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Water stress vulnerability of four *Banksia* species in contrasting ecohydrological habitats on the Gnangara Mound, Western Australia.

By

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A thesis submitted in partial fulfilment of the requirements for the awards of Bachelor of Science (Environmental Management) Honours

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ABSTRACT

The distribution of obligate and facultative phreatophytic vegetation reflects the gradient of ecohydrological habitats in a landscape. Preliminary investigations of *Banksia* vulnerability to xylem embolism have reported that obligate phreatophytes are more susceptible to water stress than facultative phreatophytes (Froend & Drake 2006). A quantitative measure of plant susceptibility to water stress is vital when establishing environmental water requirements. This study investigated interspecific differences in vulnerability to water stress for two facultative phreatophytes (*B. attenuata* and *B. menziesii*) and two obligate phreatophytes (*B. ilicifolia* and *B. littoralis*) at the same position along an ecohydrological gradient on the Gnangara Groundwater Mound, Western Australia. In addition, intraspecific differences to water stress between populations that occupy contrasting ecohydrological habitats were also determined. Plant susceptibility to water stress was established using vulnerability curves, which demonstrate the xylem potentials at which vessels become embolised. Stem-specific and leaf-specific hydraulic conductivity, as well as Huber values (ratio of stem to leaf area), were also determined to support these findings. It was found that ecohydrological habitats are a primary determining factor of plant hydraulic architecture, particularly vulnerability to xylem embolism. At the same ecohydrological habitat, where water is readily accessible there were no interspecific differences in vulnerability to water stress. In contrast, the facultative phreatophytes, *B. attenuata* and *B. menziesii*, appeared to be plastic in vulnerability to embolism in response to developing in a more xeric environment. Both facultative phreatophyte species were found to be more resistant to xylem embolism at the more xeric dune crest site in contrast to the bottom slope site. *B. ilicifolia* did not differ in vulnerability to embolism, supporting its classification as an obligate phreatophyte. This study highlights the importance of understanding site hydrological attributes when determining environmental water allocation for obligate and facultative phreatophytes.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. Introduction

1.1 Phreatophytic Vegetation on an Ecohydrological Gradient

Phreatophytic vegetation is an example of a terrestrial ecosystem that develops a dependence upon groundwater resources (Murray *et al.* 2003). Phreatophytes have been broadly defined as plant species that depend on groundwater to some degree (Busch *et al.* 1992). As a species, an obligate phreatophyte may be defined as dependent upon groundwater, with all individuals of that species being dependent upon access to groundwater (LeMaitre *et al.* 1999; Eamus *et al.* 2006). In contrast, a species that is considered a facultative phreatophyte includes individuals that are opportunistic in their use of groundwater. If there is groundwater readily available it will become an important source for a facultative phreatophyte, however individuals of the same species will also occupy areas where groundwater is not available (Zencich 2003; O'Grady *et al.* 2006). The distribution of obligate and facultative phreatophytes can reflect a gradient of ecohydrological habitats.

Ecohydrology is considered a sub-discipline of ecology and hydrology, which recognises the bi-directional relationship between ecological and hydrologic processes (Zalewski 2002). An example of this is the redistribution of water by plant roots, and the influence this has on the hydrological cycle (Burgess *et al.* 1998; Hannah *et al.* 2004). Another, and the focus of this study, is the influence hydrology has on plant physiology including the hydraulic architecture of plants (Pockman & Sperry 2000; Gries *et al.* 2003). The concept of an ecohydrological landscape encompasses both ecological and hydrological components of a particular environment and how these differ spatially (Klijn & Witte 1999). In an undulating environment it is assumed that there are differences in depth to groundwater and soil moisture, thus there are different ecohydrological habitats. Lower regions in a landscape tend to be 'wetter' than conditions upslope. Vegetation assemblages often reflect this gradient in water availability (Hatton & Evans 1998). Thus, obligate phreatophytes are often found in low-lying areas, whilst facultative phreatophytes are distributed throughout the landscape, irrespective of depth to groundwater (Zencich *et al.* 2002). This demonstrates phreatophytic vegetation distribution at different habitats along an ecohydrological landscape.

It has been observed that the distribution of species across an ecohydrological landscape and differences in water availability is reflected by a plants hydraulic architecture (Gries *et al.* 2003; Maherali *et al.* 2004; Hukin *et al.* 2005). Hydraulic architecture considers the functioning of plants as a hydraulic system (Cruiziat *et al.* 2002). To study this system the hydraulic architecture of plants is divided into a number of components. The most commonly studied are; hydraulic conductivity; specific conductivity; leaf specific conductivity; Huber value (ratio of stem cross-sectional area to leaf area); water storage capacitance and; vulnerability to embolism (Tyree & Ewers 1991). Vulnerability to embolism has become established as a means to determine the water stress vulnerability of different plants (Tyree & Ewers 1991). In an ecohydrological gradient, habitats that are further from groundwater (or at the top end of the gradient) tend to be drier, or more xeric, than habitats at the bottom of the gradient. The plants that occupy these different habitats experience differences in water stress. It may therefore be the case that there are differences in water stress vulnerability between plants that occur in xeric ecohydrological habitats and those that occur in more mesic habitats.

1.2 Plant water availability and xylem embolism

To understand plant response to water stress it is important to have an understanding of xylem cavitation and embolism. Water in plants is in constant flux, as it moves from the soil, through the plant, and to the atmosphere. Water movement is driven by evapotranspiration combined with the cohesive forces of water. The cohesion-tension theory (C-T theory) first suggested by Dixon & Joly (1895) is generally accepted as the best explanation for the ascent of sap through a plant (Sperry *et al.* 2003). Water has strong cohesive forces, meaning that its molecules are strongly attracted to each other. Water also has strong adhesive abilities, 'sticking' to walls of xylem. This allows a continuous column of sap, from the roots of a plant to its leaves. The second component of the C-T theory is tension, which is negative pressure that occurs in xylem due to evaporation. Water moves from high pressures to low pressures and transpiration creates lower pressures in the xylem than that of the surrounding environment. Failures in the water column occur if tensions get too high (ie. if xylem potential gets too low) due to the tensile strength of water (Zimmermann 1983).

As part of his 'air-seeding hypothesis' Zimmermann (1983) proposed that plant cell walls allow the water column to break at particular tensions. These breaks in the water column are referred to as xylem cavitations and are a consequence of both water under tension being in a metastable state and the structure of xylem. The vapour pressure of water at 20°C is -0.0023 MPa, whilst significantly lower xylem potentials are required to move water to the tops trees (Zimmermann 1983). The assumption that water is at significantly negative pressures in xylem is one of the main arguments against C-T theory, due to it having to be in a metastable state (Zimmermann *et al.* 1994; Canny 1995). However, it has also been demonstrated empirically that this can occur (Pockman *et al.* 1995). Water in xylem is thermodynamically unstable, wanting to change from a liquid to a vapour phase. The air seeding hypothesis suggests that the catalyst for this change in phase is the introduction of air into a plant cell from the outside, which 'seeds' cavitation (Zimmermann 1983). Xylem pores can pull air in as xylem potential drops, forming a small bubble (Fig. 1.1). This admission of gas to the vessel seeds the change in state of the xylem sap, resulting in the metastable water changing to gas. The vapour fills the xylem, resulting in a cavitation (L. *cavus*, hollow). Cavitation is a break in the water column, blocking the ascent of sap through the vessel. This obstruction is referred to as an embolism (Gr. e*mbolus*, stopper).

The air-seeding hypothesis is supported by observations that pore sizes and properties differ between species and individuals, which supports the observations that plants cavitate at different xylem potentials. The hydraulic vulnerability curve has become established as a means to compare plant susceptibility to xylem cavitation (Tyree & Ewers 1996). Sperry *et al.* (1988) demonstrated a method to quantify the degree of embolism that has occurred in a section of plant material. Emboli result in a decrease in hydraulic conductance (the inverse of hydraulic resistance), as they block the movement of water through xylem. It was demonstrated that emboli could be removed, by flushing xylem at higher pressures, the increased conductivity therefore being a measure of maximum hydraulic conductance in the absence of emboli (Sperry *et al.* 1988). They also devised a flow-meter that uses low, gravity induced flows to measure the conductance of plant material, without removing emboli.

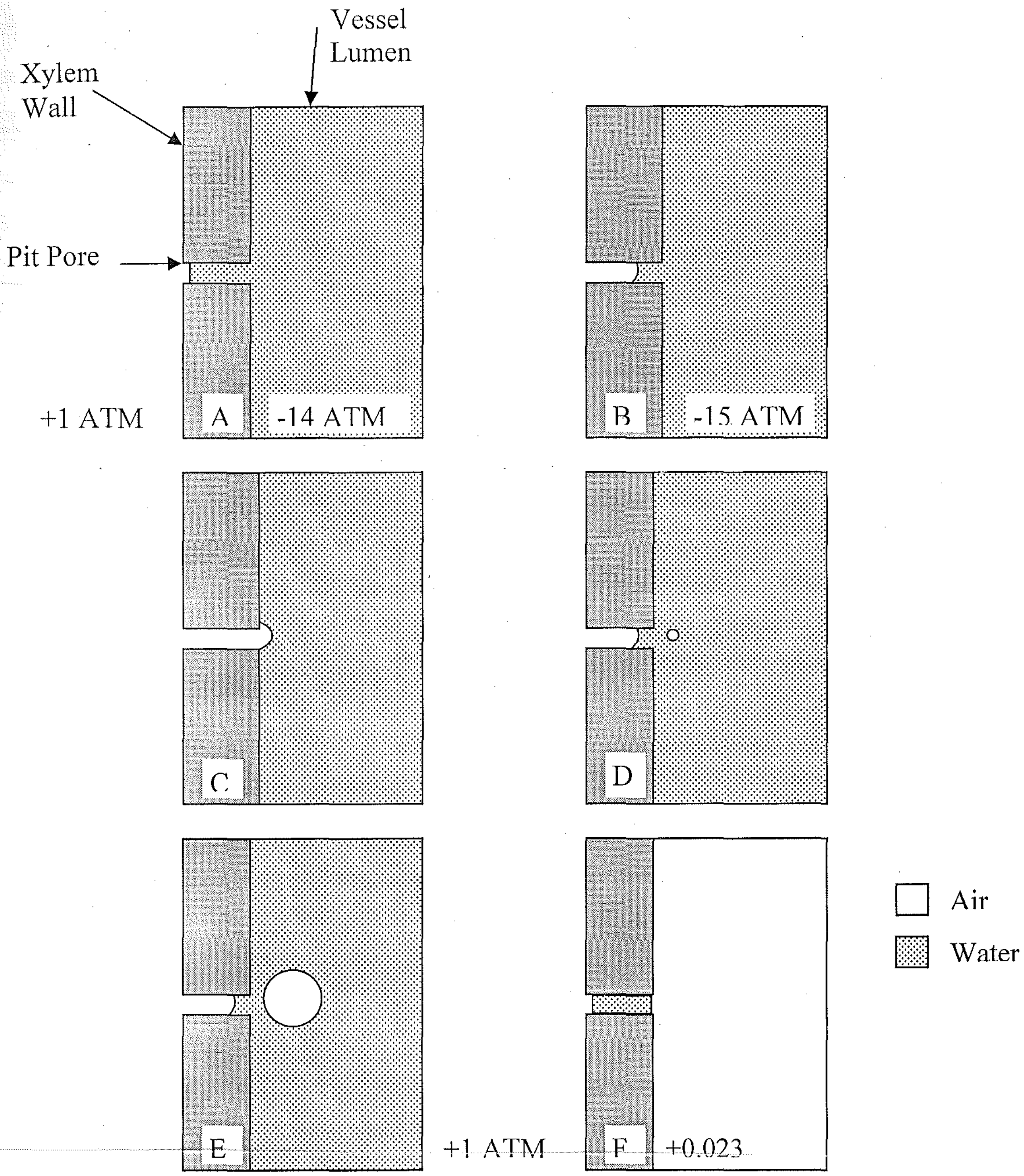

Figure. 1.1. A representation of a water filled xylem undergoing air-seeding leading to embolism of the vessel. The water filled xylem is under lower pressure than the surrounding atmosphere (A). As the pressure drops further, such as what occurs during water stress, air is pulled through the xylem pit pore (B). The meniscus reaches the lumen of the xylem (C), which acts as a catalyst to the metastable water. Water evaporates into the aspired air, creating a bubble (D). The size of the bubble increases, raising the pressure inside the xylem, resealing the pore (E). The bubble, or cavitation, continues to grow until the xylem is completely vapour-filled (F) creating an embolism and preventing the conduction of water through that conduit (adapted from Zimmermann 1983).

1.3 Hydraulic vulnerability Curves

A way to measure emboli without removing them has been established, as have methods for inducing cavitation in plant material. Three main methods have been developed: air-drying, air-injection and centrifugal force (Alder *et al.* 1997). The air-drying method involves dehydrating stems under laboratory conditions.
Xylem potential is then determined using a Scholander-type pressure chamber and the hydraulic conductivity of the stem determined using a flow-meter based on the design of Sperry *et al.* (1988). This method entails drying stems out, which is a relatively time consuming process. It also requires a number of stems to achieve one vulnerability curve, as it is difficult to judge what the xylem potential is going to be at any particular state of drying (Alder *et al.* 1997).

The air-injection method was developed as an alternative means of inducing cavitation in xylem. Stems are inserted into a specialised chamber and exposed to known pressures, which correlate with xylem potential (Cochard *et al.* 1992). Thus, using this technique lower xylem potentials can be achieved quicker than using the dehydration technique. There is also an advantage in knowing what xylem potential the stem is being exposed to (Sperry & Saliendra 1994). More recently centrifugal force has been used to induce xylem cavitation. This method involves placing plant stems in a centrifuge rotor, then spinning them on their long axis (Alder *et al.* 1997). The centrifugal force exerted on the plant stems once again corresponds with xylem potentials.

A number of studies have compared results using these different methods to evaluate the effectiveness of their use. Sperry and Saliendra (1994) evaluated the air-injection method comparing it with air drying, using stems from *Betula occidentalis*. It was found that the air-injection method gave results comparable with those determined using the air-drying method. This is interesting because the air-drying method involves the aspiration of air into the xylem, through pit pores, as described by Zimmermann's air-seeding hypothesis (Zimmermann 1983). That is, air is pulled into the xylem due to decreasing xylem potentials. In contrast the air-injection method pushes the air in the xylem using increasing air pressures. It has been concluded that the two methods produce comparable results because it takes the same pressure differences to pull air into the xylem under negative xylem potentials as it does to push it in under positive air pressures (Sperry & Saliendra 1994). Alder *et al.* (1996) evaluated the centrifugal force technique, comparing it with both the air-injection and air-drying techniques. This study found that the use of centrifugal force to induce xylem emboli in stems gave vulnerability curves identical to those developed using the other two techniques. It was suggested that the mechanism behind cavitation is the same using centrifugal force as it is for the air-drying method; that is, air is pulled into vessels due to negative xylem potentials as explained by the air-seeding hypothesis.

The vulnerability curve quantifies the degree of embolism that occurs in a plant at decreasing xylem potentials (therefore increasing tension on the water column). Using maximum hydraulic conductance it is possible to determine the percentage loss of conductance (PLC) that occurs as a result of emboli that are induced at decreasing xylem potentials. PLC is then plotted against xylem potential. Pammenter and Vander Willigen (1998) suggested an exponential-sigmoidal model to describe vulnerability data (Fig. 1.2). This function incorporates the xylem potential at which 50% loss of conductance occurs (PLC_{50}) and coefficient a, which relates to the gradient of the curve at PLC_{50}. Coeffecient a is useful as it relates xylem pit pore size to the slope of the curve. This relates to the air-seeding hypothesis, which states that larger pit pores are more vulnerable to embolism (Zimmermann 1983). Thus PLC_{50} values and coefficient a values are often used to compare vulnerability to water stress between plants.

Hydraulic conductivity measurements, particularly stem-specific conductivity (K_s) and leaf-specific conductivity (K_L) are often used as a measurement of plant hydraulic architecture (Tyree & Ewers 1991). K_s gives a measure of hydraulic efficiency, taking into consideration the length and cross-sectional area of stem. It is often reported that higher K_s values are associated with plants that occur in areas of high water availability (Kolb & Davis 1994; Martinez-Vilalta *et al.* 2002). K_L gives a measure of hydraulic 'sufficiency' of a stem to supply water to leaves. The Huber value can also be related to water availability and tree hydraulic architecture. Huber (1928) demonstrated that the investment of stem material is related to the total leaf area that the stem supplies. The Huber value (as it has since been termed) is a ratio of the amount of conducting material to the leaf area, given as a dimensionless figure (Zimmermann 1983). A higher Huber value implies that there is more wood per unit of leaf area. Plants that occur in climates where drought is common have generally been found to have higher Huber values than those found in wetter climates and higher Huber values suggest improved plant water and nutrient storage capacity (Callaway *et al.* 1994; Cruiziat *et al.* 2002). In a study investigating inter- and intraspecific differences between species that occupy different ecohydrological niches, the Huber value and hydraulic conductivity parameters may provide further insight in differences in hydraulic architecture.

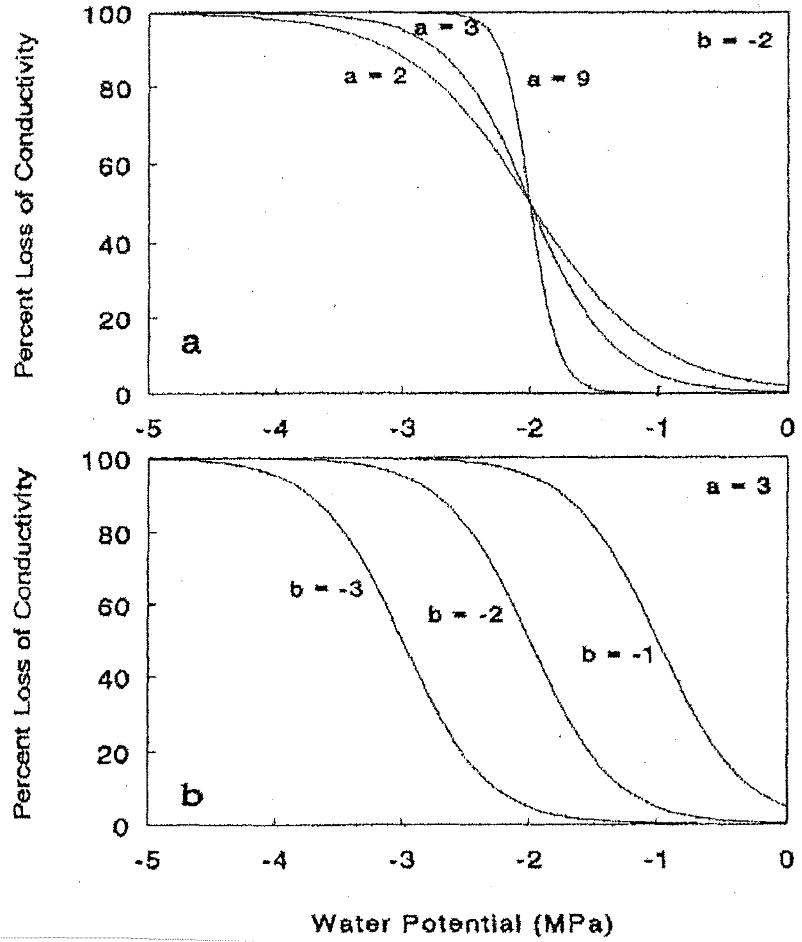

Figure. 1.2. The sigmoidal-exponential model developed by Pammenter and VanderWilligen (1998) to describe plant hydraulic vulnerability curve data. Equation for the line is: PLC = $100/\{1+\exp[a(\Psi_x-b)]\}$ where a = the gradient of a linear transformation, Ψ_x is xylem potential and b is the is Ψ_x where PLC = 50%. (a) demonstrates the effect of changing coefficient a; (b) demonstrates the effect of changing parameter b (Pammenter & Vander Willigen 1998).

Previous studies have investigated differences in hydraulic architecture between invasive weeds and native vegetation (Pratt & Black 2006); interspecific differences in contrasting climates (Maherali & DeLucia 2000); intraspecific differences along a soil-moisture gradient (Alder *et al.* 1996; Lopez *et al.* 2005); and interspecific differences between obligate phreatophytic and facultative phreatophytic vegetation (Froend & Drake 2006). Xylem structure, and hence vulnerability to embolism, appears to be related to water availability. This is particularly relevant in environments where water is the primary limiting factor, such as that found in Mediterranean climates characterised by long periods of drought (Martinez-Vilalta *et al.* 2002; Vilagrosa *et al.* 2003). In these climates, soil water availability is low during drought, meaning species are either adapted to xeric conditions, or utilise groundwater if it is available. It has been established that this is the case for a number of *Banksia* woodland species that occur over the Gnangara Mound on the Swan Coastal Plain, south-west Western Australia (Dodd, *et al.* 1984; Zencich *et al.* 2002).

1.4 Phreatophytes of the Gnangara Mound

There is a gradient in ecohydrological habitats along the Swan Coastal Plain, overlying the Gnangara Groundwater Mound, with xeric conditions found at dune crest sites and more mesic conditions found at low-lying areas, where groundwater is more readily utilised by plants. The Gnangara Mound is a shallow, unconfined aquifer, covering an area of 2,140 km^2 bordered by Moore River in the north, Darling Scarp to the east, Swan River to the south and the Indian Ocean on the west. The climate can be described as warm Mediterranean (Gentilli 1972). The long-term average rainfall for the Swan Coastal Plain is 780 mm with around 85% of rainfall received between May and October (Bureau of Meteorology 2006). Between May and August pan evaporation is lower than the rainfall received and it is during these months that recharge of the Gnangara aquifer occurs (Allen 1981). In contrast, during the hotter, drier months of summer, pan evaporation exceeds rainfall, with the average annual pan evaporation for Perth standing at 1890 mm compared to 780 mm average rainfall. It therefore becomes evident that water sources are a significant limiting factor for vegetation on the Swan Coastal Plain. This is compounded by the low water holding capacity of the soils, as the Swan Coastal Plain is largely comprised of deep quartzite sand dunes with low nutrient and water-holding capacities (McArthur & Bettenay 1974).

Groundwater can provide a permanent water source for vegetation if they are able to access it. Accessibility of groundwater can be determined by the permeability of sediments, rooting structure of plants and the overlying topography of the landscape. The undulating topography of the landscape overlying the Gnangara Mound is therefore significant in an ecohydrological sense with distribution of vegetation reflecting depth to groundwater (Zencich *et al.* 2002). Vegetation is influenced by a number of edaphic factors including climate, soil type, topography and water availability. The vegetation on the Gnangara Mound has developed on low nutrient soils, with low water holding capacity under a Mediterranean climate typified by long, hot summers (Beard 1990). Open *Banksia* woodlands dominate the Bassendean Dune system, with *Banksia attenuata* and *Banksia menziesii* the dominant tree species (Havel 1968). In low-lying regions *Banksia ilicifolia* and *Melaleuca preissiana* are common, demonstrating the influence of topography and depth to groundwater on the distribution of species.

Many of the species found at low-lying positions in the landscape over the Gnangara Mound are considered obligate phreatophytes, with their roots in contact with the capillary fringe (Kite & Webster 1989; Zencich *et al.* 2002). Previous water source partitioning studies have demonstrated variation in groundwater use at contrasting ecohydrological habitats (Zencich *et al.* 2002). In low-lying areas, groundwater is utilised throughout the seasonal cycle by individuals of both obligate and facultative phreatophyte species (Zencich *et al.* 2002). Intraspecific differences in groundwater use were also observed at different locations along the ecohydrological gradient. At a site located high in the landscape groundwater was not utilised by *B. attenuata,* with stored soil moisture from depths of approximately 8 m being the primary water source (Zencich *et al.* 2002). In periods of low rainfall, stored soil water can become depleted as it declines through evapotranspiration with decreased recharge from precipitation (Sharma & Craig 1989). It is during such periods that plants become water stressed. There are therefore distinct differences in plant water sources and the reliability of these sources at contrasting locations. Over the Gnangara Mound there is a gradient in ecohydrological habitats, as reflected by the distribution of the different phreatophyte species. It is assumed that low-lying populations will experience less water stress due to their proximity to the reliable groundwater resource than the populations occupying the dune crest populations that are reliant upon stored soil water.

The need for *Banksia* vulnerability curves to quantify the physiological threshold to water stress has been suggested previously (Lam *et al.* 2004). Froend & Drake (2006) conducted a preliminary investigation of vulnerability curves for three *Banksia* species and a *Melaleuca* species. It was found that the obligate phreatophyte species (*B. ilicifolia* and *M. preissiana*) were more vulnerable to water stress than the facultative species (*B. attenuata* and *B. menziesii*). This is important for *Banksia* species on the Gnangara Mound where water availability is a significant limiting factor and groundwater management is an issue. In addition, there is a paucity of literature regarding intraspecific differences in vulnerability to water stress at contrasting ecohydrological habitats, particularly comparing obligate and facultative phreatophytes.

1.5. Significance and aims of this study

This study aims to determine the inter- and intraspecific differences in vulnerability to water stress for two obligate and two facultative phreatophyte species. Interspecific comparisons are made at the same ecohydrological habitat to minimise the influence of site conditions so that any observed differences may be attributable to differences inherent to a species. Froend and Drake (2006) suggested that vulnerability for a species may differ according to the availability of a consistent water source, such as that afforded by shallow depth to groundwater. This study determines intraspecific differences using individuals of the same species at the limits of their ecohydrological distribution. Individuals from an ecohydrological habitat where groundwater is easily accessible are compared with individuals that occur in a more xeric habitat, where water availability is less reliable. The primary objective was to establish vulnerability curves to test these differences, though K_s, K_L and Huber values were also investigated to support these findings.

The specific hypotheses tested are:

1. Obligate phreatophytes will demonstrate a higher PLC_{50} value than facultative phreatophytes in the same ecohydrological habitat.
2. Plants in a more mesic habitat will demonstrate a higher PLC_{50} value than individuals of the same species found higher in the landscape in a more xeric ecohydrological habitat.

2. Materials and Methods

2.1 Species descriptions

This study investigated the hydraulic architecture of four congeneric *Banksia* species; *Banksia attenuata* R.Br., *Banksia menziesii* R.Br., *Banksia ilicifolia* R.Br. and *Banksia littoralis* R.Br. According to the taxonomy established by George (1981) three of the species are from the same subgenus (ie. Banksia); *B. attenuata* (section Banksia; series Cyrtostylis), *B. menziesii* (section Banksia; series Banksia) and *B. littoralis* (section Oncostylis; series Spicigerae). *B. ilicfolia* belongs to the subgenus *Isostylis*.

The four study species were selected for their contrasting distribution and phreatophytic traits. Two obligate phreatophyte species, *B. ilicifolia* and *B. littoralis*, and two facultative phreatophyte species, *B. attenuata* and *B. menziesii*, have been selected for this study. *B. littoralis* has the narrowest distribution of the study species as it is confined to damplands throughout the Swan Coastal Plain, occurring only in areas of shallow depth to groundwater (Groom *et al.* 2001). *B. ilicifolia* is generally found to be occurring at locations where depth to groundwater is less than 8 m (Arrowsmith 1992), thus its distribution is also restricted by groundwater availability. In contrast, the two facultative phreatophyte study species occupy areas where groundwater depth is in excess of 30 m. The four study species therefore occupy contrasting ecohydrological habitats.

2.2 Site descriptions – Contrasting ecohydrological habitats

An ecohydrological habitat refers to the hydrological and edaphic attributes that influence vegetation at a specific location in a landscape. A particular habitat can be defined by rainfall, recharge sources, depth to groundwater and soil matric potential (Eamus *et al.* 2006). Rainfall refers to both the quantity and seasonality of precipitation. Water recharge sources can differ between sites with some habitats receiving only localised recharge from precipitation, whilst other sites can receive regional recharge in the form of lateral groundwater flow and run-on, as well as localised precipitation recharge. Depth to groundwater refers to the proximity of the saturated zone to the soil surface, and thus plant roots (Groom 2004). Depth to groundwater also determines the volume of the unsaturated zone within which plant rhizospheres can develop. Spatial and temporal dynamics in soil matric potential determines the accessibility, or ease of which a plant can take up water from soil. These parameters all contribute to plant available water content of the soil and therefore are the key hydrological attributes that influence vegetation.

Sampling was conducted at six sites within a 3 km radius in the Lexia wetland system over the Gnangara Mound (Fig. 2.1). The study area is located on the Bassendean dune system, and the undulating geomorphology has resulted in differing ecohydrological habitats. The study required contrasting ecohydrological habitats to be defined. It has been identified that terrestrial soil water retention properties (and therefore matric potential of the soil at a given water content) and precipitation does not vary significantly in the Lexia area of the Gnangara Groundwater Mound (Lam *et al.* 2004). Thus, the defining attributes of the different ecohydrological habitats in this landscape are depth to groundwater and recharge sources. Sampling was conducted at sites that represent contrasting ecohydrological habitats within a 3 km radius (Fig. 2.1).

Hypothesis one, investigating interspecific differences in vulnerability to xylem cavitation required a site with all four study species present in the same ecohydrological habitat, to minimise environmental influences. The selected bottom slope site had a shallow water table, due to its low-lying position in the landscape. By virtue of its position in the landscape, it is assumed this site receives regional recharge, via lateral flow of groundwater, as well as surface run-on and localised recharge from precipitation that falls directly on the site. Groundwater potentially provides a seasonally consistent water source for plants at this site. The implication is that water is available at high water potentials (closer to 0, ie. free water) for the majority of the seasonal cycle. The bottom slope site is situated within close proximity to a dampland, a seasonally moist, low-lying area, characteristic of interdunal wetlands that occur along the Swan Coastal Plain. *B. littoralis* were located within the dampland, whilst *B. attenuata, B. menziesii* and *B. ilicifolia* were located on the dampland embankment. Groundwater depth was measured using pre-existing piezometers, with depth to groundwater ranging from 1.5 m next to the *B. littoralis* to 3.2 m at the embankment (Fig. 2.2).

To address hypothesis two, which investigates intraspecific differences in vulnerability to xylem embolism, contrasting ecohydrological habitats were selected. *B. attenuata* and *B. menziesii* were investigated at a dune crest site with a depth to groundwater of more than 30 m, as measure using piezometers.

Banksias at the dune crest site are likely to possess more extensive root systems than the bottom slope site plants, due to the relatively large volume of the unsaturated soil (Fig. 2.2), facilitating, as well as promoting, more extensive root growth. However, *B. attenuata* and *B. menziesii* roots have been previously measured and it was found that root abundance was very low beyond a depth of 8 m (Farrington *et al.* 1989). Groundwater depths beyond 8-9 m are therefore considered inaccessible to these species and they are instead reliant upon water from the unsaturated zone (Groom 2004). The plants at the dune crest site only receive localised recharge of their water source through precipitation. This has been demonstrated quantitatively through water source partitioning studies (Zencich *et al.* 2002), which showed a marked difference in the water availability and plant water sources at the dune crest site in contrast to the bottom site.

Banksia ilicifolia is rarely found at higher elevations, and thus greater depths to groundwater, due to it being an obligate phreatophyte. As a consequence of its patchy distribution at higher locations, it was difficult to locate sufficient plants to sample at one site. Samples were therefore taken from single plants at four different sites within a 1 km area. These four sites are together referred to as the mid-slope site (Fig. 2.2). It has previously been observed that this species generally does not occupy areas where depth to groundwater is greater than 8 m (Arrowsmith 1992). Therefore, though it has been attempted to sample the individuals at the limits of their ecohydrological range it is likely that the roots of these species are still in contact with the capillary fringe and/or groundwater.

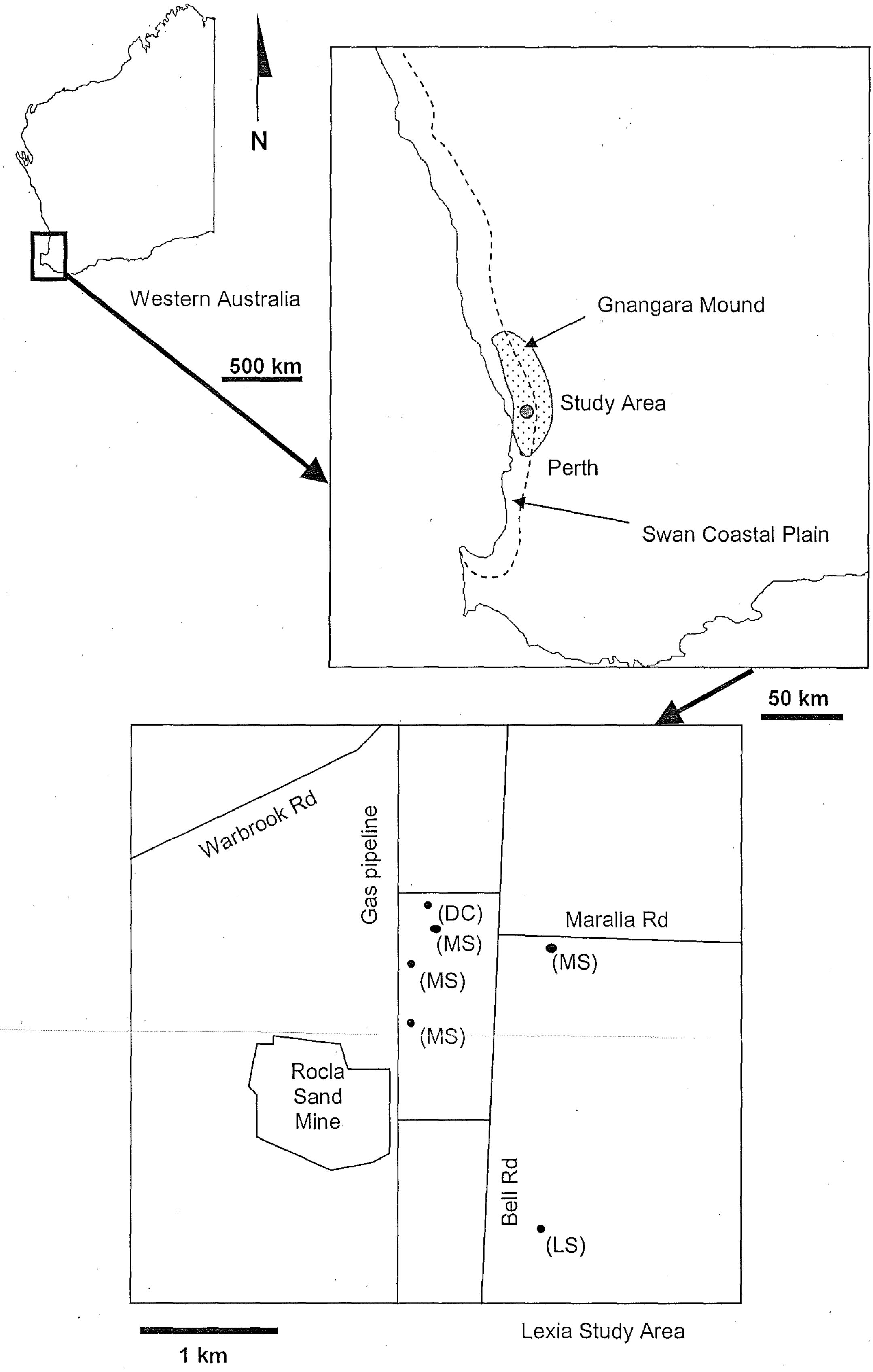

Figure 2.1. Location of specific study sites at the Lexia study site in relation to the Swan Coastal Plain. DC =Dune Crest, MS = Midslope, BS = Bottom Slope.

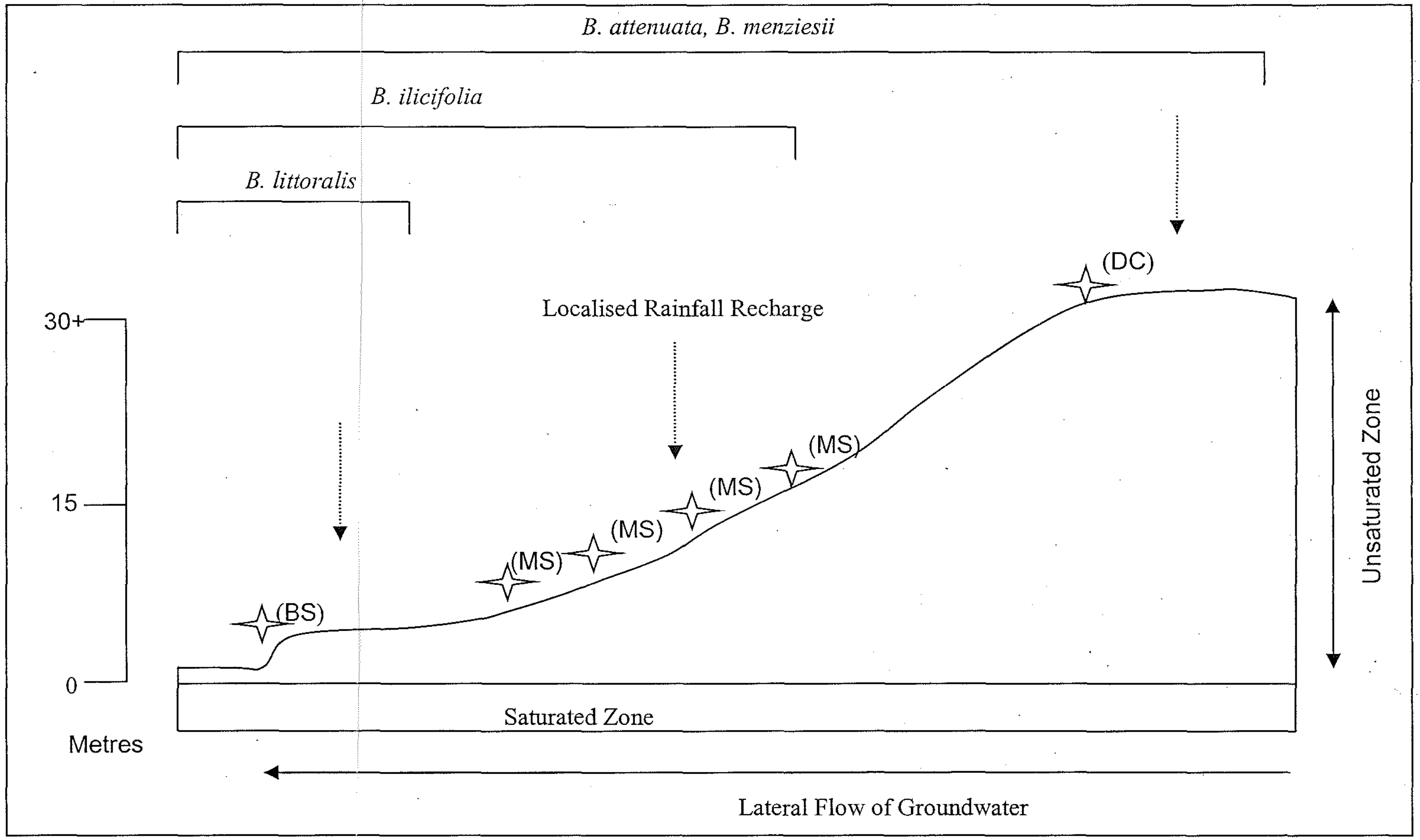

Figure 2.2. A representation of the landscape, or ecohydrological gradient, of the Lexia study site, demonstrating the distribution of study species and study sites; Bottom Slope (BS), Midslope (MS) and Dune Crest (DC). The facultative phreatophytes, *B. attenuata* and *B. menziesii* have the widest distribution, whilst the obligate phreatophytes, *B. ilicifolia* and *B. littoralis* are constrained to areas with shallower depth to groundwater. Recharge of water resources is also demonstrated with localised recharge occurring at all sites through rainfall. In addition, there are differences in regional recharge between the contrasting sites, with the bottom slope site receiving the most recharge through lateral flow of groundwater. The dune crest site does not receive regional recharge, with vegetation at this site reliant upon localised recharge of soil moisture.

2.3 Vulnerability Curves

Sampling was conducted over a 4 month period from May to August, 2006. Stems with 4-6 budscars, thus ranging in age from 4 to 6 years were collected (Lamont 1985; Fig. 2.3). Younger stems were generally found to be too short, too thin, or still carrying leaves and therefore not appropriate for the techniques used. Stem segments approximately 25cm in length were collected from each individual and were double-bagged in moist plastic bags, placed in a black plastic bag, kept cool and transported to the laboratory. They were then kept in the bags in a refrigerator at 4°C until they were used. At each sampling trip 3 trees were sampled, with 3 stems taken from each tree to allow for problems that were often experienced in the analysis process (such as stems snapping or excessively low maximum conductivity values). On sampling days, 1 stem was processed in the laboratory and on the following day, 2 stems were processed, with a total of 3 stems being processed per trip. Any left-over samples were discarded within 48 hours of collection. In total 10 vulnerability curves each were developed for *B. attenuata* at the bottom slope and the dune crest sites, 10 for *B. ilicifolia* at the bottom and midslope sites, 5 for *B. menziesii* at the bottom slope and dune crest sites and 5 for *B. littoralis* at the bottom slope site.

In the laboratory stems were trimmed using secateurs under 1mM KCl in degassed double-distilled water. Bark was removed from the stem, once again immersed in the KCl solution and trimmed one final time with a sharp razor blade. Parafilm® was wrapped around the ends of the stem to provide a seal for the pressure collars, which were then attached. Bubbles were removed from the pressure collars and they were attached to a steady state flow meter (SSFM). The SSFM is based upon that used by Feild *et al.* (2001) and Froend and Drake (2006), using a pressure drop across Peek™ tubing of a known diameter in series with the stem segment (Fig 2.4).

Using the SSFM, maximum conductivity was determined by flushing stems to remove native emboli. These are emboli that are present in the stem when it is collected and can be quantified by measuring the conductivity of the stem before it is flushed and comparing with the conductivity after flushing. Stems were initially flushed with a perfusion solution of 1mM KCl in degassed DDI water (Zwieniecki, *et al.* 2001) at 100 kPa for 15 minutes, as described by Froend and Drake (2006). After flushing, the conductivity of the stem was measured. Conductivity measurement with the SSFM involves using a head pressure of less than 5kPa to pass the perfusion solution through a stem in series with the Peek^{TM} tubing. When the conductance was measured after flushing it was often found that it was lower than the conductance measured with native emboli present. Thus, flushing at 50 kPa for 15 minutes was used and this resulted in conductivities that were higher than the initial values recorded, suggesting that this was a more appropriate flushing pressure.

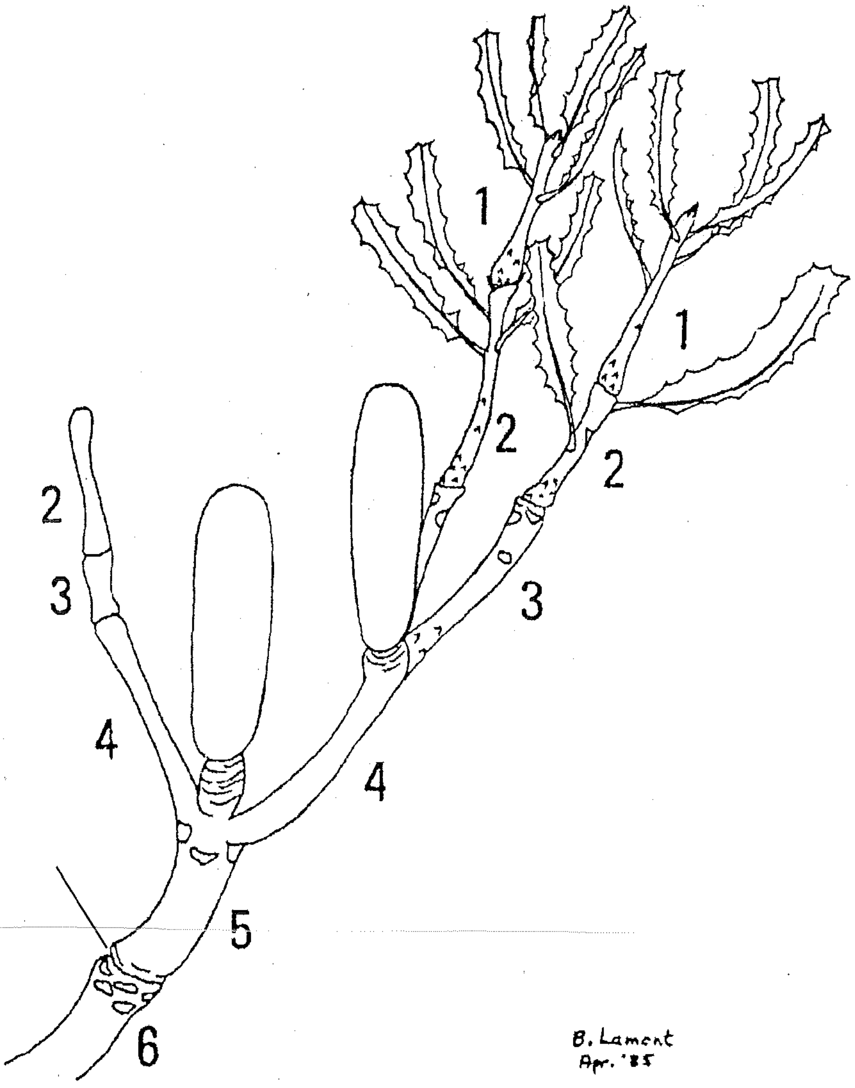

Figure 2.3. Annual incremental growth of stems in *Banksia menziesii*. By counting the budscars, the age of the stems can be determined. (adapted from Lamont 1985)

Emboli were induced by applying pressure to stems using a cavitation chamber, which is an adaptation of the Scholander-type pressure chamber (PMS instruments, Oregon, USA, Model 3005). Pressures applied to the stems are the inverse to the xylem pressure potential. Nitrogen gas was used to apply a desired pressure to the stem for 15 minutes. The pressures used to construct the vulnerability curves were 0.5, 1, 1.5, 2, 2.5, 3, 4 and 5 MPa. After applying pressure, stems were left for approximately 5 minutes to equilibrate then reattached to the SSFM. The percentage loss of conductance (PLC) was then calculated:

$$PLC = \frac{100(k_{\max} - k_h)}{k_{\max}} \tag{1}$$

where k_h is the volume flux density at a given xylem potential and $k_{\max}$ is the maximum volume flux density after the stem has been flushed.

Percentage loss of conductance was plotted against the xylem potential and an exponential-sigmoidal function (Pammenter & Vander Willigen 1998) fitted:

$$PLC = 100/\{1 + \exp[a(\Psi_x - b)]\} \tag{2}$$

where Ψ_x is xylem potential, a is related to the gradient at PLC_{50} and b is Ψ_x where PLC equals 50%.

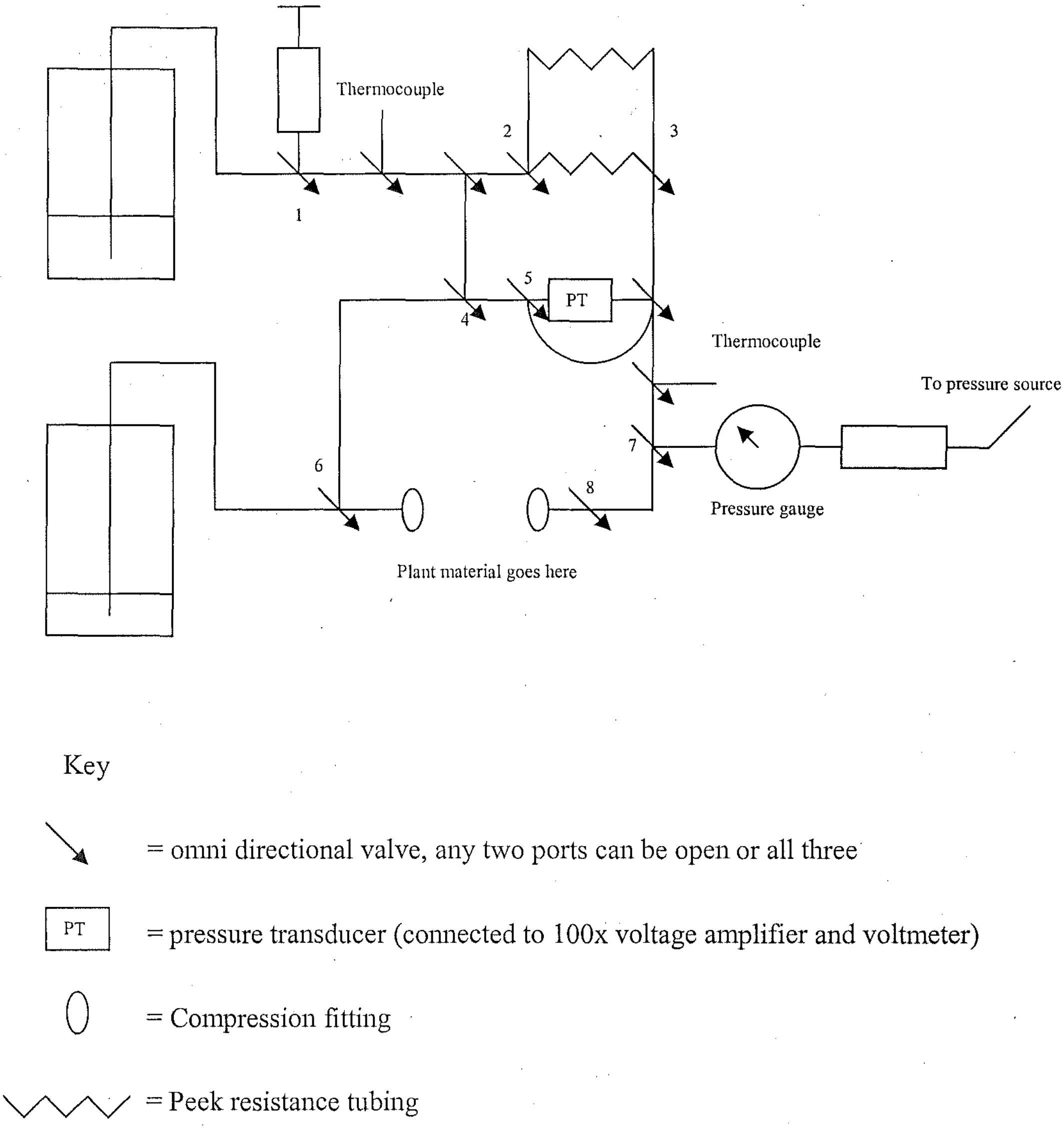

Figure 2.4. Schematic representation of the steady-state-flow meter. To measure stem conductivity a perfusion solution of 1 mM KCl in degassed, double-distilled water is passed through the stem and Peek resistance tubing in series at a head pressure below 5 kPa. To remove native emboli a pressure source is used to push the perfusion solution through the stem at a pressure of 50 kPa for 15 minutes, flushing the stem.

A number of studies have used PLC_{50} to compare vulnerability to water stress, in particular Pammenter and Vander Willigen (1998) who incorporated it into the exponential-sigmoidal function to describe plant vulnerability data. Froend and Drake (2006) identified PLC_{20} as a more appropriate measure of differences between facultative and obligate phreatophytic *Banksia* species. Thus PLC_{20} and PLC_{50} were used here to compare xylem embolism as well as coefficient a (gradient of a linear transformation of data, derived from the fitted exponential-sigmoidal function).

To examine interspecific differences in these parameters at the bottom slope site (hypothesis 1) comparisons were made by one-way analysis of variance (ANOVA) using SPSS v.14.0 software. The PLC values at the different applied xylem potentials were also compared using one-way ANOVA, to see if they correlated with the results obtained from the fitted data and to more accurately determine whether species differed in PLC at all or part of the range in applied pressure. If significant differences were found Tukey HSD post-hoc analysis was applied.

To contrast intraspecific PLC values at different ecohydrological habitats (hypothesis 2) t-tests were performed using SPSS v.14.0 software. Data from the bottom slope site for *B. attenuata* were compared with the dune crest data for *B. attenuata*. This was repeated for *B. menziesii* and *B. ilicifolia*. The a, PLC_{20} and PLC_{50} values, as well as the PLC at the different xylem potentials were once again used for these comparisons.

2.4 Hydraulic Conductivity and Huber Values

In addition to vulnerability curves, hydraulic architecture was investigated by determining stem-specific conductivity (K_s), leaf-specific conductivity (K_L) and Huber values (cross-sectional area of stem per area of leaves). Maximum hydraulic conductivity is determined by flushing native emboli from the stem, then conductance is measured as previously described. Leaf area was also determined. Leaf material was removed from the stems and placed into separate plastic bags during sampling. Leaf area was measured in the laboratory using a WinDIAS image analysis system, which is a digitizing tablet that captures the image of the leaves, then calculates their area.

K_s gives a measure of hydraulic conductivity in relation to the length and diameter of the stem. It was determined from hydraulic conductivity (K_h) which is calculated as:

$$K_h = F/(dP/dx) \tag{3}$$

where F is the flow (kg/s) through the plant material, dP is the pressure gradient (MPa) and dx is the length of the stem (m).

K_s was determined by dividing K_h by the cross-sectional area of stem. Digital callipers were used to measure the diameter of the stems with bark removed and cross-sectional area determined.

The Huber value was determined using the cross-sectional area (A_x) and leaf area (A_l):

$$H = A_x / A_l \tag{4}$$

K_L takes into consideration the size of the leaf area that the stem is supplying water to and was determined as

$$K_L = K_s \times H \tag{5}$$

Interspecific comparisons of these K_s, K_L and Huber values between these four species at the same ecohydrological habitat (the bottom site) were made using one-way ANOVA. If significant differences were found, Tukey HSD post-hoc analysis was performed. t-tests were used to analyse intraspecific differences in K_s, K_L and Huber values at contrasting ecohydrological habitats differences in Huber values within a species at different locations in the landscape. All statistical analyses were undertaken using SPSS v. 14 software.

3. Results

3.1 Interspecific differences

A comparison of four congeneric species at the same ecohydrological habitat (bottom slope site) found that there were no significant differences in vulnerability to xylem cavitation (Fig. 3.1; Table 3.2). The slope of the curve, as measured by the coefficient *a* was also similar between each of the species (P=0.580), reflecting the similarities between the PLC_{20} and PLC_{50} values (Table 3.1). There was some variation, with PLC_{20} ranging from -0.41 ± 0.06 MPa for *B. menziesii* to -0.52 ± 0.09 MPa for *B. attenuata* and -0.52 ± 0.11 MPa for *B. littoralis*. PLC_{50} values ranged from -1.29 ± 0.19 MPa for obligate phreatophyte *B. ilicifolia* to -1.44 ± 0.16 MPa for the facultative phreatophyte *B. attenuata*, though these were not significantly different (P = 0.931).

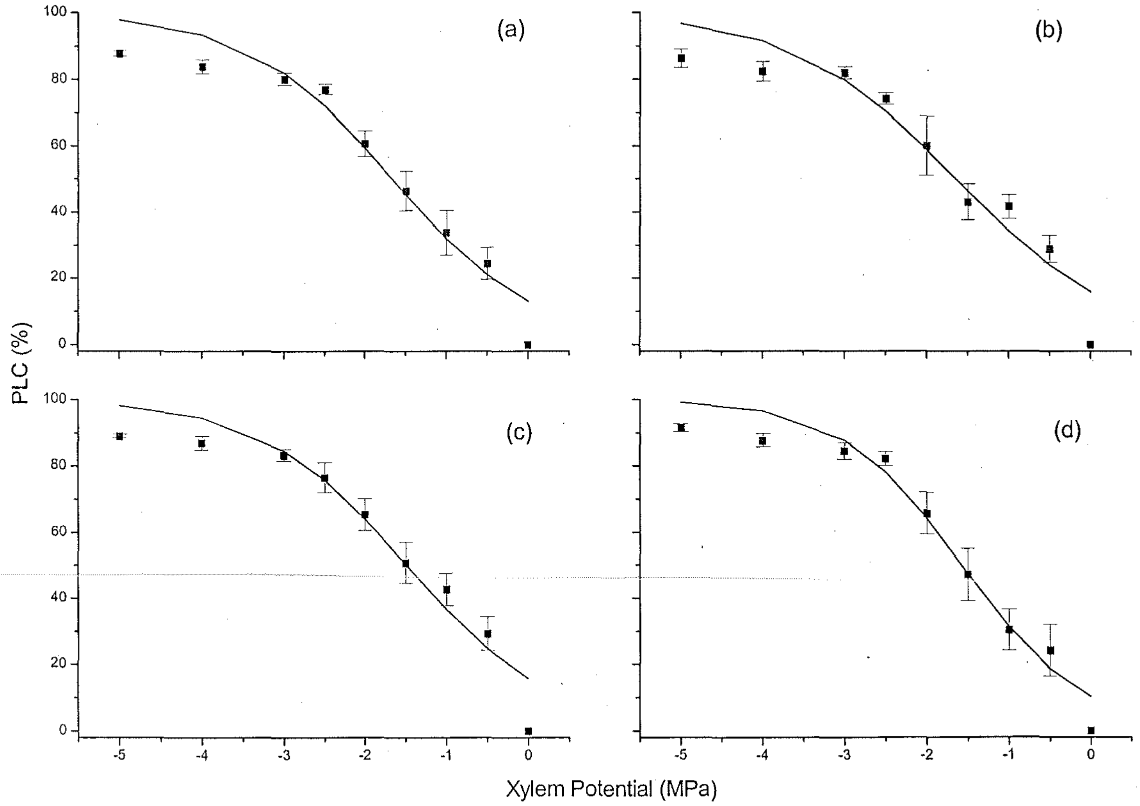

Figure 3.1. Vulnerability curves for (a) *Banksia attenuata* (r^2=0.83; n=10), (b) *Banksia menziesii* (r^2=0.84; n=5), (c) *Banksia ilicifolia* (r^2=0.80; n=10) and (d) *Banksia littoralis* (r^2=0.87; n=5) at low site. Data plotted is the mean ± 1SE. PLC is % loss of conductivity. Fitted curves are exponential-sigmoidal: PLC = $100/\{1+\exp[a(\Psi_x-b)]\}$ where a = gradient of a linear transformation, Ψ_x is xylem potential and b is the is Ψ_x where PLC = 50%

Table 3.1. Mean (±1SE) values for *a* (gradient) and PLC_{20} (xylem potential at which % loss of conductance = 20) and PLC_{50} from exponential-sigmoidal curve fitted to data for *Banksia attenuata* (*n*=10), *Banksia menziesii* (*n*=5), *Banksia ilicifolia* (*n*=10) and *Banksia littoralis* (*n*=5) at the low site.

	a	**PLC_{20} (MPa)**	**PLC_{50} (MPa)**
B. attenuata	1.38 ± 0.13	-0.52 ± 0.09	-1.44 ± 0.16
B. menziesii	1.13 ± 0.23	-0.41 ± 0.06	-1.40 ± 0.13
B. ilicifolia	1.37 ± 0.12	-0.43 ± 0.07	-1.29 ± 0.19
B. littoralis	1.47 ± 0.16	-0.52 ± 0.11	-1.41 ± 0.21

Table 3.2. One-way analysis of variance (ANOVA) results comparing the parameters *a* (gradient), PLC_{20} (xylem potential at which % loss of conductance = 20), PLC_{50} and PLC_{80} from exponential-sigmoidal curves fitted to data for *B. attenuata, B. menziesii, B. ilicifolia and B. littoralis* at the low site.

	df	**F**	**sig.**
a	3,26	0.666	0.580^{ns}
PLC_{20}	3,26	0.390	0.761^{ns}
PLC_{50}	3,26	0.147	0.931^{ns}

The values of the applied pressures corresponded well with the PLC_{20} and PLC_{50} values derived from the fitted exponential-sigmoidal function. Statistical analysis of PLC at each applied pressure found that there were no significant differences (Table 3.3). This reflects the no significant differences found for data obtained from the fitted curve.

Table 3.3. One-way ANOVA results comparing % loss of conductance data at each applied xylem potential for *B. attenuata, B. menziesii, B. ilicifolia and B. littoralis* the low site. Values assigned ^{ns} signifies no significant differences found.

Xylem Potential (MPa)	**d.f**	**F**	**sig.**
-0.5	3,25	0.251	0.860^{ns}
-1	3,26	0.893	0.458^{ns}
-1.5	3,24	0.232	0.873^{ns}
-2	3,26	0.283	0.837^{ns}
-2.5	3,20	0.832	0.492^{ns}
-3	3,26	0.768	0.523^{ns}
-4	3,26	0.993	0.412^{ns}
-5	3,20	0.515	0.676^{ns}

Stem-specific conductivity (K_s) was highest for the two obligate phreatophyte species in contrast to the facultative phreatophytes (Fig 3.2), though no statistically significant differences were observed (*P*= 0.252).

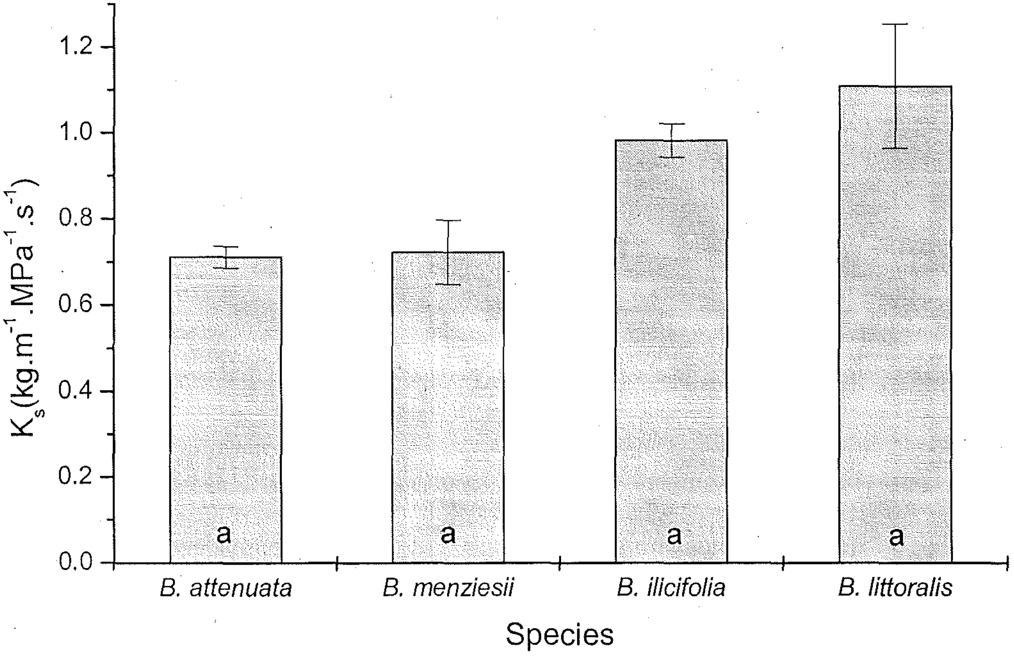

Figure 3.2. Stem-specific conductivity (K_s) values for each study species at the bottom slope site. Plotted values are means ± 1 SE. Same letters indicate no significant differences between species (df= 3,26; F =1.445; sig. 0.252)

There were no clear trends evident in leaf-specific conductivity and Huber values in regards to obligate and facultative phreatophytes. Leaf-specific conductivity (K_L) was highest for the *B. ilicifolia* ($7.87 \times 10^{-4} \pm 4.1 \times 10^{-5}$ Kg.m^{-1}.MPa.s^{-1}), though the lowest K_L value was for *B. littoralis* (3.65×10^{-4} 3.1×10^{-5} Kg.m^{-1}.MPa.s^{-1}), both of which are obligate phreatophytes (Fig. 3.3). However, no statistically significant differences were identified through analysis using one-way ANOVA (*P*=0.073). A comparison of Huber values for each of the four species investigated found that the obligate phreatophyte *B. littoralis* was significantly different from *B. ilicifolia* and *B. attenuata* (*P*=0.028). The facultative phreatophyte was neither different from *B. attenuata* nor *B. littoralis* (Fig. 3.4).

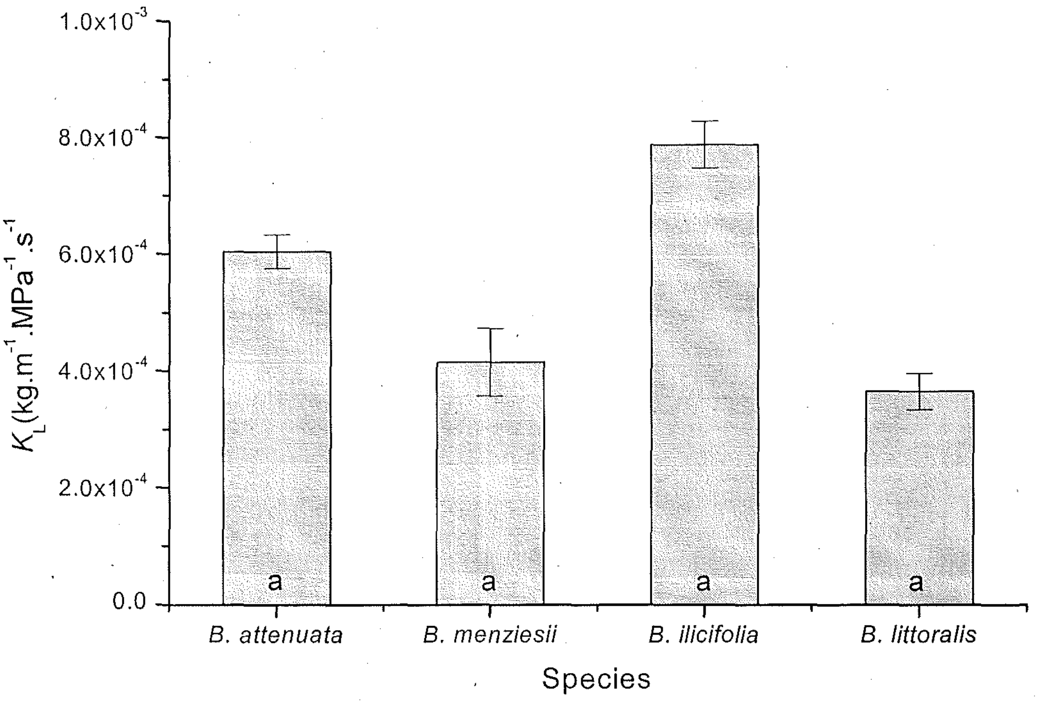

Figure 3.3. Leaf-specific (K_L) values for *Banksia attenuata* (*n*=10), *Banksia menziesii* (*n*=5), *Banksia ilicifolia* (*n*=10) and *Banksia littoralis* (*n*=5) at the bottom slope site. Plotted values are means ± 1 SE. Same letters indicate no significant differences between species (df. = 3, 26; F = 2.163; sig. = 0.073)

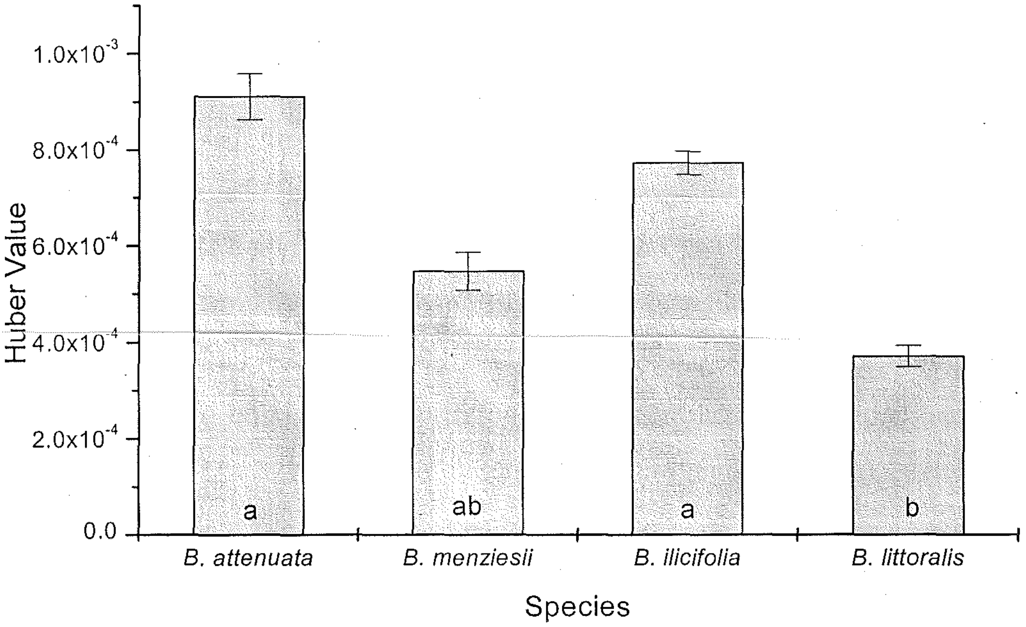

Figure. 3.4. Huber values (ratio of twig cross-sectional area to leaf area) for *Banksia attenuata* (*n*=10), *Banksia menziesii* (*n*=5), *Banksia ilicifolia* (*n*=10) and *Banksia littoralis* (*n*=5) at the low site. Values are means ± 1 SE. One-way ANOVA found significant differences between species (df. = 3,26; F = 3.552; sig. = 0.028) Treatments with the same letters did not differ significantly (P >0.05).

3.2 Intraspecific Differences

Intraspecific differences in hydraulic architecture of Banksias located in contrasting ecohydrological habitats were investigated to address hypothesis two of this study. The shape of the vulnerability curves were all similar, as demonstrated by the similar values for the coefficient a (Table 3.4). No significant differences were identified for a though intraspecific differences were evident at both PLC_{20} and PLC_{50} (Table 3.5). The two facultative phreatophyte species, *B. attenuata* and *B. menziesii* were found to have an increased resistance to xylem cavitation at the more xeric dune crest site (Fig. 3.5; Table 3.4). At the dune crest site PLC_{20} was higher for both *B. attenuata* (-1.34 ± 0.15) and *B. menziesii* (-1.23 ± 0.13) in contrast to the individuals at the more mesic bottom slope site (Table 3.4). The PLC_{50} value for *B. attenuata* was -1.44 MPa at the bottom slope site, in contrast to -2.51 MPa at the dune crest site ($P = 0.000$; Table 3.4). This pattern was similar for *B. menziesii* which had a PLC_{50} value of -1.41 MPa at the low site and -2.23 MPa at the dune crest site ($P = 0.005$). In contrast to the results for the facultative phreatophyte species, the obligate phreatophyte, *B. ilicifolia,* did not demonstrate any significant differences to water stress between individuals sampled at the limits of their ecohydrological distribution. No significant differences were observed (Table 3.5) though PLC_{20} and PLC_{50} were both lower at the mid slope site (Table 3.4).

PLC values derived from an exponential-sigmoidal function fitted to the data corresponded with PLC values at each applied pressure. For the two facultative phreatophyte species, significant differences occurred at higher (less negative) xylem potentials. For *B. attenuata* significant differences were found at -1 to -3 MPa and for *B. menziesii* at -0.5, -1, -2.5 and -3 MPa (Table 3.6 & 3.7). The obligate phreatophyte, *B. ilicifolia* showed no significant differences between the bottom slope and midslope sites (Table 3.8). At lower xylem potentials (ie. -4 and -5 MPa), there were no significant differences for any of the species.

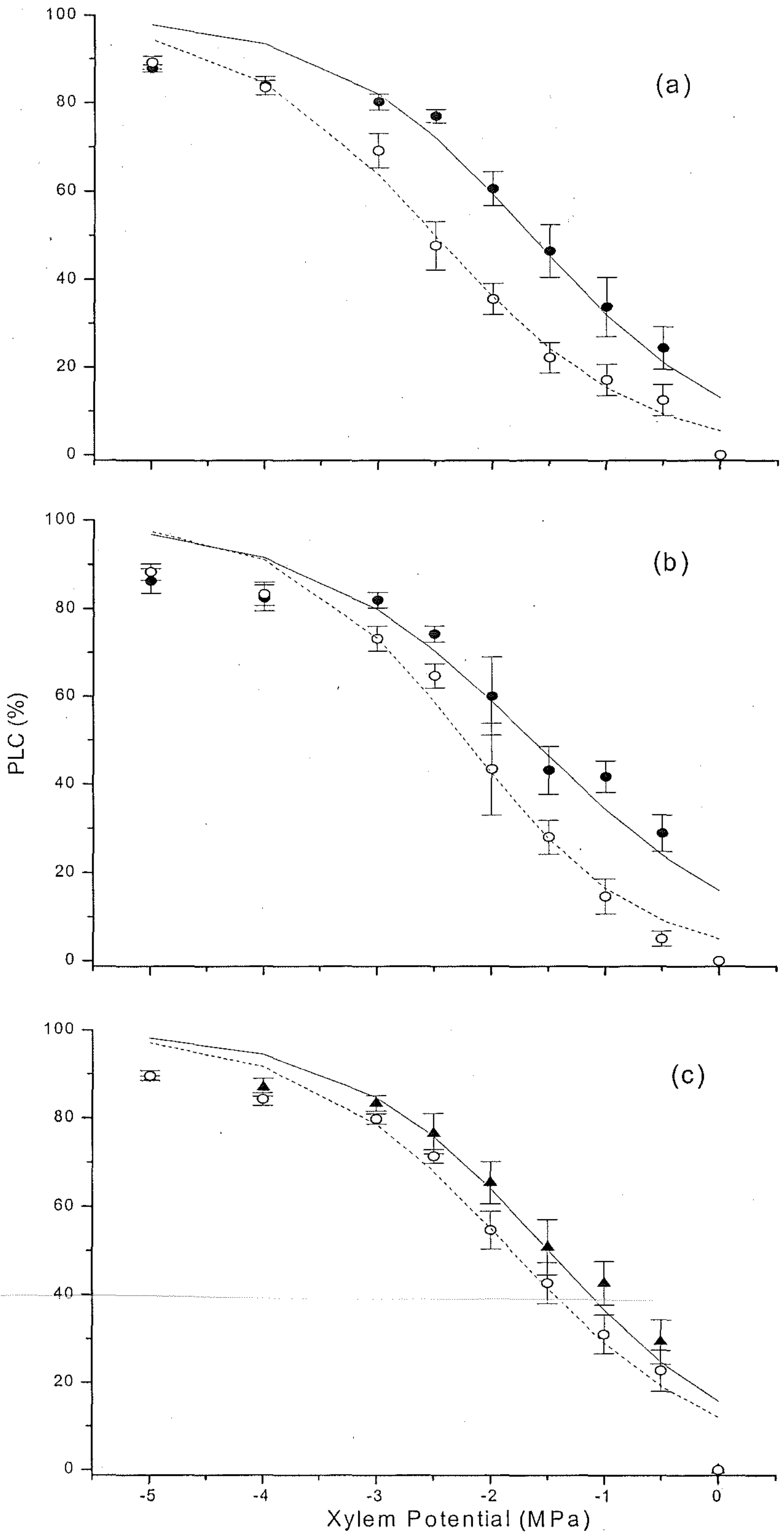

Figure 3.5. Vulnerability curves for bottom slope (open circles), dune crest (solid circles) for (a) *B. attenuata* (r^2=0.83; r^2=0.90; *n*=10) (b) *B. menziesii* (r^2=0.84; r^2=0.91; *n*=5) and bottom slope (open circles and mid slope (solid triangles) for (c) *B. ilicifolia* (r^2=0.80; r^2=0.87; *n*=10). Data plotted is the mean ± 1SE. PLC is % loss of conductivity. Fitted curves are exponential-sigmoidal: PLC = $100/\{1+\exp[a(\Psi_x-b)]\}$ where *a* = the gradient of a linear transformation, Ψ_x is xylem potential and *b* is the is Ψ_x where PLC = 50%.

Table 3.4. Mean (±1SE) values for *a* (gradient) and PLC_{20} (xylem potential at which % loss of conductance = 20) and PLC_{50} from exponential-sigmoidal curves fitted to data for *Banksia attenuata* bottom slope and dune crest sites (*n*=10), *B. menziesii* bottom slope and dune crest sites (n=5) and *B. ilicifolia* bottom slope and mid slope sites (*n*=10).

	a	**PLC_{20}**	**PLC_{50}**
B. attenuata **Low Site**	1.39 ± 0.13	-0.52 ± 0.09	-1.44 ± 0.16
B. attenuata **Dune crest**	1.24 ± 0.10	-1.34 ± 0.15	-2.51 ± 0.12
B. menziesii **Low Site**	1.14 ± 0.23	-0.42 ± 0.06	-1.41 ± 0.13
B. menziesii **Dune crest**	1.40 ± 0.67	-1.23 ± 0.13	-2.23 ± 0.16
B. ilicifolia **Low Site**	1.37 ± 0.12	-0.44 ± 0.07	-1.30 ± 0.19
B. ilicifolia **Top Site**	1.22 ± 0.08	-0.60 ± 0.15	-1.78 ± 0.13

Table. 3.5. Results from t-tests comparing mean values for PLC_{20} (xylem potential at which % loss of conductance = 20), PLC_{50} and PLC_{80} from exponential-sigmoidal curve fitted to data for *Banksia attenuata* bottom slope and dune crest sites (*n*=10), *B.menziesii* bottom slope and dune crest sites (n=5) and *B. ilicifolia* bottom slope and mid slope sites (*n*=10).

Species		**df**	**t**	**sig.**
B. attenuata				
	a	18	-0.904	0.378 ^{ns}
	PLC_{20}	18	-4.828	0.000
	PLC_{50}	18	-5.203	0.000
B. menziesii				
	a	8	-1.091	0.307 ^{ns}
	PLC_{20}	8	5.790	0.000
	PLC_{50}	8	3.900	0.005
B. ilicifolia				
	a	18	1.113	0.280 ^{ns}
	PLC_{20}	18	0.966	0.347 ^{ns}
	PLC_{50}	18	2.151	0.050 ^{ns}

Table 3.6. A comparison of % loss of conductance values at different xylem potentials, using t-tests to compare *Banksia attenuata* data from the bottom slope with *B. attenuata* data from the dune crest site.

Xylem potential (Mpa)	**d.f**	**t**	**sig.**
-0.5	17	2.004	0.061 ^{ns}
-1	18	2.181	0.043
-1.5	15	3.628	0.002
-2	18	4.841	0.000
-2.5	8.5	2.320	0.001
-3	11.5	2.582	0.025
-4	18	0.160	0.875 ^{ns}
-5	17	-0.529	0.604 ^{ns}

Table. 3.7. A comparison of % loss of conductance values at different xylem potentials, using t-tests to compare *Banksia menziesii* data from the bottom slope with *B. menziesii* data from the dune crest site.

Xylem potential (MPa)	**d.f**	**t**	**sig.**
-0.5	8	5.345	0.001
-1	8	5.060	0.001
-1.5	8	2.276	0.052 ^{ns}
-2	8	1.217	0.258 ^{ns}
-2.5	8	2.877	0.021
-3	8	2.636	0.030
-4	8	-0.236	0.819 ^{ns}
-5	7	-0.578	0.581 ^{ns}

Table 3.8. A comparison of % loss of conductance values at different xylem potentials, using t-tests to compare *Banksia ilicifolia* data from the bottom slope with *B. ilicifolia* data from the *B. ilicifolia* high sites.

Xylem potential (MPa)	**d.f**	**t**	**sig.**
-0.5	18	0.953	0.353 ^{ns}
-1	18	1.756	0.096 ^{ns}
-1.5	18	1.037	0.313 ^{ns}
-2	17	1.651	0.117 ^{ns}
-2.5	8.5	1.065	0.316 ^{ns}
-3	18	7.490	0.153 ^{ns}
-4	18	1.066	0.300 ^{ns}
-5	6	-0.129	0.902 ^{ns}

Stem-specific conductivity (K_s) was highest at the more xeric site for each of the study species. This difference was statistically significant for *B. menziesii* with a higher K_s at the dune crest site in contrast to the bottom slope site (P=0.009). There were no significant differences found for *B. attenuata* or *B. ilicifolia*, though K_s was higher for each of these species at the dune crest and mid slope site respectively (Fig. 3.6).

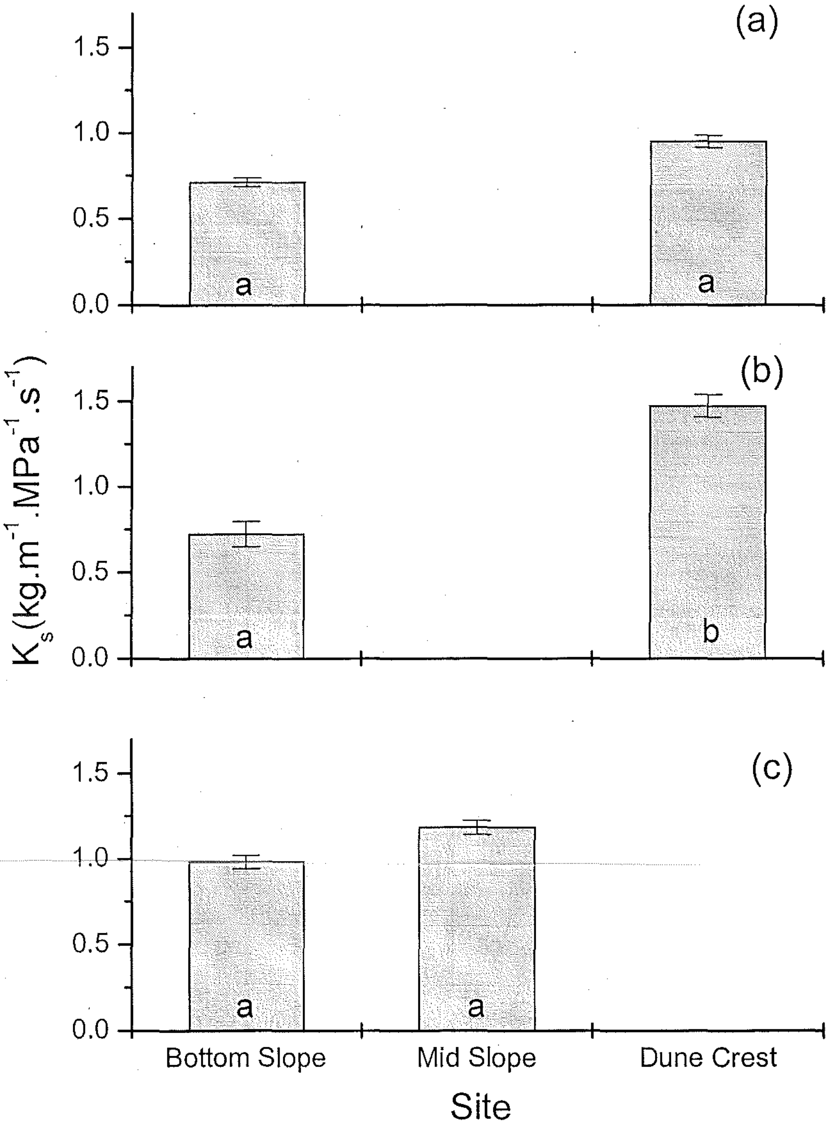

Figure 3.6. Stem-specific hydraulic conductivity (K_s) for (a) *Banksia attenuata* (n=10), (b) *Banksia menziesii* (n=5) and (c) *Banksia ilicifolia* (n=10) at contrasting ecohydrological habitats. Plotted values are means ± 1SE. Different letters indicate significant differences between sites, *B. menziesii* (df. = 8; t = -3.391; sig. = 0.009) and no significant differences for *B. attenuata* (df. = 18; t = -1.754; Sig. = 0.096) and *B. ilicifolia* (df. = 18; t = -1.754; Sig. = 0.096)

Leaf-specific conductivity (K_L) values was determined to be slightly higher at the dune crest site for the two facultative phreatophyte species (*B. attenuata* and *B. menziesii*). For the obligate phreatophyte, *B. ilicifolia*, K_L was lower at the mid-slope site in contrast to the bottom slope site for the (Fig. 3.7). These differences were not found to be statistically significant.

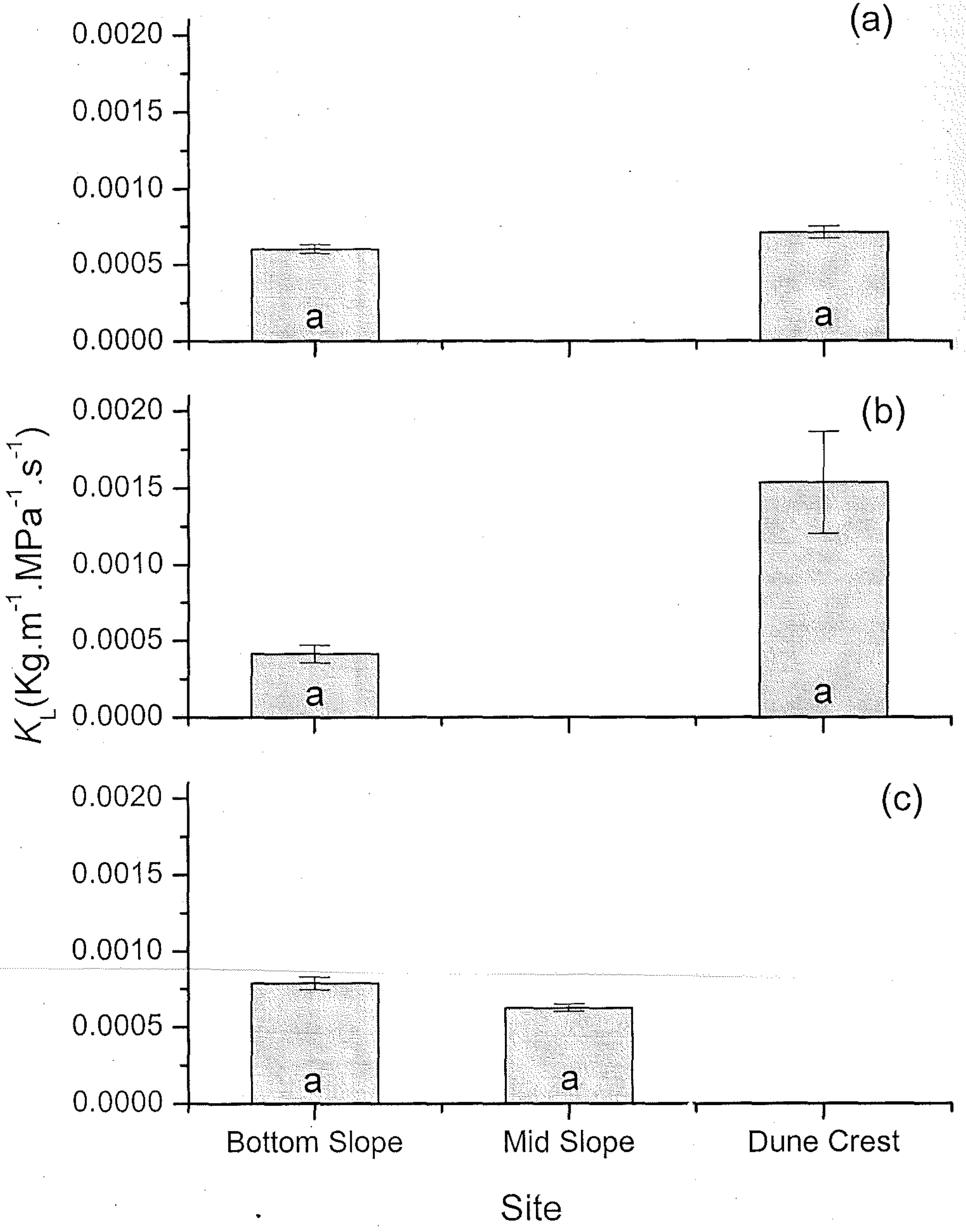

Figure 3.7. Leaf-specific hydraulic conductivity (K_L) for (a) *Banksia attenuata* (*n*=10), (b) *Banksia menziesii* (*n*=5) and (c) *Banksia ilicifolia* (*n*=10) at contrasting ecohydrological habitats. Plotted values are means ± 1SE. Same letters indicate no significant differences being identified between sites: *B. attenuata* (df. = 18; t = -0.696; sig. = 0.495); *B. menziesii* (df. = 8; t = -1.49; sig. = 0.175) and *B. ilicifolia* (df. = 17; t = 1.044; sig. = 0.311).

Similar to K_L values, Huber values were determined to be higher at the dune crest site in comparison to the bottom slope site for the two facultative phreatophyte species (Fig. 3.8). The Huber value was lower for *B. ilicifolia* at the mid-slope site in contrast to the bottom slope site, also reflecting the results for K_L values. However, none of these differences were determined to be statistically significant.

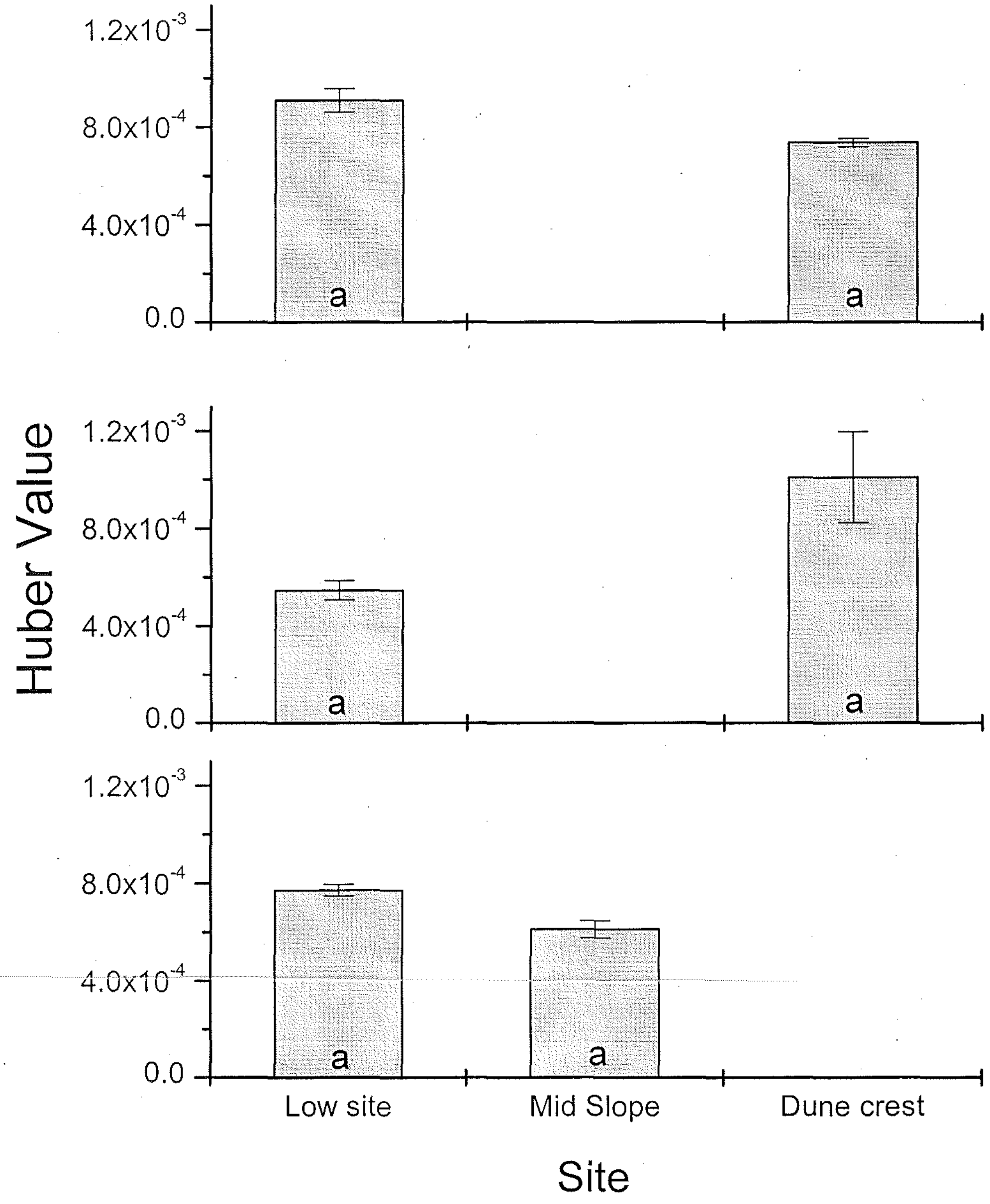

Figure 3.8. Huber values (ratio of twig cross-sectional area to leaf area) for (a) *Banksia attenuata* (n=10), (b) *Banksia menziesii* (n=5) and (c) *Banksia ilicifolia* (n=10) at contrasting locations in the landscape in relation to the ecohydrological gradient. Plotted values are means ± 1SE. Same letters indicate no significant differences between sites: *B. attenuata* (df. = 18; t = 1.090; sig. = 0.290); *B. menziesii* (df. = 8; t = -1.089; sig. = 0.308) and *B. ilicifolia* (df. = 17; t = 1.234; sig. = 0.234)

4. Discussion

4.1 Interspecific differences in vulnerability to xylem cavitation

The hydraulic architecture of individuals of four *Banksia* species was investigated at the same ecohydrological habitat (bottom slope site) to minimise confounding environmental factors. Many studies on hydraulic architecture have compared riparian species with more xeric species and there is general agreement that riparian species are more vulnerable to xylem cavitation (Tyree *et al.* 1992; Lopez, *et al.* 2005). There have been suggestions that this pattern might also apply to obligate and facultative phreatophytes (Froend & Drake 2006). Froend and Drake (2006) reported that the obligate phreatophyte species *B. ilicifolia* and *Melaleuca preissiana* were more vulnerable to xylem embolism than the facultative phreatophyte species *B. attenuata* and *B. menziesii*. However, contrary to the preliminary findings of Froend and Drake (2006), this more comprehensive study showed both congeneric obligate and facultative phreatophyte species, in the same ecohydrological habitat, have the same vulnerability to xylem embolism.

Preliminary findings also demonstrated consistently lower PLC values than those determined in this study (Froend & Drake 2006). Froend and Drake (2006) suggested that PLC_{20} is a more appropriate point of comparison. However, this current study compared the actual values determined for each applied pressure and found that the differences (or lack thereof) that were evident at PLC_{20} were also present at PLC_{50}. Since the majority of other studies compare PLC_{50} values (Pammenter & Vander Willigen 1998; Martínez-Vilalta *et al.* 2002; Maherali *et al.* 2004), these are used to make comparisons in this study. PLC_{50} for *B. attenuata* was determined to be -2.69 MPa by Froend and Drake (2006), compared to -1.44 MPa and -2.51 MPa at the bottom slope and dune crest, respectively. The discrepancies were larger still for *B. menziesii* and *B. ilicifolia*. Froend and Drake (2006) reported a PLC_{50} of -3.24 MPa for *B. menziesii*, much lower than the -2.23 MPa at the dune crest site in this study. This was similar for *B. ilicifolia*, with a PLC_{50} value of -2.84 MPa compared to -1.78 MPa at the mid-slope site in this current study. The differences in vulnerability curves may be an artefact of the different methods used to induce embolism in stems. The previous study used the air-drying method to determine vulnerability curves, meaning that several stems ($n = 4$) were used to produce one curve. In contrast, the air-injection method used in this study allows for a curve to be made from one stem through a series of different applied pressures. More replications of full vulnerability curves can therefore be made in the same amount of time it takes to make one full curve using the air-drying method. Correspondingly, the differences may be attributed to the disparity in number of replicates for each point on the curve. For example, at -1 MPa on the bottom slope *B. ilicifolia* vulnerability curve there are 10 replicates. In contrast, at this xylem potential on the curve for the same species in the Froend and Drake (2006) study only one data point is determined.

Others have also found that vulnerability curves determined using the air-drying method give more resistant curves than those developed using the air-injection method. Pockman and Sperry (2000) compared the two methods when determining the vulnerability curve for *Larrea tridentata.* The air-injection method gave a more vulnerable curve than the air-drying method. Similarly Lopez *et al.* (2005) found that PLC_{50} was lower using the air-drying method in contrast to the air-injection method when determining the vulnerability curves for *Cordia alliodora.* However, Sperry and Saliendra (1994) found that vulnerability curves determined with both techniques were "indistinguishable" from each other. Therefore, air-injection is a valid method for inducing xylem cavitation, though vulnerability curves may vary from those obtained using air-drying or centrifugal force and this should be acknowledged in the interpretation of results.

It should also be noted that in the current study, there was consistently more variation (ie. larger standard error) in PLC at the higher xylem potentials. This has generally not been addressed in the literature and does not always appear to occur, irrespective of the method used to induce cavitation. Similar to the results of this current study, Hacke *et al.* (2006) reported vulnerability curves that had larger standard errors at less negative xylem potentials, though they used centrifugal force. Vulnerability curves for *Acer grandidentatum* determined using the air-injection method had consistently large standard errors at each xylem potential (Alder *et al.* 1996). Vilagrosa *et al.* (2003) used the air-drying method and reported vulnerability curves that had larger standard errors in the middle section of the curve (approx. PLC_{20} to PLC_{80}). There may therefore be no significance in the pattern of standard errors observed in this current study.

The age of the stems used may have contributed to the relatively high PLC_{50} values reported in this study in comparison to the results of Froend and Drake (2006). It has been reported that embolised xylem can become refilled and that they often undergo a cavitation and refilling cycle. Cavitation fatigue has been observed previously, with stems that have undergone a cavitation-refilling cycle being found to be more vulnerable than stems that had not experienced water-stress. It has been suggested that this is due to weakened interfibrillar bonds in the pit membrane of xylem that have been exposed to water-stress (Hacke & Sperry 2001). It may be the stems used in this current study (aged 4 to 6 years) demonstrated some degree of cavitation fatigue. However, as it was a comparative study and used similar aged plant material to make inter- and intraspecific comparisons, the influence of cavitation fatigue on the interpretation of results should be minimal.

The absence of interspecific differences between obligate and facultative species shown in this study may be further explained. The distinction between phreatophyte types could be confounded if the study species are congeneric with associated similarities in xylem structure. Cavender-Bares *et al.* (2004) suggested that closely related species that inhabit similar environmental conditions will exhibit similar phenotypic traits. The four species in this study show different distributional ranges along the ecohydrological gradient. However, at a single ecohydrological habitat the effects of site conditions on plant development appear to be important. At the bottom slope site all four species, both obligate and facultative phreatophytes, developed in conditions where access to water is relatively unrestricted, with water available at higher water potentials (closer to 0 MPa) for the majority of the seasonal cycle. Water is less likely to be a limiting factor at the lower ecohydrological habitat and as a consequence there may be no selection pressure for xylem resistant to cavitation (Lopez *et al.* 2005). It is probable the relatedness of the species, combined with them all having the same exposure to water stress (ie. at the same ecohydrological habitat), is the strongest explanation for the lack of variation in vulnerability to xylem embolism.

There was some interspecific variation in the other parameters of hydraulic architecture that were measured. Huber values were found to be significantly lowest for *B. littoralis*, which suggests that this species invests less sapwood in proportion to leaf area. This is supported by the low K_L values observed for *B. littoralis*. Edwards (2006) reported that there was no correlation between *K*s and K_L for *Pereskia* species. It was suggested that Huber value, and thus carbon allocation, may be more closely related to K_L than hydraulic architecture, as measured by K_s (Edwards 2006). *B. littoralis* may therefore be relatively efficient in water transport without having to invest in wood (ie. increase its stem diameter). In contrast *B. attenuata* had a relatively low K_s value, but a higher K_L and a statistically significant higher Huber value than *B. littoralis*. This suggests that *B. attenuata* has invested more stem area to support its leaves. These differences demonstrate that there is some interspecific variation in hydraulic architecture, despite there being no significant differences in vulnerability to xylem cavitation.

4.2 Intraspecific differences in vulnerability to xylem embolism

To address hypothesis 2 of the study, intraspecific differences in hydraulic architecture, particularly vulnerability to xylem embolism, associated with different ecohydrological habitats were determined. Other studies have suggested that plants that occupy drier habitats are more resistant to xylem cavitation (Engelbrecht *et al.* 2000; Piñol & Sala 2000; Froend & Drake 2006). It was thus expected that trees sampled at the dune crest site (the drier habitat) would be more resistant to xylem cavitation. The results obtained generally agree with this hypothesis, with both facultative phreatophyte species (*B. attenuata* and *B. menziesii*) being more resistant at the dune crest site compared to the bottom slope site. However, the obligate phreatophyte, *B. ilicifolia*, was found to have no significant intraspecific differences in vulnerability at two contrasting ecohydrological habitats. The primary difference between these sites is the availability of a reliable water source, in particular groundwater. Individuals of the two facultative species at the dune crest site have experienced drier conditions, having to access water at lower potentials than the individuals at the bottom slope site. Plants at the dune crest site are reliant upon precipitation, with localised recharge of stored soil moisture being their only water source. Over summer this water source is depleted through transpiration and is not recharged again until the next precipitation event and it is during this period that plants experience the most water stress. Even during wetter months, water is less accessible to plants at the dune crest site compared to the bottom slope site (Zencich *et al.* 2002). At the dune crest site water is quick to infiltrate, with the coarse sands having a low water-holding capacity. There is therefore less plant available water, particularly during summer, at the dune crest site than the bottom slope site. A higher resistance to xylem cavitation is a beneficial trait for an individual that occurs in an environment where water availability is more limiting (Maherali *et al.* 2004). *B. attenuata* and *B. menziesii* at the dune crest site have developed in conditions where water is less available and more variable in contrast to individuals of the same species at the bottom slope site and have xylem that are more resistant to water stress induced cavitation. It appears that these two species have been able to adjust their hydraulic architecture to be able to inhabit this more variable and xeric environment, as indicated by decreased vulnerability to water stress. It may be inferred that ontogeny, rather than phylogeny, is having a greater influence on plant vulnerability to water stress as illustrated by the *B. attenuata* and the *B. menziesii* that established in the drier, water limited environment being more resistant.

Water availability has become established as a determining factor of hydraulic architecture with many studies demonstrating interspecific differences in vulnerability to xylem embolism in relation to water availability. Hacke *et al.* (2000) conducted a study similar to this current one, comparing inter- and intraspecific differences in vulnerability to xylem embolism for plants that experience varying degrees of water stress. The phreatophytic species *Chrysothamnus nauseosus* was compared with a 'summer green' group and a drought deciduous group that drops its leaves during summer drought. It was found that the phreatophytic species was most vulnerable to embolism and this was attributed to it not having experienced drought, as it has access to a reliable water source. In contrast, *C. viscidiflorus*, a shallow rooted summer evergreen which has limited access to water over summer, was more resistant to xylem cavitation. This supports the observations in this study that the facultative phreatophyte species at the dune crest site were more resistant to cavitation than individuals of the same species at the bottom slope site. The primary difference between the populations is access to a reliable water resource and it appears that some plants are able to adjust their vulnerability to xylem cavitation in response to water availability. Similarly, a comparison of sub-species of *Artemisia tridentate* in the Great Basin of North America found that there was a gradient of resistance to xylem embolism in correlation with water availability (Kolb & Sperry 1999). The sub-species that occurred in an arid environment was significantly less vulnerable to xylem cavitation than the sub-species that occurred in a mesic environment. Brodribb and Hill (1999) compared hydraulic architecture of 10 conifer species that occupied different climates. At one extreme *Actinostrobus acuminatus*, a semi-arid shrub from Western Australia, which occupies a habitat where mean quarterly rainfall is 14 mm had a PLC_{50} value of -14.2 MPa. In contrast *Dacrycarpus dacrydiodes,* a rainforest tree from New Zealand which grows in an areas with a mean quarterly rainfall of 378 mm had a PLC_{50} value of -2.3 MPa. This pattern was similar for the other 8 species in the study, which suggests that water availability is a primary determining factor of vulnerability to xylem cavitation.

The majority of studies, such as the ones previously discussed, investigate differences in vulnerability to water stress between species in contrasting environments. More relevant to this current study are investigations of *intraspecific* differences in vulnerability to water stress. Alder *et al.* (1996) found that there was an increase in root cavitation resistance for *Acer grandidentatum* at a more xeric upslope site in contrast to individuals of the same species at a more mesic downslope site. Similarly, Pockman & Sperry (2000) compared riparian and upland populations of *Baccharis sarothroides* and *Prosopis velutina.* It was once again found that the upland populations of each species were less vulnerable to xylem embolism than the populations at the 'wetter' riparian site. Holste *et al.* (2006) conducted a glasshouse experiment, testing the effect of different environmental conditions on the hydraulic architecture of *Phaseolus vulgaris.* The study found that plants exposed to dry conditions, therefore water stress, during development were less susceptible to xylem cavitation than the well-watered control plants. There is thus a strong trend between water availability and resistance to xylem embolism and the results for *B. attenuata* and *B. menziesii* in this current study agree with this trend.

The lack of significant differences between the populations of *B. ilicifolia* may be due to of a number of factors. *B. ilicifolia* does not occupy areas that are vastly different from each other in terms of water availability since it has a limited distribution. It only occurs in areas where depth to groundwater less than about 8 m (Arrowsmith 1992). It may be that this species does not experience the same degree of water stress as *B. attenuata* and *B. menziesii* due to its relative proximity to groundwater and the capillary fringe. It may be expected that the similarity of water availability at sites colonized by *B. ilicifolia* and its year round use of groundwater (Zencich *et al.* 2002) is reflected in the similarities in vulnerability to xylem embolism. However, the more constrained distribution of *B. ilicifolia* may in fact be a consequence of a lack of plasticity in its ability to alter xylem structure. These results may further confirm the classification of *B. ilicifolia* as an obligate phreatophyte, since it appears that it requires a reliable water source due to its inability to alter its xylem structure.

The vulnerability curves determined in this study were all of a similar shape, as reflected by coefficient a values. The coefficient a relates to xylem structure, particularly the distribution of the largest pit pore in each vessel, whilst PLC_{50} relates to the mean maximum size of pit pores for all vessels (Pammenter & Vander Willegen 1998). The similarities in a observed in this study may reflect the relatedness of the congeneric species used, indicating that xylem structure is similar between species. It may be inferred that the main difference between the more resistant dune crest individuals and the bottom slope trees is the mean maximum pit pore size. For example *B. attenuata* at the dune crest site may have larger pit pores than bottom slope *B. attenuata* (as indicated by PLC_{50}), but the distribution of pores is similar (as indicated by a).

It was originally expected that the trees that have a more reliable access to water would exhibit higher K_s values, in accordance with the theory of safety vs. conducting efficiency (Zimmermann 1983). A number of studies have found that in habitats where water availability is not a limiting factor species will exhibit high K_s values (Kolb & Davis 1994; Martinez-Vilalta *et al.* 2002). It is thought that higher specific conductivity in plants is related to increased susceptibility to xylem embolism. High specific conductivity can be related to wider xylem vessels and larger interconduit pit pores. According to the air seeding hypothesis, larger pores are more likely to permit air into a vessel as xylem potential decreases, thus making xylem with larger pores more likely to cavitate (Zimmermann 1983; Hacke *et al.* 2006). Contrary to expectation, the dune crest populations all exhibited higher K_s values than the bottom slope trees, with *B. menziesii* in particular having statistically significant higher K_s values. However, others have reported similar results. Pockman and Sperry (2000) found that *Tamarix ramosissima* was relatively resistant to xylem cavitation and had a high specific conductivity. Maherali and DeLucia (2000) found that Ponderosa pines that occurred in a desert had higher K_s than individuals of the same species that grew in a montane habit. However, no significant differences in vulnerability to xylem embolism were found. It was suggested that the K_s values were associated with the higher evaporative demand at the desert site, thus a need to transport water more efficiently. It may be noted that there is no strong evolutionary relationship between specific conductivity (K_s) and vulnerability to embolism in angiosperms, despite the fact that there is an established relationship between pit pore size and vulnerability to embolism (Maherali *et al.* 2004). This has been attributed to the influence of other traits, which also determine specific conductivity, but do not necessarily relate to xylem cavitation, such as increased vessel length. It has been proposed that in some cases a correlation between resistance to xylem embolism and high specific conductivity is due to an increase in hydraulic efficiency (Maherali *et al.* 2004). This allows a plant to maintain the water column at increasingly negative xylem potentials, avoiding cavitation (Maherali & DeLucia 2000). Thus, this study of *Banksia* agrees with earlier studies that showed no trend between K_s and vulnerability to xylem embolism (Kavanagh, *et al.* 1999; Maherali *et al.* 2004).

Xylem reinforcement may be better correlated with vulnerability to embolism than the diameter of the conduit, with studies reporting that plants with more resistant xylem also have thicker secondary xylem walls (Hacke & Sperry 2001; Holste *et al.* 2006). This suggests that the facultative phreatophyte species (*B. attenuata* and *B. menziesii*) at the dune crest site may have thicker secondary xylem walls, or more reinforcing, than the individuals of the same species at the bottom slope site. The reinforcement of xylem may help increase resistance to xylem cavitation, particularly for plants that experience regular water stress (Holste *et al.* 2006). However, it is often found that K_s decreases as a trade-off to thickened xylem walls (Pitterman *et al.* 2006). This was not found in this study, since the more resistant xylem at the dune crest habitat were also found have higher K_s values. It may therefore be inferred that xylem wall thickening was not associated with the increased resistance to xylem cavitation, though this would have to be confirmed through further studies of xylem anatomy.

There was no clear trend in an intraspecific comparison of Huber values and leaf specific hydraulic conductance between the bottom slope, mid slope and dune crest sites. It was expected that a higher Huber value would be associated with increased resistance to xylem embolism. A high Huber value means that there is more conducting wood per unit of leaf material (Tyree & Ewers 1991). If xylem vessels are reinforced to provide better resistance to cavitation, then it would be expected that there is more wood as this material provides the reinforcement. Also, it is often found that plants in more xeric environments have smaller leaf area, in response to less water availability

(Mencuccini 2003). This would also increase Huber values such as those found for *B. menziesii* at the dune crest site; however this was not statistically significant. There was little difference in Huber values between the dune crest and bottom slope sites for *B. attenuata* and the mid and bottom slope sites for *B. ilicifolia.* Leaf-specific conductivity gives a measure of the sufficiency of a stem to supply water to the leaves distal to the stem. *B. menziesii* had the largest K_L out of the study species, which correlates with the Huber value that was observed. It appears that *B. menziesii* at the dune crest site has a large investment in wood area in relation to leaf area. The large Huber value may be required to maintain the large supply of water to the leaves, as indicated by K_L. It should be noted that K_L and Huber values for *B. menziesii* had a large standard error and that these differences were not found to be significantly different.

4.3 Phenotypic Plasticity

The overall appearance of an organism is a result of its genotype and its interaction with the environment during development; resulting in its phenotype. Phenotypic plasticity refers to the ability of an organism to modify the expression of its genotype in response to its environment (Bradshaw 1965). The dry conditions experienced by the *B. attenuata* and *B. menziesii* populations at the dune crest site appear to have contributed to increased resistance to xylem cavitation. Water availability is not a limiting factor for trees at the bottom slope site and there may be no selection pressure for more resistant xylem. In contrast, resistant xylem is beneficial at a site where water availability is an issue, such as at the dune crest site. Facultative phreatophytes, *B. attenuata* and *B. menziesii,* appear to be more 'plastic' in response to environmental conditions in contrast to obligate phreatophyte species.

A limited number of studies have suggested phenotypic plasticity in vulnerability to xylem embolism in response to a gradient of water availability and/or climate. Sperry and Ikeda (1997) found that roots of *Psuedotsuga menziesii* at a more xeric upslope site were more vulnerable than roots from a down-slope site. It was suggested that this species is plastic in its ability to make adjustments to vulnerability to xylem embolism. Maherali *et al.* (2002) found that differences in hydraulic architecture in *Ponderosa* pines in contrasting climates are not due to ecotypic divergence. It was found that there was some genetic differentiation among populations, but it was not attributable to geographic distance or differences in climate (Maherali *et al.* 2002). It was instead concluded that differences in the hydraulic architecture between populations was due to phenotypic plasticity in response to different environmental conditions.

The Lexia study site is a heterogenous environment with regards to the undulating sand dunes over the superficial aquifer. There are differences in the depth to groundwater and the distribution of the dominant canopy species reflects this. It appears that *B. attenuata* and *B. menziesii* have sufficient plasticity in hydraulic architecture, in particular xylem resistance to embolism, to occupy a broader range in ecohydrological habitat. The obligate phreatophyte, *B. ilicifolia* does not demonstrate this plasticity. Facultative phreatophytic species, such as *B. attenuata* and *B. menziesii*, may be able to occupy areas without access to groundwater since they are able to adjust their hydraulic architecture. In contrast, the vulnerability curve for *B. ilicifolia* did not differ between sites, suggesting that this obligate phreatophyte is less plastic in its hydraulic architecture.

4.4 Conclusions

This study has shown that ecohydrological habitats can determine hydraulic architecture, particularly vulnerability to xylem embolism. At the same ecohydrological habitat where water is readily accessible (bottom slope site) there were no interspecific differences in vulnerability to water stress. It appears that there is no selection pressure for resistant xylem in the more mesic environment where plants experience little water stress. In contrast, the facultative phreatophytes, *B. attenuata* and *B. menziesii*, appeared to be plastic in vulnerability to embolism in response to developing in a more xeric environment. *B. ilicifolia* did not differ in vulnerability to embolism, supporting its classification as an obligate phreatophyte.

4.5 Consequences for management

An understanding of phreatophyte response to decreased water availability is essential for determining ecological water requirements for environmental water allocation. The results of this study suggest that vulnerability curves may be usefully incorporated into future research that contributes to groundwater management. Vulnerability curves are an inexpensive and logistically viable surrogate to *in situ* groundwater drawdown experiments to get an indication of plant response to decreased water availability (Froend & Drake 2006). Vulnerability curves provide a quantitative measure of relative vulnerabilities to water stress, data that is necessary for the quantitative modelling of *Banksia* woodland response to groundwater drawdown. This quantitative measure of plant susceptibility to groundwater drawdown combined with quantitative measurements of environmental attributes provides for a more sophisticated capacity for vegetation response modelling and water resource management.

This study has built upon the results of Froend and Drake (2006), providing a more comprehensive study of interspecific differences in xylem cavitation for four co-occurring *Banksia* species. This current study, contrary to preliminary investigations, demonstrates that at the same location all four *Banksia* species have the same response to water stress. In an ecohydrological habitat where a reliable water source is almost constant, such as that afforded by the bottom slope site, there is insufficient antecedent environmental pressure to develop significant differences in vulnerability to xylem cavitation between species. Development of hydraulic architecture in such a habitat confers an elevated susceptibility to cavitation if and/or when water availability is reduced. Thus, all four *Banksia* species are relatively vulnerable to any future decreases in groundwater levels. If changes in water availability persist the data from this study suggest that obligate phreatophytes would be lost from low-lying areas, whilst facultative phreatophytes would re-establish under these drier conditions. Long-term monitoring of Banksia woodland on the Gnangara Mound has previously identified changes in the overstorey composition in response to a gradual decline in groundwater (Groom *et al.* 2000, Groom *et al.* 2001). In particular, a decline in the obligate phreatophyte, *B. ilicifolia,* and the replacement of this species with the more water stress tolerant facultative phreatophytes, *B. attenuata* and *B. menziesii* has been observed (Groom *et al.* 2000). It may be inferred that this is in part due to the lack of plasticity in hydraulic architecture for *B. ilicifolia* as suggested by the results obtained in this study.

Froend and Drake (2006) also suggested that there may be intraspecific variation in vulnerability to water stress in relation to different locations on an ecohydrological gradient. This study has demonstrated that there are intraspecific differences, highlighting the importance of site hydrological attributes in determining plant vulnerability to water stress, as was also inferred by Zencich *et al.* (2002). The results from this study may feed into the larger research programme investigating *Banksia* response to groundwater decline over the Gnangara Mound. The main contribution of this study is an understanding of intraspecific differences for facultative phreatophytes and the importance of site hydrological attributes in determining plant susceptibility to decreased water availability.

4.5 Study limitations and suggestions for further research

This study focussed on determining the vulnerability curves for obligate and facultative phreatophytes. Vulnerability curves give an indication of how plants may respond to water stress, but as discussed previously, there are limitations in the methods used to determine them. As demonstrated, there can be discrepancies between studies that used different methods to induce cavitation, such as the differences between Froend and Drake (2006) and this current study. These differences should be acknowledged in the interpretation of data. Also, this study only looked at stem vulnerability to embolism. It is often noted that roots are more vulnerable to xylem embolism and may be more limiting to a plant than xylem in the stem (Tyree & Ewers 1991; Sperry & Ikeda 1997; Kavanagh *et al.* 1999). Future studies may wish to investigate this further to determine if *Banksia* roots are more vulnerable to embolism than the stems and to determine if this is more limiting to the plant.

There are a number of parameters that may be measured to support the findings of this study. Previous studies have investigated the relationships between water availability and vessel characteristics (February *et al.* 1995). An investigation of xylem structure, particularly diameter and secondary xylem wall thickening would provide considerable support for the observations made in this study and provide more detail on the hydraulic architecture of *Banksia* species. The methodologies used for this could be similar to Pittermann *et al.* (2006). Transverse hand-sections of xylem should be made, dyed with toluidene blue, mounted on a slide and analysed under a microscope. Images of xylem tissues should be taken with microscope mounted camera. Image analysis software may then be used to measure xylem diameter and xylem wall thickness. This information then may be correlated with the vulnerability to xylem cavitation observed in this study.

Whilst xylem structure is extremely relevant in determining plant vulnerability to water stress, it has been demonstrated that stomatal control can also prevent embolism (Davies & Zhang 1991). It has been previously demonstrated that there can be a correlation between greater stomatal control and resistance to xylem embolism (Rood & Mahoney 1990; Sparks & Black 1999). Further studies may investigate if this correlation exists for the more resistant dune crest *Banksia* populations, determining if they have increased stomatal control in response to a drier environment.

Further research into the phenotypic plasticity in hydraulic architecture of *Banksia* species in response to decreased water availability may also be desirable. A relatively simple way of doing this would be a glasshouse trial, similar to that conducted by Holste *et al.* (2006). Individuals of *B. attenuata*, *B. menziesii* and *B. ilicifolia* should be grown in a controlled environment and in the same soil type. The control plants would be kept well watered, whilst the treatment plants would be kept under dry conditions. Vulnerability curves and xylem anatomy measurements could then be made. Based on the observations made in this study it would be hypothesized that individuals of the facultative species, *B. attenuata* and *B. menziesii*, grown under dry conditions will be more vulnerable to xylem embolism, whilst the obligate phreatophyte may not survive in the drier conditions, or does not differ between the control and the treatment.

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