* from Peruvian montane cloud forest; *Atherosperma moschatum* and *Eucalyptus coccifera* from Tasmanian montane rainforest. Curves fitted are sigmoidal functions. Solid vertical lines indicate the water potential at 50% loss of $K_{leaf}$ ($P50_{leaf}$).

Across the Tasmanian species sample, including the dry forest species, a significant relationship was found between $P50_{leaf}$ and the 5th percentile of mean annual rainfall across each species geographic distribution (Fig. 4.2). Species with low leaf vulnerability showed climatic limits that extended into drier regions, while species with more vulnerable leaves were geographically restricted to areas with high rainfall. Each species sampled from dry sclerophyll forest in Tasmania was less vulnerable to xylem cavitation compared to its paired montane rainforest close relative (Fig. 4.2),
Chapter 4 – *Adaptive significance of P50*\textsubscript{leaf}*

regardless of its generic ecological affinity (Table A4.1). A test of the phylogenetically independent relationship between vulnerability and climate for the species pairs was highly significant (*P* < 0.01; Fig. 4.2).

Table 4.1 Compiled data of leaf hydraulic vulnerability (*P50*\textsubscript{leaf}) for all species sampled in the study. *P50*\textsubscript{leaf} values for the Tasmanian species are drawn from previous work by our laboratory group (Blackman et al., In press). Dry forest species paired with closely related montane rainforest species within Tasmania are denoted by asterisks.

<table>
<thead>
<tr>
<th>family</th>
<th>species</th>
<th><em>P50</em>\textsubscript{leaf} (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tasmanian montane rainforest</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asteraceae</td>
<td><em>Olearia pinifolia</em> (Hook.f.) Benth.*</td>
<td>−1.71 ± 0.03</td>
</tr>
<tr>
<td>Atherospermataceae</td>
<td><em>Atherosperma moschatum</em> Labill.</td>
<td>−1.48 ± 0.03</td>
</tr>
<tr>
<td>Ericaceae</td>
<td><em>Cyathodes straminea</em> R.Br.</td>
<td>−2.00 ± 0.15</td>
</tr>
<tr>
<td>Ericaceae</td>
<td><em>Gaultheria hispida</em> R.Br.</td>
<td>−1.32 ± 0.04</td>
</tr>
<tr>
<td>Ericaceae</td>
<td><em>Richea scoparia</em> Hook.f.</td>
<td>−1.41 ± 0.09</td>
</tr>
<tr>
<td>Myrtaceae</td>
<td><em>Eucalyptus coccifera</em> Hook.f.**</td>
<td>−2.65 ± 0.15</td>
</tr>
<tr>
<td>Nothofagaceae</td>
<td><em>Nothofagus cunninghamii</em> (Hook.) Oerst.</td>
<td>−1.70 ± 0.11</td>
</tr>
<tr>
<td>Nothofagaceae</td>
<td><em>Nothofagus gunnii</em> (Hook.) Oerst.</td>
<td>−1.53 ± 0.04</td>
</tr>
<tr>
<td>Pittosporaceae</td>
<td><em>Pittosporum bicolor</em> Hook.***</td>
<td>−1.87 ± 0.08</td>
</tr>
<tr>
<td>Proteaceae</td>
<td><em>Hakea lissosperma</em> R.Br.****</td>
<td>−2.85 ± 0.24</td>
</tr>
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<td>Proteaceae</td>
<td><em>Lomatia polymorpha</em> R.Br.******</td>
<td>−1.57 ± 0.12</td>
</tr>
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<td>Proteaceae</td>
<td><em>Orites diversifolius</em> R.Br.</td>
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</tr>
<tr>
<td>Proteaceae</td>
<td><em>Telopea truncata</em> (Labill) R.Br.</td>
<td>−1.58 ± 0.08</td>
</tr>
<tr>
<td>Rubiaceae</td>
<td><em>Coprosma nitida</em> Hook.f.</td>
<td>−1.95 ± 0.04</td>
</tr>
<tr>
<td>Winteraceae</td>
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<td>−1.56 ± 0.13</td>
</tr>
<tr>
<td><strong>Tasmanian dry sclerophyll forest</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asteraceae</td>
<td><em>Olearia hookeri</em> (Sond.) Benth.*</td>
<td>−2.36 ± 0.11</td>
</tr>
<tr>
<td>Myrtaceae</td>
<td><em>Eucalyptus pulchella</em> Desf.**</td>
<td>−4.31 ± 0.36</td>
</tr>
<tr>
<td>Pittosporaceae</td>
<td><em>Bursaria spinosa</em> Cav.***</td>
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</tr>
<tr>
<td>Proteaceae</td>
<td><em>Hakea microcarpa</em> R.Br.****</td>
<td>−3.96 ± 0.22</td>
</tr>
<tr>
<td>Proteaceae</td>
<td><em>Lomatia tinctoria</em> (Labill) R.Br.*****</td>
<td>−2.08 ± 0.13</td>
</tr>
<tr>
<td><strong>Peruvian montane rainforest</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloranthaceae</td>
<td><em>Hedyosmum anisodorum</em> Todzia</td>
<td>−1.14 ± 0.07</td>
</tr>
<tr>
<td>Chloranthaceae</td>
<td><em>Hedyosmum lechleri</em> Solms</td>
<td>−1.09 ± 0.07</td>
</tr>
<tr>
<td>Clusiaceae</td>
<td><em>Clusia ducuoides</em> Engl.</td>
<td>−1.25 ± 0.09</td>
</tr>
<tr>
<td>Clusiaceae</td>
<td><em>Clusia elliptica</em> Kunth</td>
<td>−1.19 ± 0.13</td>
</tr>
<tr>
<td>Cunoniaceae</td>
<td><em>Weinmannia lechleriana</em> Engl.</td>
<td>−1.27 ± 0.12</td>
</tr>
<tr>
<td>Cunoniaceae</td>
<td><em>Weinmannia microphylla</em> Kunth</td>
<td>−1.03 ± 0.04</td>
</tr>
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<td>Lauraceae</td>
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<td>−1.52 ± 0.06</td>
</tr>
<tr>
<td>Melastomataceae</td>
<td><em>Miconia adinantha</em> Wurdack</td>
<td>−1.91 ± 0.05</td>
</tr>
<tr>
<td>Melastomataceae</td>
<td><em>Miconia aprica</em> Gleason</td>
<td>−1.55 ± 0.20</td>
</tr>
<tr>
<td>Melastomataceae</td>
<td><em>Miconia barbeyana</em> Cogn</td>
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</tr>
<tr>
<td>Meliaceae</td>
<td><em>Cedrela montana</em> Moritz ex Turez</td>
<td>−1.36 ± 0.16</td>
</tr>
<tr>
<td>Myrsinaceae</td>
<td><em>Myrsine pellucida</em> (Ruiz &amp; Pav) Spreng.</td>
<td>−1.48 ± 0.10</td>
</tr>
<tr>
<td>Myrtaceae</td>
<td><em>Eugenia pubescens</em> (Kunth) DC.</td>
<td>−1.55 ± 0.10</td>
</tr>
<tr>
<td>Theaceae</td>
<td><em>Frezierea uncinata</em> A.L. Weitzman</td>
<td>−1.20 ± 0.24</td>
</tr>
</tbody>
</table>
The range of within-site leaf vulnerability to decreasing water potential was significantly different between the Peruvian and Tasmanian montane rainforest communities. In Peru, $P_{50\text{leaf}}$ ranged from $-1.03$ MPa in *Weinmannia microphylla* to the least vulnerable Peruvian species *Miconia adinantha*, with $P_{50\text{leaf}}$ recorded at $-1.91$ MPa (Table 4.1). In comparison, the distribution of $P_{50\text{leaf}}$ for Tasmanian montane rainforest species overlapped with that of Peru at the most vulnerable end of the range but showed a markedly longer tail at the less vulnerable end of the range: it thus ranged from $-1.25$ MPa in *Orites diversifolius* to the most drought resistant species *Hakea lissosperma*, with $P_{50\text{leaf}}$ recorded at $-2.85$ MPa (Table 4.1). The significant overlap of Tasmanian species at the most vulnerable end of the range was largely comprised of species with unequivocal rainforest ancestry whereas the tail at the less vulnerable end was represented by species from dry forest clades (Table A4.1). Mean vulnerability for the component of the Tasmanian community with true rainforest ancestry (mean = $-1.49$ MPa ± 0.14) was not significantly different (t-test, $P < 0.23$) to the Peruvian species (mean = $-1.38$ MPa ± 0.25).

**Figure 4.2** The relationship between leaf vulnerability to cavitation ($P_{50\text{leaf}}$) and the 5th percentile of mean annual rainfall across each of the Tasmanian species' geographic distribution. Solid grey symbols represent montane rainforest species while open symbols represent dry sclerophyll species. A hyperbolic curve was fitted through all the Tasmanian species data based on a theoretical intercept at $-22$ MPa (see text). Species pairs are denoted by enlarged symbols and connected by solid regression lines; hexagon, *Bursaria spinosa* and *Pittosporum bicolor*; square, *Lomatia*; upward triangle, *Olearia*; diamond, *Hakea*; downward triangle, *Eucalyptus*. A significant phylogenetically independent relationship was recorded between leaf vulnerability and climate (t-test; $P < 0.01$).
A greater range of $P50_{\text{leaf}}$ in the Tasmanian montane rainforest sample compared with the Peruvian community was confirmed by a significant inequality of variance in $P50_{\text{leaf}}$ between the two sites (Bartlett’s test for homogeneity of variance: $P \leq 0.03$; Fig. 4.3). Box plots in Figure 4.3 illustrate the distributions of $P50_{\text{leaf}}$ values among species within each community and imply greater functional convergence of leaf vulnerability among species of montane cloud forest in Peru (mean = $-1.38 \text{ MPa} \pm 0.25$) compared to those of montane rainforest in Tasmania (mean = $-1.76 \text{ MPa} \pm 0.46$).

**DISCUSSION**

*Adaptive significance of $P50_{\text{leaf}}$*

The strong relationship between $P50_{\text{leaf}}$ and the bioclimatic limits of the Tasmanian species (Fig. 4.2) indicates a central adaptive role for leaf xylem vulnerability in defining species’ distribution patterns. This adaptive significance of $P50_{\text{leaf}}$ is further supported by the phylogenetically independent comparisons showing that species from
dry sclerophyll forest produced leaves that were consistently less vulnerable to drought induced hydraulic dysfunction than their wetter montane rainforest species pairs (Fig. 4.2). This analysis contained species pairs with generic ecological affinities based in both wet and dry forests. This implies bidirectional evolution, with evolution of both increased drought resistant leaves under drying climate and reduced drought resistance in response to more moist conditions. As shown in stems, this suggests that the trade-off associated with drought resistance in leaves might come at the cost of extra investment in xylem reinforcement and reduced growth rate (Hacke et al., 2001; Poorter et al., 2010). This concept is re-enforced by evidence that leaf hydraulic vulnerability is strongly correlated with anatomical properties of the xylem that relate to water stress-induced conduit collapse (Blackman, 2010 #1634). Overall, the consistency of these relationships adds to growing evidence that xylem resistance to hydraulic failure is subject to strong selection across the rainfall spectrum (Brodribb & Hill 1999; Maherali et al., 2004; Pockman & Sperry 2000).

The current study is the first to show a direct link between leaf hydraulic vulnerability to dysfunction and species’ distributions in angiosperms, and is among the first to investigate the adaptive significance of hydraulic dysfunction in leaves (Blackman et al., 2009; Brodribb & Cochard 2009; Hao et al., 2008). This is surprising considering that leaf hydraulic systems tend to be relatively vulnerable and rate limiting in the whole-plant hydraulic continuum (Sack & Holbrook 2006). These results extend recent observations that leaf vulnerability is intimately linked to seedling survivorship in response to drought in a number of conifer (Brodribb & Cochard 2009) and woody angiosperm species (Blackman et al., 2009). Taken together, these results point to leaf hydraulic vulnerability as a measurable and fundamental indictor of plant performance in relation to increasing aridity.

_Ecological implications of contrasting diversity in P50_{leaf}_.

In view of its adaptive significance, ecologically deterministic models of trait-based community assembly should predict \( P50_{leaf} \) to converge in our two ever-wet plant communities, despite the phylogenetic origin of the plant lineages involved (Keddy 1992). Such trait convergence has been shown to arise via the influence of habitat filtering, which acts to restrict the range of trait values that are viable under certain environmental conditions (Diaz et al., 1998; Weiher & Keddy 1995). Recent studies of tropical plant systems have highlighted the role of environmental filtering in driving community assembly (Lebrija-Trejos et al., 2010), although others have emphasised specific trait-based strategy differentiation in promoting species co-existence (Kraft et
Our data from the tropics supports the former, suggesting that the relatively small range of leaf vulnerabilities in Peruvian cloud forest is evidence of convergent evolution of this key adaptive trait. Indeed, the ever-wet climate in these montane rainforest communities, combined with the effects of regular cloud immersion, leaves little latitude for niche differentiation based on variation in drought resistance. Hence the long-term stability and high species diversity of the Peruvian cloud forest (Urrego et al., 2005) may have helped drive convergence in $P50_{\text{leaf}}$.

Further evidence of convergent evolution of high leaf hydraulic vulnerability among rainforest species is provided by the strong overlap in $P50_{\text{leaf}}$ between the Peruvian species and the Tasmanian species with true rainforest ancestry. However, the overall greater variability in leaf hydraulic vulnerability values shown in Tasmanian montane rainforest suggests that additional processes to those related to ecological determinism and habitat filtering have had an effect on the phylogenetic and functional composition of the regional flora. One plausible explanation is that the Tasmanian montane rainforest may have greater spatial heterogeneity in seasonal water availability, thus permitting occupation by species with different water-stress physiologies. Such spatial heterogeneity has been linked to substantial variability in stem hydraulic vulnerability that reflects differences in rooting depth among co-occurring species in Mediterranean-type climates (Jacobsen et al., 2007; Pockman & Sperry 2000). However, considering that montane rainforest in Tasmania can receive more than 3000 mm of rainfall spread uniformly throughout the year, the almost ever-wet conditions in these communities should leave little latitude for the development of strong seasonal moisture gradients in the soil profile. Greater seasonal water-stress in montane rainforest in Tasmania may result from slightly higher summer temperatures leading to higher evaporative demand than in Peru, where montane rainforest is regularly immersed in cloud, resulting in low transpiration rates (Motzer et al., 2005) and reduced risk of water deficit (Cavelier 1990). However, although these processes may induce lower vulnerability in the Tasmanian plants, they provide a poor explanation for the large differences in variability.

The greater variability in $P50_{\text{leaf}}$ among co-occurring species in Tasmanian montane rainforest may also be related to climate variability over longer time-scales. Indeed, Tasmania experiences regular drought during El Niño events, and, in addition, a number of multi-year dry episodes have occurred in south-eastern Australia within the last 10 000 years (Hunt 2009). In contrast, the persistent cloudiness of tropical montane rainforests is likely to have buffered the affects of such long-term climate
variability. Taken together, these climatic conditions suggest that montane rainforest in Tasmanian may experience more severe drought episodes than the Peruvian forest studied here. As a result, Tasmanian montane rainforest is likely to occur within a more complex temporal landscape of water availability, which as a result promotes the co-existence of species with a variety of ecological strategies. In particular, this temporal climate variability may influence each species' regeneration niche (Grubb 1977) whereby episodic drought favours the recruitment and establishment of more drought tolerant species in otherwise high rainfall environments.

An alternative explanation of the high variability of $P50_{\text{leaf}}$ among Tasmanian montane rainforest species centres on the idea that community assembly is partly shaped by parochial historical constraints (Ricklefs 2008). The lowest leaf vulnerabilities observed in the Tasmanian montane rainforest were in species from clades that apparently evolved in response to drier climatic conditions. Thus, phylogenetic ancestral state reconstruction of bioclimatic traits provides independent evidence that the least vulnerable species, *Hakea lissosperma*, was derived from dry climate lineages (Jordan *et al.*, 2008). The same is likely to be true for the second least vulnerable species, *Eucalyptus coccifera*, which is phylogenetically nested within a clade dominated by dry climate species (Steane *et al.*, 1999). The presence of such species in high rainfall sites that should select against low hydraulic vulnerability suggests that historical processes may have influenced the range of trait values allowed to co-exist within this community. Indeed, the widespread extinctions of the Tasmanian rainforest flora caused by repeated episodes of glacial aridity during the Pleistocene (described in Materials and Methods) may have led to reduced inter-species competition and high levels of potential niche opportunity, thus allowing relatively drought tolerant species to invade high-rainfall sites. A key factor in creating reduced competition may have been the selective elimination of specialist species through the cyclic, high amplitude, climatic perturbation of the Pleistocene (Dynesius & Jansson 2000). This process appears to have resulted in virtually all Tasmanian montane rainforest being climatic generalists, as indicated by wide ecological ranges, typically spanning lowland forests to treeline habitats (Jarman *et al.*, 1999). This inference is supported by the slight displacement toward lower $P50_{\text{leaf}}$ among the ‘true’ Tasmanian rainforest species compared to those in Peru (Fig. 4.3). This lack of high-rainfall specialists among the Tasmanian montane rainforest species may explain why they do not competitively exclude more drought tolerant species in this environment.
Our results have potential implications for plant community responses to current climate change. Because the Tasmanian montane rainforest contains a high number of generalist species, the community is likely to be more resilient to extinction than habitat specialists. Thus, we propose that if current climate change results in more frequent and severe drought (IPCC 2007), tropical plant communities that exhibit narrow ranges of drought related trait variation may be more susceptible to extinction than mid-high latitude plant communities that retain strong ecological signals of long-term climate variability and/or drought induced Pleistocene extinctions. This process may be exacerbated in tropical mountain forests by predictions of reduced cloudiness and humidity (Foster 2001). Thus, diverse plant communities where excessively drought tolerant species are competitively excluded are potentially at risk from local extinction if the regional climate perturbation proves to be dry and hostile.

Conclusion

We highlight leaf hydraulic vulnerability to water-stress-induced dysfunction as a key adaptive trait that plays a central role in defining species’ climatic limits in terms of water availability. However, our results suggest that the distribution of adaptive trait values within local communities may be influenced not only by local ecological processes but also by long-term climate variability and/or historical constraints unique to different regional floras. Any attempt to predict community-scale impacts of the current major climatic perturbation will require careful consideration of within-site functional trait distributions in relation to the influence of both historical as well as contemporary ecology.

REFERENCES


Chapter 4 – Adaptive significance of $P_{50}^{leaf}$


Chapter 4 – Adaptive significance of P50leaf


### Table A4.1

Generic ancestral habitat affinity for the Tasmanian species (R = rainforest; DF = dry forest; A = ambiguous) and climate data in terms of minimum (5<sup>th</sup> percentile) and maximum (95<sup>th</sup> percentile) mean annual rainfall (mm) across each of the Tasmanian species’ current geographic distribution.

<table>
<thead>
<tr>
<th>species</th>
<th>ancestral habitat</th>
<th>reference</th>
<th>5&lt;sup&gt;th&lt;/sup&gt; percentile rainfall (mm)</th>
<th>95&lt;sup&gt;th&lt;/sup&gt; percentile rainfall (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>montane rainforest</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><em>Atherosperma moschatum</em></td>
<td>R</td>
<td>(Renner et al., 2000)</td>
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<td>2266</td>
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<tr>
<td><em>Coprosma nitida</em></td>
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<td>(Robbrecht &amp; Manen, 2006)</td>
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<td><em>Eucalyptus coccifera</em></td>
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<td>(Crisp et al., 2004)</td>
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<td><em>Hakea lissosperma</em></td>
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<td>(Hill, 1991)</td>
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<td><em>Tasmaninia lanceolata</em></td>
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<td><em>Telepea truncata</em></td>
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</tr>
<tr>
<td><em>Eucalyptus pulchella</em></td>
<td>DF</td>
<td>(Crisp et al., 2004)</td>
<td>597</td>
<td>1045</td>
</tr>
<tr>
<td><em>Hakea microcarpa</em></td>
<td>DF</td>
<td>(Jordan et al., 2008)</td>
<td>614</td>
<td>1546</td>
</tr>
<tr>
<td><em>Lomatia tinctoria</em></td>
<td>R</td>
<td>(Jordan et al., 2005)</td>
<td>616</td>
<td>1444</td>
</tr>
<tr>
<td><em>Olearia hookeri</em></td>
<td>A</td>
<td></td>
<td>589</td>
<td>868</td>
</tr>
<tr>
<td><em>Bursaria spinosa</em></td>
<td>A</td>
<td></td>
<td>351</td>
<td>1204</td>
</tr>
</tbody>
</table>


Figure A4.1 A phylogeny showing the phylogenetic distribution of montane rainforest species used in the study. Black bars represent Tasmanian species; green bars represent Peruvian species.
Chapter 5

Two measures of leaf capacitance: insights into the water transport pathway and hydraulic conductance in leaves

Blackman CJ, Brodribb TJ

ABSTRACT

The efficiency and stress tolerance of leaf water transport are key indicators of plant function, but our ability to assess these processes is constrained by gaps in our understanding of the water transport pathway in leaves. A major challenge is to understand how different pools of water in leaves are connected to the transpiration stream and hence determine leaf capacitance ($C_{\text{leaf}}$) to short and medium term fluctuations in transpiration. Here, we examine variation across an anatomically and phylogenetically diverse group of woody angiosperms in two measures of $C_{\text{leaf}}$ assumed to represent bulk-leaf capacitance ($C_{\text{bulk}}$) and the hydraulic capacitance of actively transpiring tissues ($C_{\text{transp}}$), respectively. Among species, $C_{\text{bulk}}$ was significantly correlated with leaf mass per unit area, while $C_{\text{transp}}$ was independently related to leaf lignin content (%) and the saturated mass of leaf water per unit dry weight. Rehydration and steady-state measurements of leaf hydraulic conductance agreed if $C_{\text{transp}}$ was used rather than $C_{\text{bulk}}$ suggesting that the leaf tissue in some species is hydraulically compartmentalised and that only a proportion of total leaf water is hydraulically well connected to the transpiration stream. These results indicate that leaf rehydration kinetics can accurately measure $K_{\text{leaf}}$ with knowledge of the capacitance of the hydraulic pathway.
INTRODUCTION

Recent investigations of leaf hydraulic conductance ($K_{\text{leaf}}$) and leaf hydraulic vulnerability to water-stress-induced dysfunction ($P50_{\text{leaf}}$) have accelerated our understanding of the processes that define plant function and drive differences in species performance and ecological strategy (Aasamaa et al., 2001; Sack et al., 2002; Brodribb & Holbrook 2003; Sack et al., 2003; Brodribb et al., 2005; Nardini et al., 2005; Hao et al., 2008; Brodribb & Cochard 2009; Blackman et al., 2009; Brodribb et al., 2010). $K_{\text{leaf}}$ describes the flow of water through leaves, from the point of entry in the petiole to the sites of evaporation at a given water potential difference ($\Delta \Psi_{\text{leaf}}$), while $P50_{\text{leaf}}$ describes the ability of plants to maintain $K_{\text{leaf}}$ under conditions of water stress. However, our capacity to comprehensively explore the implications of how these traits influence whole-plant function and are coordinated with leaf structure remains limited because the techniques used to assess these two hydraulic traits are often incompatible.

The rehydration kinetics of $\Psi_{\text{leaf}}$ for partially desiccated leaves is a technique that has long been used to determine water movement in leaves (Weatherly 1963; Boyer 1974). This technique has more recently been used to measure leaf hydraulic vulnerability by assessing the percentage loss of $K_{\text{leaf}}$ from maximum values as water potentials decline (Brodribb & Holbrook 2003). However, this method remains untested against standard methods for calculating absolute $K_{\text{leaf}}$ that involve pushing, evaporating, and pulling water out of the leaf (Tyree et al., 1993; Kolb et al., 1996; Sack et al., 2002; Brodribb & Holbrook 2006). These standard methods have been shown to produce similar $K_{\text{leaf}}$ measurements for a particular species (Sack et al., 2002), and all determine $K_{\text{leaf}}$ directly as measured flow rate ($F_{\text{leaf}}$) divided by the driving force for flow ($\Delta \Psi_{\text{leaf}}$). In contrast, the rehydration kinetics technique estimates $K_{\text{leaf}}$ by assuming leaf rehydration is equivalent to the charging of a capacitor through a resistor (Brodribb & Holbrook 2003), a formulation that requires knowledge of leaf water storage capacitance ($C_{\text{leaf}}$).

Leaf water storage capacitance has been correlated with leaf hydraulic conductance (Sack et al., 2003; Hao et al., 2008) and it is thought that increased capacitance may be involved in desiccation avoidance (Lamont & Lamont 2000) and/or buffering against rapid water potential fluctuations (Sack & Tyree 2005). $C_{\text{leaf}}$ is typically determined from the initial slope of the relationship between $\Psi_{\text{leaf}}$ and water volume in excised leaves. Because this pressure-volume relationship is determined from leaf samples that
are slowly desiccated using the bench drying technique (Tyree & Hammel 1972), it is assumed that the pressure-volume properties of the different cell types within the leaf are equilibrated and that the calculation of $C_{\text{leaf}}$ represents bulk-leaf capacitance. However, the kinetics of leaf rehydration suggests that leaves should not be treated as a single capacitor or pool of water (Tyree et al., 1981; Zwieniecki et al., 2007). Indeed, recent work suggests that the biphasic rehydration kinetics of leaves in a large number of plant species reflects the presence of two spatially/anatomically distinct regions within the leaf, only one of which is hydraulically well connected to the transpiration stream (Zwieniecki et al., 2007). This implies that the bulk-leaf capacitance determined by pressure-volume analysis may not be appropriate for the calculation of $K_{\text{leaf}}$ from leaf rehydration kinetics. Rather, a more specific measure of the capacitance of the leaf tissues tightly connected to the transpiration stream is required.

In this study we compare two measures of leaf capacitance assumed to represent bulk-leaf capacitance ($C_{\text{bulk}}$) and the capacitance of the leaf tissues that are hydraulically well connected to the transpiration stream ($C_{\text{transp}}$), respectively, in relation to differences in anatomy and leaf structure across a diverse group of cool temperate woody angiosperms. We also examine whether these different measures of capacitance influence the determination of absolute $K_{\text{leaf}}$ using the rehydration kinetics of $\Psi_{\text{leaf}}$ relaxation in relation to more standard methods for determining $K_{\text{leaf}}$. Specifically, we aim to test whether the rehydration-kinetics technique in conjunction with the capacitance of the transpiration stream can accurately determine absolute $K_{\text{leaf}}$. Finally, we test for leaf structural and functional trait associations with maximum $K_{\text{leaf}}$ in order to further enhance our understanding of this key hydraulic property in relation to hydraulic design and plant function.

MATERIALS AND METHODS

Plant species and material

We sampled 20 phylogenetically disparate cool temperate woody angiosperm species from montane rainforest (15 species) and dry sclerophyll forest (five species) in Tasmania, Australia (Table 5.1). These species exhibited a variety of leaf morphologies, ranging in their degree of scleromorphy (as reflected by leaf mass per unit area) from 137 g m$^{-2}$ in the relatively broad leaves of the rainforest species *Atherosperma moschatum* to 772 g m$^{-2}$ in the extremely scleromorphic needles of *Hakea lissosperma*. Included in the species group were the winter deciduous species *Nothofagus gunnii* and the vessel-less species *Tasmannia lanceolata*. The species also varied in their ecological
tolerance in terms of minimum water availability (as reflected by the 5th percentile of mean annual rainfall across each species distribution), ranging from 351 mm per year for the dry forest species *Bursaria spinosa* to 1268 mm per year for the montane rainforest species *Orites diversifolius* (CJ Blackman *et al.*, unpublished). Across species, these climatic limits have been shown to correspond to interspecific variation in leaf xylem vulnerability to drought-induced hydraulic dysfunction (CJ Blackman *et al.*, unpublished). Branch and leaf material were sampled during the summer months (between December and February) from the sun-exposed part of the plant of each of five individuals per species.

**Table 5.1** Compiled data of maximum leaf hydraulic conductance calculated using a modified rehydration technique ($K_{\text{dyn}}$, see materials and methods), and maximum assimilation ($A_{\text{max}}$) across all species in the study.

<table>
<thead>
<tr>
<th>Family</th>
<th>Species</th>
<th>Code</th>
<th>$K_{\text{dyn}}$ (mmol m$^{-2}$ s$^{-1}$ MPa)</th>
<th>$A_{\text{max}}$ (µmol m$^{-2}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>montane rainforest</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asteraceae</td>
<td><em>Oleraria pinifolia</em> (Hook.f.) Benth.</td>
<td>Op</td>
<td>2.4 ± 0.3</td>
<td>8.9 ± 1.5</td>
</tr>
<tr>
<td>Atherospermataceae</td>
<td><em>Atherosperma moschatum</em> Labil.</td>
<td>Am</td>
<td>4.8 ± 1.0</td>
<td>7.0 ± 0.2</td>
</tr>
<tr>
<td>Ericaceae</td>
<td><em>Cyathodes straminea</em> R.Br.</td>
<td>Cs</td>
<td>3.9 ± 1.1</td>
<td>4.2 ± 0.9</td>
</tr>
<tr>
<td>Ericaceae</td>
<td><em>Gaultheria hispida</em> R.Br.</td>
<td>Gh</td>
<td>6.3 ± 1.1</td>
<td>11.4 ± 1.5</td>
</tr>
<tr>
<td>Ericaceae</td>
<td><em>Richea scoparia</em> Hook.f.</td>
<td>Rs</td>
<td>2.6 ± 0.2</td>
<td>9.0 ± 0.9</td>
</tr>
<tr>
<td>Myrtaceae</td>
<td><em>Eucalyptus coccifera</em> Hook.f.</td>
<td>Ec</td>
<td>6.1 ± 0.5</td>
<td>15.6 ± 2.5</td>
</tr>
<tr>
<td>Nothofagaceae</td>
<td><em>Nothofagus cunninghamii</em> (Hook.) Oerst.</td>
<td>Nc</td>
<td>3.7 ± 0.6</td>
<td>6.9 ± 0.4</td>
</tr>
<tr>
<td></td>
<td><em>Nothofagus gunnii</em> (Hook.) Oerst.</td>
<td>Ng</td>
<td>3.9 ± 0.6</td>
<td>8.0 ± 1.6</td>
</tr>
<tr>
<td>Pittosporaceae</td>
<td><em>Pittosporum bicolor</em> Hook.</td>
<td>Pb</td>
<td>4.9 ± 1.1</td>
<td>11.5 ± 2.8</td>
</tr>
<tr>
<td>Proteaceae</td>
<td><em>Hakea lissosperma</em> R.Br.</td>
<td>Hi</td>
<td>13.2 ± 2.7</td>
<td>25.5 ± 4.2</td>
</tr>
<tr>
<td>Proteaceae</td>
<td><em>Lomatia polymorpha</em> R.Br.</td>
<td>Lp</td>
<td>4.6 ± 0.7</td>
<td>9.5 ± 1.4</td>
</tr>
<tr>
<td>Proteaceae</td>
<td><em>Orites diversifolius</em> R.Br.</td>
<td>Od</td>
<td>10.6 ± 2.7</td>
<td>8.3 ± 2.0</td>
</tr>
<tr>
<td>Proteaceae</td>
<td><em>Telopea truncata</em> (Labill) R.Br.</td>
<td>Tt</td>
<td>9.8 ± 2.2</td>
<td>10.5 ± 1.4</td>
</tr>
<tr>
<td>Rubiaceae</td>
<td><em>Coprosma nitida</em> Hook.f.</td>
<td>Cn</td>
<td>9.1 ± 1.4</td>
<td>11.3 ± 1.3</td>
</tr>
<tr>
<td>Winteraceae</td>
<td><em>Tasmannia lanceolata</em> (Poir) A.C.Sm.</td>
<td>Tl</td>
<td>6.2 ± 1.0</td>
<td>5.2 ± 0.5</td>
</tr>
<tr>
<td><strong>dry sclerophyll</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asteraceae</td>
<td><em>Olearia hookeri</em> (Sond.) Benth.</td>
<td>Oh</td>
<td>7.9 ± 1.3</td>
<td>14.4 ± 1.8</td>
</tr>
<tr>
<td>Myrtaceae</td>
<td><em>Eucalyptus pulchella</em> Desf.</td>
<td>Ep</td>
<td>7.0 ± 1.3</td>
<td>18.2 ± 0.5</td>
</tr>
<tr>
<td>Pittosporaceae</td>
<td><em>Bursaria spinosa</em> Cav.</td>
<td>Bs</td>
<td>5.0 ± 0.4</td>
<td>9.8 ± 0.6</td>
</tr>
<tr>
<td>Proteaceae</td>
<td><em>Hakea microcarpa</em> R.Br.</td>
<td>Hm</td>
<td>13.5 ± 1.7</td>
<td>20.4 ± 1.2</td>
</tr>
<tr>
<td>Proteaceae</td>
<td><em>Lomatia tinctoria</em> (Labill) R.Br.</td>
<td>Lt</td>
<td>4.0 ± 0.6</td>
<td>9.1 ± 0.8</td>
</tr>
</tbody>
</table>

$C_{\text{bulk}}$ determined by P-V analysis

The relationship between $\Psi_{\text{leaf}}$ and water volume in the leaf (pressure-volume analysis) was quantified by allowing detached leaves to slowly desiccate on the laboratory bench.

85
during which time leaf weight and $\Psi_{\text{leaf}}$ were periodically measured (Tyree & Hammel 1972). $C_{\text{bulk}}$ was calculated in terms of relative water content (RWC) from the initial slope (pre-turgor loss) of the relationship between $\Psi_{\text{leaf}}$ and RWC (Figure 5.1a).

To calculate $K_{\text{leaf}}$ from the kinetics of leaf water potential relaxation ($K_{\text{leaf}, \Psi_{\text{relax}}} \text{ mmol m}^{-2} \text{s}^{-1} \text{MPa}$), leaf capacitance as determined from the initial slope of the pressure-volume relationship ($\Delta \text{RWC/} \Delta \Psi_{\text{leaf}}$) was expressed in absolute terms and normalised by leaf area. To do this, the values of leaf dry weight per unit area and the saturated mass of water per unit dry weight were determined for each species and used to calculate leaf area normalised absolute capacitance:

$$C_{\text{bulk}} = \Delta \text{RWC/} \Delta \Psi_{\text{leaf}} \cdot (\text{DW/LA}) \cdot (\text{WW/DW})/M \quad (5.1)$$

where DW = leaf dry weight (g); LA = leaf area (m²); WW = saturated mass of leaf water at 100% RWC (g); M = molar mass of water (g mol⁻¹).

**Figure 5.1** Illustration of the two measures of leaf capacitance for a typical woody angiosperm (a), $C_{\text{bulk}}$ and (b), $C_{\text{transp}}$. $C_{\text{bulk}}$ was calculated from the initial slope of the pressure-volume relationship between $\Psi_{\text{leaf}}$ and RWC (vertical dashed line represents the turgor loss point) expressed in absolute terms and normalised by leaf area (see equation 5.1). $C_{\text{transp}}$ was calculated as the volume of water (indicated by the shaded area beneath the data points) taken up by a rehydrating leaf connected to a flowmeter during a transition from $\Psi_o$ to $\Psi_f$ (see equation 5.2). Initial maximum flow ($F$) in these rehydration plots was determined by fitting an exponential curve through the data points and extrapolating back to the initial point of leaf excision.

**$C_{\text{transp}}$ determined by bulk flow**

Leaf capacitance was also measured directly for each species by calculating the bulk volume of water absorbed by a partially desiccated leaf or shoot while connected to a flowmeter (see methods for measuring $K_{\text{ss}}$ below). Here, leaf capacitance was
calculated as the volume of water taken up by the leaf during a transition from $\Psi_o$ to $\Psi_f$ (see Figure 5.1b):

$$C_{\text{transp}} = \Sigma F / (\Psi_o - \Psi_f) \quad (5.2)$$

where $\Sigma F =$ sum of the flow of water into the leaf during rehydration adjusted for leaf area (mmol m$^{-2}$) and temperature following Brodribb & Holbrook (2006); $\Psi_o =$ initial leaf water potential (MPa); $\Psi_f =$ final leaf water potential (MPa).

Dynamic vs steady state measures of leaf hydraulic conductance

$K_{\text{leaf}}$ was measured for each species using a modified rehydration technique under non-steady-state conditions whereby leaves were allowed to rehydrate while connected to a flowmeter ($K_{\text{dyn}}$, Brodribb & Cochard 2009). Due to small leaf size and/or reduced petiole length, plant shoots from the most recent fully expanded growth-cohort were used for each species. Branches were collected during the morning and immediately bagged to induce stomatal closure. Prior to rehydrating the sample shoot, the leaf water potential ($\Psi_{\text{leaf}}$) was determined from two neighbouring shoots or leaves from the same branch. Branches were discarded if initial $\Psi_{\text{leaf}}$ exceeded species-specific values that corresponded to reduced leaf hydraulic conductance (Blackman, 2010 #1634). The sample shoot was excised underwater and immediately connected to a flowmeter where it remained under moist paper towel (to prevent transpiration) at 20°C with flow rate logged every 5 s for 60 s or until flow rate decayed by half from maximum as shoots rehydrated. Subsequently, the shoot was disconnected and immediately wrapped in moist paper towel and transferred to a Scholander pressure chamber (PMS, Albany, OR, USA) for determination of final $\Psi_{\text{leaf}}$. $K_{\text{dyn}}$ was calculated at the two instantaneous points corresponding to the initial and final $\Psi_{\text{leaf}}$ using equation 5.3:

$$K_{\text{dyn}} = - \frac{I}{A \cdot \Psi_{\text{leaf}}} \quad (5.3)$$

where $I =$ instantaneous flow rate into the leaf (mmol s$^{-1}$); $A =$ projected leaf area. Initial and final $K_{\text{leaf}}$ values did not vary significantly and hence were combined to produce a mean maximum leaf hydraulic conductance for each species.

In order to test whether maximum rates of leaf hydraulic conductance were consistent under non-steady-state and steady-state conditions, we measured $K_{\text{ss}}$ for a subset of six species using the evapotranspiration method by allowing a shoot to reach steady-state conditions while connected to a flowmeter measuring the transpirational flux (Sack et al., 2002; Brodribb & Holbrook 2006). Shoots were excised under water between 0900
h and 1200 h and immediately connected to a flow meter under full-sun conditions (1500 µmol quanta m\(^{-2}\) s\(^{-1}\)). During the measurement, shoot temperature was maintained between 20 and 24°C by directing a stream of heated air uniformly across the shoot sample. This temperature range was monitored by two thermocouples pressed against the abaxial surface of two leaves. After 3-5 minutes at steady-state, the shoot was removed, wrapped in moist paper towel and water potential measured using a pressure chamber. The leaf hydraulic conductance was calculated as the ratio of transpiration flux to leaf water potential, and was both standardised to the viscosity of water at 20°C and adjusted for leaf area. A minimum of five shoots per species were measured and the maximum hydraulic conductance under steady-state conditions calculated as the y-intercept of a plot of water potential verses hydraulic conductance in the range of 0 – 1 MPa (Brodribb & Holbrook 2006).

\(K_{\text{leaf}}\) determined by the rehydration kinetics of \(\Psi_{\text{leaf}}\)

Leaf hydraulic conductance was also determined by assessing the kinetics of \(\Psi_{\text{leaf}}\) relaxation upon leaf rehydration (\(K_{\text{relax}}\); Brodribb & Holbrook 2003)). This method has previously been applied to the current species sample where it was used to construct leaf vulnerability curves (Blackman, 2010 #1634), although the \(K_{\text{relax}}\) data for the deciduous species *Nothofagus gunnii* was omitted from the current study due to differences in the seasonal timing of plant sampling that affected leaf function. Essentially it calculates \(K_{\text{leaf}}\) from the rate of \(\Psi_{\text{leaf}}\) relaxation in leaves excised from the stem underwater over a predetermined period of time. Importantly, this calculation requires knowledge of leaf capacitance so that:

\[
K_{\text{relax}} = C_{\text{leaf}} \ln[\Psi_o/\Psi_f]/T
\]

where \(\Psi_o\) = initial leaf water potential (MPa); \(\Psi_f\) = final leaf water potential (MPa); \(T\) = duration of rehydration (s); \(C_{\text{leaf}}\) = leaf capacitance (mmol m\(^{-2}\) MPa\(^{-1}\)).

Leaf structural traits

Lignin content was determined by the Klason procedure (Hatfield & Fukushima 2005) involving 1 g of dried and milled leaf samples sealed within filter bags (ANKOM Technology; Macedon, NY, USA) and suspended in 72% H\(_2\)SO\(_4\) acid for 3 h at room temperature. Prior to lignin determination, samples were pre-washed at 100±0.05°C for 1 hr in each of Neutral Detergent Solution and Acid Detergent Solution using a digestion instrument (ANKOM Technology), in order to remove materials insoluble in the acid solution.
Leaf mass per unit area was calculated for each of the current species as described by previous work (Blackman et al., In press). The saturated mass of water per unit dry weight (g) was calculated for each species by calculating the difference between wet weight and dry weight, divided by dry weight.

**Assimilation**

$A_{\text{max}}$ was measured on three healthy leaves from each of five plants per species using a portable gas analyser (Li-COR 6400; Li-Cor, Lincoln, NE, USA). All species were measured on plants in the field under conditions of both high soil water availability and high light intensity. During all measurements conditions within the cuvette were maintained such that they approximated ambient temperature, vapour-pressure difference, and CO$_2$. The resultant temperature remained at between 20 and 28°C, vapour-pressure difference across leaves was 1- 2 kPa, while CO$_2$ ranged from 375-390 µmol mol$^{-1}$. All measurements were made between 0900 h and 1100 h when CO$_2$ uptake was maximal, with a light intensity of 1500 µmol quanta m$^{-2}$ s$^{-1}$. Where species had small leaves, portions of plant shoots containing a number of leaves were measured. For other species $A_{\text{max}}$ was measured on leaves removed from the plant and immediately secured in the gas analyser cuvette. In all cases, $A_{\text{max}}$ was adjusted for leaf area.

**Vein density**

Vein density (VD) was quantified by measuring the total length of veins (mm) within an area (mm$^2$) of the leaf lamina. For species with flattened leaves, VD was measured from paradermal sections. To do this, windows were cut into the epidermis by a razor, and then cleared in 1 M KOH. After clearing, the sections were placed in toluidine blue to highlight the lignified xylem in the veins. Three images of vein architecture at either 10 or 20 x magnification were captured from each of five leaves (five individuals) per species using a digital camera attached to a light microscope. VD was measured using image analysis software (Image J, National Institutes of Health, Bethesda, MD, USA). For species with terete or highly revolute leaves, and/or with parallel venation and highly lignified bundle sheath extensions, VD was measured by calculating the ratio of leaf width to total vein number in cross-section.

**Trait correlations**

Linear regression analysis was used to test for key inter-specific trait correlations (Sigmaplot; SPSS Inc., Chicago, IL, USA). Log transformations were made to data that
showed skewed distributions. Regressions or differences were considered to be
significant if \( P \leq 0.05 \).

RESULTS

Leaf capacitances

Species-specific leaf capacitance values determined by pressure volume analysis (\( C_{bulk} \)) were higher than the bulk flow of water into the leaf during rehydration (\( C_{transp} \)) in all but one of the species studied, and these differences were significant for most of the species (Figure 5.2). These differences were most apparent in species such as *Olearia pinifolia* where \( C_{bulk} (1510 \pm 236 \text{ mmol m}^{-2} \text{ MPa}^{-1}) \) was nearly five times higher than \( C_{transp} (315 \pm 11 \text{ mmol m}^{-2} \text{ MPa}^{-1}) \), and the two *Hakea spp.* where values of \( C_{bulk} \) were about twice those of \( C_{transp} \). However, \( C_{leaf} \) values in some species were not significantly different (e.g. *Eucalyptus pulchella*, \( C_{bulk} 647 \pm 105 \text{ mmol m}^{-2} \text{ MPa}^{-1}; C_{transp} 560 \pm 82 \text{ mmol m}^{-2} \text{ MPa}^{-1} \)) (Figure 5.2).

![Figure 5.2 A plot illustrating the degree of similarity between the two measures of leaf capacitance for each of the species in the current study, \( n = 4-5 \) (open circles represent differences with a statistical significance of \( P \leq 0.01 \); grey circles represent differences with a statistical significance of \( P \leq 0.05 \); black circles represent no statistical difference). Species codes are detailed in Table 5.1. The dashed line represents a 1:1 relationship.](image)

Across species, \( C_{bulk} \) was correlated with leaf structural traits such as leaf mass per unit area (LMA; Figure 5.3a), while \( C_{transp} \) was negatively correlated with leaf lignin content.
(%; Figure 5.3b) and positively correlated with water-flux traits such as the ratio of the saturated mass of water in the leaf (g) per unit leaf dry weight (Figure 5.3c). The species in which the differences in leaf capacitance values were large tended to have leaves with distinct regions of lignified tissue located outside of the venation system (e.g. *Olearia pinifolia* and *Hakea lissosperma*; Figure 5.4a,b), while the species that recorded similar leaf capacitance values tended to have leaves with small quantities of lignin and minimal differentiation between spongy and palisade mesophyll across the leaf (e.g. *Eucalyptus pulchella*; Figure 5.4c).

![Figure 5.3 Plots showing the significant relationships between a, the log of $C_{\text{bulk}}$ and the log of leaf mass per unit area (LMA) and b, the log of $C_{\text{transp}}$ and the log of the saturated mass of water per unit dry weight. Levels of significance are shown (*, $P \leq 0.05 > 0.01$; **, $P \leq 0.01$).](image)
Figure 5.4 Images of leaf cross-sections for three woody angiosperms illustrating different leaf anatomies that influence the two measures of $C_{\text{leaf}}$. The two measures of $C_{\text{leaf}}$ differed significantly in species such as *Olearia pinifolia* and *Hakea lissosperma* that tended to contain a substantial proportion of lignified tissue (stained blue). The anatomy of *O. pinifolia* (image a) was characterised by a heavily lignified endodermis, while *H. lissosperma* (image b) was characterised by heavily lignified core-tissue, as well as an abundance of lignified sclereids embedded in the mesophyll. In contrast, the two measures of $C_{\text{leaf}}$ were similar in species that had minimal amounts of leaf lignin outside of the veins and showed little anatomical segregation between spongy and palisade tissues within the mesophyll (e.g. *Eucalyptus pulchella*; image c). Scale bars are 100 microns.

$K_{\text{leaf}}$ and the effect of leaf capacitance

Leaf hydraulic conductance determined during non steady-state flow ($K_{\text{dyn}}$) varied widely among the current species sample ranging from a minimum of 2.4±0.3 mmol m$^{-2}$ s$^{-1}$ MPa$^{-1}$ in the sub-alpine species *Olearia pinifolia* to a maximum rate of 13.5±1.7 mmol m$^{-2}$ s$^{-1}$ MPa$^{-1}$ in the dry forest species *Hakea microcarpa* (Table 5.1). A highly significant linear relationship was observed between leaf hydraulic conductance values measured on a sub-sample of species under both non steady-state ($K_{\text{dyn}}$) and steady-state ($K_{\text{ss}}$) conditions (Figure 5.5a). The slope of this relationship was not significantly different to a 1:1 correspondence.
Figure 5.5 Comparisons between techniques (a); the relationship between $K_{\text{dyn}}$ measured in a sub-sample of species using the modified rehydration technique (Brodribb and Holbrook 2009) and $K_{\text{leaf,ss}}$ measured using the evaporative flux technique under steady-state conditions (Sack et al 2002; Brodribb and Holbrook 2006). Species codes are detailed in Table 5.1. (b); the relationship between the two measures of leaf capacitance and maximum leaf hydraulic conductance across all species. Open circles represent the log of $C_{\text{bulk}}$; black circles represent the log of $C_{\text{transp}}$. (c); the strong and significant relationship between maximum leaf hydraulic conductance ($K_{\text{dyn}}$) and $K_{\text{relax}}$ measured using the rehydration kinetics of $\Psi_{\text{leaf}}$ relaxation (Brodribb and Holbrook 2003) in conjunction with $C_{\text{transp}}$ across all species. Levels of significance for all relationships are shown (ns, not significant; ** $P \leq 0.01 > 0.001$; *** $P \leq 0.001$).
Across species, only $C_{\text{transp}}$ was significantly correlated with maximum leaf hydraulic conductance (Figure 5.5b). Further to this, an almost 1:1 relationship ($r^2 = 0.85$) was shown between $K_{\text{dyn}}$ and $K_{\text{relax}}$ when $C_{\text{transp}}$ was used in equation 5.4 (Figure 5.5c). The more standard method of using $C_{\text{bulk}}$ in this equation yielded poor correspondence between $K_{\text{relax}}$ and either $K_{\text{dyn}}$ or $K_{\text{ss}}$ (data not shown).

**Coordination of $K_{\text{leaf}}$ with other leaf traits**

Maximum rates of assimilation varied among species ranging from $4.2 \pm 0.9 \mu\text{mol m}^{-2}\text{s}^{-1}$ in the species *Cyathodes straminea* to $25.5 \pm 4.2 \mu\text{mol m}^{-2}\text{s}^{-1}$ in the species *Hakea lissosperma* (Table 5.1). Across species, significant positive relationships were found between $K_{\text{dyn}}$ and both maximum rates of assimilation (Figure 5.6a) and vein density (Figure 5.6b).

![Figure 5.6](image)

**Figure 5.6** Plots of the relationship between maximum leaf hydraulic conductance and maximum assimilation (a) and vein density (b) across all species. Significance levels are shown (**, $P \leq 0.01 > 0.001$; ***, $P \leq 0.001$).**

**DISCUSSION**

Leaf capacitance is an important parameter in leaves that defines the dynamics of how leaves respond to fluctuations in transpiration rate or upstream water potential. We
found that two measures of leaf capacitance detailed in the current study produced very different values, probably because they measure the capacitance of different tissue regions within the leaf. Both capacitances are functionally informative and related to different aspects of leaf construction, but we found that only one capacitance can be used to calculate leaf hydraulic efficiency.

Variation in the two measures of $C_{\text{leaf}}$

Our results show that $C_{\text{leaf}}$ determined from pressure-volume analysis where leaf samples are slowly dried on the laboratory bench was relatively large because all leaf tissues contribute to the capacitance measurement regardless of the strength of their connection to the transpiration stream. Hence $C_{\text{bulk}}$ is assumed to reflect bulk leaf capacitance. In contrast, $C_{\text{transp}}$ determines the leaf capacitance in response to short-term dynamic changes in leaf hydration and thus is likely to reflect the capacitance of tissues closely connected to the transpiration stream. Recent work has shown that differences in leaf anatomy can influence the hydraulic supply in leaves. Zwieniecki et al., (2007), for example, showed that the bi-phasic rehydration kinetics of leaf water potential in many species reflects the rehydration of two anatomically distinct (hydraulically disjunct) regions within the leaf. These authors suggest that the leaves of such species are hydraulically compartmentalised and that only a portion of the leaf tissue is well connected to the transpiration stream. The data here support this interpretation, suggesting that divergence between different measures of leaf capacitance occurs due to this influence of hydraulic compartmentalisation.

Across species, $C_{\text{transp}}$ was independently and negatively correlated with lignin content (Fig 5.3b). This relationship suggests that lignified tissues may be hydraulically isolated from other tissues within the leaf and furthermore reduce the volume of water that readily exchanges with the transpiration stream. The potential effect of these differences in leaf anatomy is also reflected by anecdotal evidence that the two measures of $C_{\text{leaf}}$ were significantly different in species with leaves that contained distinct regions of lignified tissue outside of the venation system and/or had a high proportion of water conducting sclereids embedded in the mesophyll (see Figure 5.4a,b). Also, in hypostomatic leaves with well-developed palisade, it has been suggested that the transpiration stream largely bypasses the photosynthetic palisade mesophyll (Zwieniecki et al., 2007). However in species where $C_{\text{bulk}}$ and $C_{\text{transp}}$ are similar we conclude that most of the water in the leaf exchanges with the transpiration stream over the period of seconds to minutes. These species were characterized by
amphistomatic leaves that showed little anatomical differentiation within the mesophyll (e.g. *Eucalyptus pulchella*; Figure 5.4c).

In addition to being linked to hydraulic compartmentalisation, our results show that the two measures of $C_{\text{leaf}}$ are coordinated with other leaf structural properties. Across species, $C_{\text{bulk}}$ was independently correlated with leaf mass per unit area (LMA). This relationship is consistent with previous findings that bulk leaf capacitance is coordinated with leaf thickness (Sack *et al.*, 2003) on the basis that leaf thickness is an important component of LMA (Niinemets 1999). It is also consistent with evidence that LMA is directly affected by leaf water content (Shipley 1995), implying that $C_{\text{bulk}}$ may reflect the saturated mass of water per unit dry weight in leaves. However, across our species sample, $C_{\text{bulk}}$ was not correlated with leaf water content per unit dry weight (data not shown), which instead was significantly and positively correlated with $C_{\text{transp}}$. This relationship between leaf water content per unit dry weight and $C_{\text{transp}}$ suggests that dense tissues such as heavily lignified cell walls effectively displace space occupied by mesophyll water which, unlike sclerified tissue, exchanges readily with the transpiration stream. Furthermore, because leaf water content per unit area is largely a function of leaf thickness (Shipley 1995; Vendramini *et al.*, 2002), it also suggests that leaf water content per unit dry mass may be a better indicator of the leaf water contained within leaf tissues that actively exchange with the transpiration stream.

The possible physiological consequences of the relationship between leaf water content and $C_{\text{transp}}$ relates to short and long-term buffering of changes in leaf water potential. Leaf water storage capacitance is widely thought to act as a buffer against transient changes in leaf water potential (Sack & Tyree 2005) and has also been associated with leaf desiccation avoidance after the stomata have closed (Lamont & Lamont 2000). Because the water within leaf tissues that actively exchange with the transpiration stream is released over a matter of seconds, it may buffer rapid changes in leaf water potential associated, for example, with fluctuations in transpiration. But it is unlikely to have any long-term benefits to a plant facing increased desiccation. Instead, this role of delaying leaf desiccation may rely on the water storage capacitance of the hydraulically more isolated leaf tissues that release stored water more slowly.

*K_{\text{leaf}} and the effect of different leaf capacitances*

While $K_{\text{leaf}}$ is typically measured directly using techniques that involve pushing, evaporating and pulling water out of the leaf (Tyree *et al.*, 1993; Kolb *et al.*, 1996; Sack *et al.*, 2002; Brodribb & Holbrook 2006), we determined maximum leaf hydraulic
conductance ($K_{\text{dyn}}$) using a modified rehydration technique that measures the rehydration kinetics of leaves connected to a flowmeter (Brodribb & Cochard 2009). Across our species sample, these values of $K_{\text{leaf}}$ are consistent with previous measurements of $K_{\text{leaf}}$ in temperate woody angiosperm species (Sack & Holbrook 2006). Further to this, we observed a significant relationship in a sub-sample of species between $K_{\text{dyn}}$ and leaf hydraulic conductance measured using the evaporative flux method under steady-state conditions ($K_{\text{ss}}$; (Sack et al., 2002; Brodribb & Holbrook 2006)). This consistency in species-specific $K_{\text{leaf}}$ values indicates that the modified rehydration technique used here provides an accurate measure of maximum leaf hydraulic conductance.

Across species values of $K_{\text{dyn}}$ were correlated with $C_{\text{transp}}$ but not $C_{\text{bulk}}$ indicating that the capacitance of the leaf tissues that exchange readily with the transpiration stream is more highly coordinated with leaf hydraulic conductance than bulk leaf capacitance. Furthermore, it adds support to the idea that leaves can be hydraulically compartmentalised and that some of the leaf tissue is hydraulically poorly connected to the transpiration stream. In addition, we measured leaf hydraulic conductance for each of our species using the rehydration kinetics of $\Psi_{\text{leaf}}$ relaxation ($K_{\text{relax}}$). Because this measure of leaf hydraulic conductance assumes leaf rehydration is equivalent to the charging of a capacitor through a resistor (Brodribb & Holbrook 2003), the final calculation of $K_{\text{leaf}}$ depends on using the appropriate leaf capacitance. Across species we found that $K_{\text{relax}}$ values were consistent with $K_{\text{dyn}}$ values only when $C_{\text{transp}}$ was used in conjunction with leaf water potential relaxation kinetics (equation 5.4). This indicates that absolute $K_{\text{leaf}}$ can be accurately determined using the rehydration kinetics of $\Psi_{\text{leaf}}$ relaxation but only in conjunction with the capacitance of leaf tissues that contribute to $K_{\text{leaf}}$. It also implies that previous measurements of absolute $K_{\text{leaf}}$ using the rehydration kinetics of $\Psi_{\text{leaf}}$ relaxation (Tyree & Cheung 1977; Brodribb & Holbrook 2003; Hao et al., 2008; Blackman et al. 2009) may over-estimate $K_{\text{leaf}}$ on the basis that they were calculated using bulk leaf capacitance. Consequently we suggest that any future calculation of absolute $K_{\text{leaf}}$ by this technique should be made using the capacitance of the leaf tissues that are hydraulically well connected to the transpiration stream.

**Coordination of $K_{\text{leaf}}$, gas exchange and vein density**

Across species maximum leaf hydraulic conductance was significantly and positively correlated with maximum rates of assimilation, indicating that gas exchange may be constrained by leaf hydraulic conductance in these species. In addition, the significant and positive relationship between $K_{\text{dyn}}$ and vein density adds to a growing realisation...
that whole-leaf hydraulic efficiency depends on the properties of the leaf venation (Sack & Tyree 2005; Brodribb et al., 2007; Brodribb & Feild 2010). Vein density in particular has recently been identified as a key driving force linked to greater hydraulic efficiency and higher photosynthetic capacity in the evolution of angiosperm species (Brodribb & Feild 2010). Although significant, the relationship between vein density and $K_{\text{leaf}}$ was rather weak, but this result was expected due to an abundance of water-transporting sclereids in many of these species (Brodribb et al., 2010).

Conclusion

Our results indicate that the two measures of leaf capacitance detailed here reflect the capacitance of different portions of the leaf tissue. $C_{\text{transp}}$ was shown to better reflect the capacitance of the leaf-tissues that exchange water readily with the transpiration stream. It was also the capacitance solely related to leaf water content, however, this relationship may be a secondary correlation based on the proportion of mesophyll tissue hydraulically well connected to the transpiration stream. Importantly, we also found that measurements of absolute $K_{\text{leaf}}$ by the rehydration kinetics of $\Psi_{\text{leaf}}$ relaxation, when calculated using $C_{\text{transp}}$ were consistent with those determined by more standard methods. Because the rehydration kinetics technique has already been successfully applied to measurements of leaf hydraulic vulnerability, we propose that it can be applied to studies that examine both $P50_{\text{leaf}}$ and absolute $K_{\text{leaf}}$.

REFERENCES


Chapter 5 – Leaf capacitance and water transport in leaves


Chapter 6

Summary and synthesis

Investigations of the hydraulic properties of leaves have increased considerably in recent years, reflecting an ever-growing realisation that leaf hydraulics plays a central role in defining plant function. These investigations have begun to reveal how leaf hydraulics is coordinated with leaf structure and function (Aasamaa et al., 2001, Brodribb et al., 2005, Sack et al., 2003) and influences plant functional responses to water-stress (Brodribb & Holbrook, 2003, Trifilo et al. 2003). However, large gaps remain in our understanding of leaf hydraulics as an important determinant of plant function and indicator of ecological strategy.

In this dissertation I investigated the leaf hydraulics of a diverse group of woody angiosperm species in order to better understand how key leaf hydraulic traits are integrated with leaf structure and function, and how leaf hydraulics can influence adaptation and ecological strategy in relation to drought. New insights derived from this investigation have important and wide-ranging implications for understanding and assessing plant function, leaf structure and function, ecological strategy, plant adaptation, species distribution patterns, and processes of community assembly. Taken together, I strongly argue that leaf hydraulics are integrated with many aspects of leaf structure and function and are key to understanding the potential impacts of increasing aridity on plant function and community dynamics. I also argue that some of these results may lead to a greater ability to predict the drought tolerance of different plant species, thus improving management practices in both natural and agricultural systems within the context of climate change.

In this thesis, I have demonstrated a series of important aspects of leaf hydraulics:

1) its significance in driving plant functional responses during drought and in subsequent recovery following rewatering (Chapter 2).

2) how variation in leaf hydraulic vulnerability ($P_{50_{leaf}}$) is linked to the structural dimensions of leaf xylem and coordinated with leaf structural and functional traits related to drought resistance (Chapter 3).
3) how variation in $P50_{\text{leaf}}$ is adaptive across broad environmental gradients and informative to our understanding of the patterns and processes of community assembly (Chapter 4).

4) how different measures of leaf capacitance ($C_{\text{leaf}}$) relate to the water transport pathway and influence the determination of maximum hydraulic conductance in leaves ($K_{\text{leaf}}$) (Chapter 5).

These major findings are combined and discussed in the following synthesis aimed at addressing a number of key research questions relating to the functional, adaptive and ecological significance of variation in leaf hydraulic characteristics, especially vulnerability under water-stress. I also consider the potential applications of variation in leaf hydraulic traits related to drought resistance.

**What is the functional significance of leaf hydraulics?**

Leaf hydraulics is now recognised as a fundamental determinant of plant function (Sack & Holbrook, 2006). However, our understanding of the significance of leaf hydraulics in defining plant function and driving differences in species function, particularly in relation to drought, is far from complete. Filling these gaps in our knowledge may be particularly significant given that the frequency and intensity of drought is predicted to increase in many parts of the world as a result of climate change (IPCC, 2007). An important step in this process will be to identify the traits that underlie plant functional responses during drought and in subsequent recovery following rewatering, and relate to variation in plant drought resistance.

The glasshouse drought experiment, detailed in Chapter 2, showed that both the vulnerability of $K_{\text{leaf}}$ to declining $\Psi_{\text{leaf}}$ and the safety margin between stomatal closure and $P50_{\text{leaf}}$ varied among four ecologically disparate woody angiosperm species. This indicated that these species vary in terms of their vulnerability to water-stress-induced hydraulic dysfunction as well as their functional response to increasing drought. However, across a larger group of species, a strong correlation was observed between $P50_{\text{leaf}}$ and turgor loss point (TLP) (Chapter 3). This suggests that, on the basis of a close correspondence between hydraulic dysfunction and turgor loss and stomatal closure (Brodribb & Holbrook, 2003), leaf hydraulic vulnerability has the potential to influence the functional response of these species to short-term water-stress.

In the field, a significant positive relationship was found between $P50_{\text{leaf}}$ and the seasonal minimum water potential ($\Psi_{\text{min}}$; Chapter 3). This result is consistent with
previous studies on hydraulic resistance in stems (Bhaskar et al., 2007, Jacobsen et al., 2007, Pockman & Sperry, 2000) and suggests that species with low $P50_{\text{leaf}}$ are able to tolerate lower leaf water potentials under drought conditions than species with high $P50_{\text{leaf}}$. Also, these species with low $P50_{\text{leaf}}$ tended to have significantly wider safety margins from hydraulic dysfunction ($\Psi_{\text{min}} - P50_{\text{leaf}}$), suggesting that variation in leaf hydraulic vulnerability may influence the range of negative leaf water potentials within which plants can remain functionally active.

Taken together, these results highlight a number of ways in which leaf hydraulics are functionally significant in plants exposed to drought stress. However, the results presented in this thesis also have implications for understanding the processes that underlie plant recovery from drought and relate to species’ absolute drought tolerance. In the glasshouse drought experiment, the recovery of gas exchange following the rewetting of severely drought-stressed plants was strongly correlated with the relatively slow recovery of $K_{\text{leaf}}$ in three of the four species (Chapter 2). This indicates conformity to a hydraulic-stomatal limitation model of gas exchange recovery in these species; although a slight change in the sensitivity of stomata to $\Psi_{\text{leaf}}$ during recovery suggests that the plant hormone ABA may also affect stomatal reopening (Lovisolo et al., 2008). This result of hydraulically mediated plant recovery in woody angiosperms adds weight to a growing body of evidence that points to leaf hydraulics as a key driver of plant functional recovery from drought (Brodribb & Cochard, 2009, Lo Gullo et al., 2003, Resco et al., 2009). However, further work is required in angiosperms to firstly tease out the relative importance of plant hormonal and hydraulic signalling during drought recovery and secondly identify the mechanisms (e.g. cavitation, cell-collapse, and/or turgor loss) that directly relate to water-stress-induced leaf hydraulic dysfunction and delimit the rate of recovery following rewetting.

Unlike in conifers (Brodribb & Cochard, 2009), drought-induced plant death in the four angiosperm species did not occur at water potentials associated with near complete leaf hydraulic dysfunction. This suggests that irrecoverable drought damage in these angiosperm species is associated with massive hydraulic failure in the stem or roots. It also suggests that compared to conifers, angiosperms have a greater capacity to overcome near complete leaf hydraulic dysfunction by either refilling embolised conduits while under negative pressure (Bucci et al., 2003, Nardini et al., 2008) or producing new leaves. However, $P50_{\text{leaf}}$ was significantly correlated with drought survivorship in the four experimental species, indicating that there might be some adaptive link between leaf hydraulic vulnerability and species’ absolute drought.
tolerance in woody angiosperms. Indeed, because drought-induced mortality in young plants is likely to be dominated by hydraulic failure, this adaptive link might be particularly strong in the early stages of plant development.

In addition to identifying the functional significance of leaf hydraulics in relation to drought-stress, the results presented in this thesis indicate that leaf hydraulics is important to other aspects of plant function. In Chapter 5 I showed that different measures of leaf capacitance can reflect the proportion of water within the leaf that readily exchanges with the transpiration stream. This result is functionally significant because it suggests that the hydraulic properties of different leaf tissues can help buffer against short- (Sack & Tyree, 2005) and long-term (Lamont & Lamont, 2000) changes in water potential. In Chapter 5 I also showed that absolute $K_{\text{leaf}}$ was correlated with maximum rates of assimilation, thus indicating the functional coordination between leaf hydraulic conductance and gas exchange in plants (Aasamaa et al., 2001, Brodribb et al., 2005, Sack et al., 2003). I also highlighted the functional significance of leaf hydraulic design in terms of vein density in determining hydraulic efficiency and photosynthetic capacity (Brodribb & Feild, 2010). However, the relationship between vein density and $K_{\text{leaf}}$ was rather weak, which suggests that the abundance of water-transporting sclereids in many of the species can enhance leaf hydraulic systems (Brodribb et al., 2010).

**What is the adaptive significance and evolutionary basis of variation in leaf hydraulic vulnerability?**

While I have provided strong evidence for the importance of leaf hydraulics in determining plant function under drought conditions, the results in this thesis also provide substantive evidence of the adaptive significance of leaf hydraulic vulnerability in relation to water-stress. Across a phylogenetically and ecologically diverse group of cool temperate woody angiosperms, a significant relationship was found between variation in $P_{50,\text{leaf}}$ and each species’ distributional climatic limits in terms of minimum water availability (Chapter 4). In general, the species with low $P_{50,\text{leaf}}$ exhibited distributions that extended into drier regions, while the species with high $P_{50,\text{leaf}}$ were geographically restricted to areas of high rainfall. The adaptive significance of $P_{50,\text{leaf}}$ was further supported using species pairs from wet and dry forest environments. Across these pairings, the wet forest species were consistently more vulnerable to water-stress-induced leaf hydraulic dysfunction. This was despite their respective generic ecological affinity based in both wet and dry forests, implying that the evolution of $P_{50,\text{leaf}}$ is bi-directional, with the evolution of both increased drought resistant leaves
under drying climate and reduced drought resistance in response to more moist conditions.

Overall, the consistency of these results suggests that xylem resistance to hydraulic failure is subject to strong selection across the rainfall spectrum (Brodribb & Hill, 1999, Maherali et al., 2004, Pockman & Sperry, 2000). This selection pressure may be particularly strong in leaves considering that leaves are often more sensitive to hydraulic dysfunction than other plant organs (Brodribb et al., 2003, Choat et al., 2005, Hao et al., 2008). As a result, I propose that $P50_{\text{leaf}}$ be used as a fundamental indicator of plant drought resistance, especially in evergreen species, and incorporated into studies aimed at understanding land-plant ecology. To date, these studies have focused on relatively easily-won functional traits such as leaf mass per unit area (LMA) and wood density. While LMA and wood density change systematically along broad environmental gradients (Chave et al., 2009, Reich et al., 1999) and have been related to increased drought survival (Niinemets, 2001) and increased cavitation resistance in stems (Hacke et al., 2001), neither was significantly correlated with $P50_{\text{leaf}}$ across the species studied here (Chapter 3). Nor were they correlated with the bioclimatic limits of each species distribution in terms of minimum water availability (Chapter 3).

Following this, care needs to be taken when using functionally correlative traits as indicators of plant adaptation to increasing aridity.

As shown in stems, the evolutionary trade-off associated with drought-resistance in leaves might come at the cost of extra investment in xylem reinforcement and reduced growth rate (Hacke et al., 2001, Poorter et al., 2010). This concept is reinforced by the strong and significant relationship shown between $P50_{\text{leaf}}$ and the dimensions of the xylem in the leaf minor veins that predict the vulnerability of conduits to water-stress-induced collapse ($t/b^3$; Chapter 3). While this relationship does not necessarily indicate a direct link between reduced leaf hydraulic conductance and conduit collapse in these woody angiosperms, hydraulic dysfunction in these species may be linked to xylem cavitation. This suggests that the strong relationship between $P50_{\text{leaf}}$ and $t/b^3$ may be due to an evolved coordination between xylem structural strength and leaf hydraulic vulnerability that provides a safety factor from conduit collapse. Because the cost of structurally reinforced leaf xylem is likely to be high, maximum carbon-economy in terms of vein synthesis will be achieved if this safety margin between xylem cavitation and collapse is minimal. Indeed, preliminary evidence indicates that the $t/b^3$ dimensionality of xylem conduits is constant throughout higher-order veins. This suggests that the relationship between xylem structure and hydraulic vulnerability in
leaves may have significant implications to both leaf carbon-economy and the potential trade-offs between plant drought resistance and growth.

How does leaf hydraulics relate to the functional composition of plant communities under current and future climates?

Because the results presented in this thesis have highlighted the significance of leaf hydraulic vulnerability in defining plant function and adaptation in relation to water-stress, they also have important implications for understanding the processes related to the functional composition of plant communities under current and future climates. In Chapter 4 I argued that the functional composition of plant communities is influenced by a combination of current climate, long-term climate variability and/or parochial historical constraints. Specifically, I found that the range of $P50_{\text{leaf}}$ values among species occurring in a Peruvian montane rainforest was narrow, indicating that there is little evolutionary amplitude for variation in adaptive traits related to drought resistance in high-rainfall communities characterised by high species diversity and a relatively stable climatic history. In contrast, the range of $P50_{\text{leaf}}$ values was significantly broader in a high-rainfall Tasmanian montane rainforest characterised by lower species diversity, greater long-term rainfall variability, and a recent history of widespread extinction of the rainforest flora. Because both communities occur in high-rainfall sites, the greater variability in $P50_{\text{leaf}}$ shown among the Tasmanian montane rainforest species suggests that processes in addition to those related to current climate can influence the distribution of adaptive traits within modern-day plant communities. Indeed, while the diversity of functional traits related to drought resistance might reflect spatial heterogeneity and niche differentiation associated with long-term climate variability and episodic drought, I also argue that the functional composition of Tasmanian montane rainforest is influenced by the unique history of its regional flora (Ricklefs, 2008).

The principal implication of this analysis of within-site trait distributions of $P50_{\text{leaf}}$ is that modern-day plant communities with different ranges of drought related trait variation will be disproportionately impacted by climate change associated with increasing aridity. Specifically, I suggest that tropical forests with a narrow range of drought related trait variation will be more sensitive to an increase in drought frequency and intensity than mid-high latitude plant communities that retain strong ecological signals of long-term rainfall variability and/or historical drought-induced extinctions. However, despite these potential differences in community-specific susceptibility to increased drought, drier climate conditions and prolonged drought
will likely cause fundamental changes in the functional composition across different plant communities. Because variation in $P_{50_{\text{leaf}}}$ was shown to define species' climatic limits in terms of minimum water availability and correspond to drought survivorship in seedlings, the functional composition of these communities under drier conditions will surely be influenced by leaf hydraulic traits that delimit each species' recruitment niche and confer increased drought resistance and survival.

Are leaf hydraulic traits useful indicators of drought resistance?

The results presented in this thesis strongly indicate that leaf hydraulic vulnerability represents a direct measure of drought resistance in cool temperate woody angiosperm species. Variation in $P_{50_{\text{leaf}}}$ was shown to influence drought survivorship in seedlings and directly reflect plant adaptation to drought by defining the climatic limits across each species distribution in terms of minimum rainfall. Therefore, leaf hydraulic vulnerability has the potential to be used as an indicator of the capacity of plants to establish and function under varying degrees of drought-stress. Thus, greater knowledge of species' leaf hydraulic constraints will be highly informative to understanding the potential impacts of increasing aridity on plant function in both natural and artificial systems.

In addition, the strong relationship between $t/b^3$ and $P_{50_{\text{leaf}}}$ suggests that the structural analysis of leaf xylem could potentially provide a relatively quick and easy estimate of species' drought resistance. This result is highly significant because our understanding of the potential impacts of drought on plant function and community dynamics is currently limited by our ability to rapidly measure drought-adapted functional traits such as $P_{50_{\text{leaf}}}$ across different plant groups. Furthermore, it implies that the dimensions of leaf xylem structure will allow us to trace the evolution of drought resistance in the fossil record and thus develop a picture of the diversification and radiation of different plant groups in response to increasing aridity.

Conclusion

Throughout this thesis I have demonstrated that by studying the hydraulic properties of leaves we can begin to understand the very nature of leaf structure and function and the processes that drive differences in species ecological strategy. The ability of plants to effectively move water through leaves not only reflects leaf hydraulic design, but constrains plant function and defines whole-plant responses both during drought and in subsequent recovery following rewatering. Furthermore, the ability of plants to resist water-stress-induced leaf hydraulic dysfunction is influenced by the structural
properties of the leaf xylem and drives differences in species drought tolerance. Further investigations of these hydraulic traits across different plant groups will enhance our understanding of the evolution of leaves and enable us to better predict the potential impacts of climate change on plant function and community dynamics.

REFERENCES


