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WOODY PLANT ADAPTATIONS TO WATER STRESS IN ARID SHRUB COMMUNITIES

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WOODY PLANT ADAPTATIONS TO WATER STRESS IN ARID SHRUB COMMUNITIES

By

Anna Linden Jacobsen

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Anna Linden Jacobsen

Plants vary greatly in the amount of water stress they experience and in their ability to maintain water transport during periods of water stress. Woody plants in arid shrub communities experience periods of extreme water stress and likely have evolved distinct mechanisms for tolerating this stress. This may be particularly important in species that maintain evergreen leaves and tolerate rather than avoid seasonal water stress. The present dissertation examines, at the cellular, tissue, and community levels, what xylem traits are associated with the xylem pressures experienced by arid woody plants and their ability to maintain a functioning water transport system in the face of water stress. Additionally, the role of xylem traits in community assembly and structure are examined. Water stress tolerance strategies among woody shrub species from Californian and South African shrub communities are compared to examine whether species from these communities are utilizing common or different water stress tolerating strategies.

Across arid communities, communities composed of woody shrub species shared a common mean water stress level although individual species varied greatly in the level of water stress they experienced. Species that experienced very low xylem water potentials seasonally had dense xylem due to reinforcement of both xylem vessel walls and the surrounding fiber matrix. These cellular traits were associated with individual and community level differences in cavitation resistance. Communities differed in the suites of traits used by species within each community, including differences in their xylem traits and in their stomatal response to water stress. These community specific water use traits may play a role in community assembly and suggest that communities may be differentially susceptible to invasion by non-native woody species. Comparable plant communities from the Mediterranean-type climate regions of California and South Africa appeared to be utilizing similar water use strategies, suggesting that there may be convergence in water use strategy for communities in comparable arid environments.

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KEY TO SYMBOLS OR ABBREVIATIONS

d	vessel diameter
d_{h}	mean hydraulic vessel diameter
g_{s}	stomatal conductance
g_{smin}	minimum seasonal stomatal conductance
g_{smax}	maximum seasonal stomatal conductance
K_{h}	hydraulic conductivity
K_{hmax}	maximum hydraulic conductivity
K_{l}	leaf specific hydraulic conductivity
K_{s}	xylem specific hydraulic conductivity
MOE	Modulus of Elasticity
MOR	Modulus of Rupture
PIC	phylogenetic independent contrast
PLC	percentage loss in hydraulic conductivity
P_{x}	xylem pressure potential
P_{min}	minimum seasonal xylem pressure potential
P_{50}	xylem pressure potential at 50% loss in hydraulic conductivity
P_{75}	xylem pressure potential at 75% loss in hydraulic conductivity
Ψ_{x}	xylem water potential
Ψ_{w}	water potential
Ψ_{min} or Ψ_{wmin}	minimum seasonal water potential
Ψ_{wmax}	maximum seasonal water potential

Ψ_{50}	water potential at 50% loss in hydraulic conductivity
Ψ_{75}	water potential at 75% loss in hydraulic conductivity
R*	level of a limiting resource at which there is no population growth
SLA	specific leaf area
$(t/b)_h^2$	theoretical vessel implosion resistance where *t* is the thickness of the double vessel wall between two vessels and *b* is the lumen breadth of a vessel near the hydraulic mean vessel diameter (h).

INTRODUCTION

Woody plant species differ in their ability to transport water and in their ability to resist failure of their hydraulic transport system during periods of water stress. Concomitantly, species also differ in the level of water stress that they experience, even among plants growing together at a common microsite. Arid woody plants, particularly evergreen species, experience extremely negative xylem pressures yet must also be able to maintain their water transport pathway to supply leaves. Thus, arid evergreen shrub species likely exhibit unique xylem adaptations in order to cope with the extreme negative pressures that can develop within their xylem during periods of limited soil moisture and high evaporative demand.

The present dissertation examines, at the cellular and tissue level, what xylem traits are associated with xylem pressures experienced by arid woody plants and their ability to maintain a functioning water transport system in the face of severe water stress. At the organismal and community levels, seasonal water relations of shrub species are compared. The relationship between plant water stress tolerance, functional traits, and the phylogenic relations among and distribution of species within and between communities is examined. Finally, water stress tolerance strategies among woody shrub species from two comparable regions global are examined as a test of how climate and plant morphological convergence may relate to how arid woody shrub species respond to water stress.

Plant water transport

Water is transported in plants under negative pressure via the soil-plant-atmosphere continuum (Cowan 1965). Water is "pulled" from the soil, through the plant, and out of leaves by the evaporative pull of the atmosphere. Water molecules are connected by cohesive forces among water molecules and adhesive forces to cell walls, allowing a column of water molecules to persist under considerable tension (*i.e.* the cohesion-tension theory of xylem sap ascent; Tyree & Zimmermann 2002).

The theoretical tensile strength of water quite is great (-130 to -150 MPa; Apfel 1972 cited in Tyree & Zimmermann 2002) and water columns have been measured withstanding pressures of -28 MPa (Briggs 1950), yet water columns within tracheary elements are subject to failure at much higher (less negative) pressures. Riparian plants may experience failure of all of the water conduits within stem xylem at relatively high pressures (> -1.0 MPa), while water columns within the xylem conduits of xeric angiosperms and many gymnosperms may remain intact at considerably lower pressures (< -12 MPa) (*e.g.* Pockman & Sperry 2000). These differences are likely due to size and structure of tracheary elements and inter-conduit pits and pit membranes (Sperry & Tyree 1988; Sperry *et al.* 1991; Sperry & Hacke 2004; Hacke *et al.* 2004). Differences may also be related to the mechanical strength of xylem vessels and their surrounding tissues, which may be susceptible to implosion under extreme negative pressures (Hacke *et al.* 2001*a*). Additionally, wall differences, damage to cell walls, or degradation or damage to vessel pits can affect the ability of the water column in a conduit to resist tension (Sperry *et al.* 1991; Hacke *et al.* 2001*a*; Tyree & Zimmermann 2002) as can changes in the concentration of solutes in the xylem which may lower the energy required to break the hydrogen bonds between water molecules (Sperry & Tyree 1988).

Hydraulic failure

Water stress induced cavitation of the xylem occurs when the cohesive bonds between water molecules fail. Because water columns are under negative pressure in the xylem, breakage of these intermolecular bonds results in rapid expansion of the gap in the water column and the filling of the created void in the water column with gas from adjacent gas filled cells or intercellular spaces and water vapor, in a process termed air-seeding (Sperry & Tyree 1988; Tyree & Zimmermann 2002). The resulting blockage of the xylem conduit by a gas bubble is a xylem embolism. Because cavitation events are rapidly followed by embolism formation, these terms are often used interchangeably.

Water stress induced failure of water transport in conduits may also occur due to mechanical implosion or inward bending of vessel or tracheid walls. Xylem conduits have been shown to collapse inward as xylem tensions increase (*i.e.* become more negative) (Cochard *et al.* 2004; Brodribb & Holbrook 2005). Resistance of xylem to failure has been shown to correlate to the mechanical strength of conduits against implosion which is estimated by the square of the thickness of the double vessel wall between two conduits (t) divided by the vessel lumen diameter (b) ($(t/b)_h^2$; Hacke *et al.* 2001a).

Cavitation and subsequent embolism can also be caused by freezing and thawing of the xylem sap, although the mechanism of embolism formation differs from that of water stress induced cavitation. Freezing-induced embolism is dependent on conduit volume (Ewers 1985; Davis *et al.* 1999b; Pittermann & Sperry 2003). When xylem sap freezes, gas that was dissolved in the xylem sap comes out of solution and forms bubbles. The larger the conduit, the larger are the bubbles which form in the xylem sap and larger bubbles take longer to dissolve back into solution upon thawing of the xylem sap increasing the likelihood that xylem tensions will pull on these bubbles causing them to expand. Xylem conduits with diameters greater that 43 to 44 μm appear to be particularly susceptible to freezing induced cavitation (Davis *et al.* 1999*b*; Pittermann & Sperry 2003).

Xylem embolism repair has been shown to occur in some species. Positive pressure of the xylem may occur through loading of solutes into the xylem when transpiration is limited. This may dissolve embolisms in the xylem, although positive root pressures are usually not great enough to dissolve embolisms in relatively tall woody plants, with few notable exceptions (Sperry *et al.* 1987; Fisher *et al.* 1997; Williams *et al.* 1997). Xylem embolism repair has also been shown to occur under negative pressures, but these pressures are generally very near to zero (Salleo *et al.* 1996; 2004; however see, Melcher *et al.* 2001). Thus, for most woody angiosperms, especially those in semi-arid or arid regions, repair of xylem embolism is unlikely and plants must either avoid high levels of embolism or be able to rapidly grow new xylem when water is available.

Woody plants vary greatly in their cavitation resistance (Maherali *et al.* 2004). The ability of woody plants to avoid cavitation can be estimated using a vulnerability to cavitation curve. Woody stems or roots can be dried down to known tensions (*i.e.* xylem pressure potentials or xylem water potentials) and the amount of embolism can be estimated by determining the loss in conductivity of the xylem. Negative xylem pressures can be generated using air-desiccation of shoots (Sperry *et al.* 1988) or by spinning stem or root segments in a centrifuge (Alder *et al.* 1997). Pressures in the xylem can also be generated using air-injection (Sperry & Saliendra 1994).

Xylem cellular and mechanical traits

Chapter 1 is a study of anatomical and biomechanical traits related to water stress tolerance in six chaparral species. This chapter investigates the possible mechanical and hydraulic costs to increased cavitation resistance. Not all plants in arid environments display high resistance to cavitation and woody angiosperms are seldom able to resist cavitation at pressures that are more negative than they regularly experience in the field suggesting that increased cavitation resistance may come at a cost.

Seasonal water relations

In Chapters 2 and 3 the seasonal water relations of shrub species in three different arid shrub communities, chaparral, coastal sage, and Mojave Desert scrub, are examined. Shrub species in these communities experience similar levels of water stress seasonally, yet differ in their resistance to cavitation. What are the xylem and leaf traits associated with different water stress tolerating strategies among species from these communities? Chapter 2 is a study of seasonal differences in cavitation resistance and seasonal hydraulics. In Chapter 3, seasonal measures of water status and stomatal conductance are compared across communities. This expands on the prior chapters, which have focused on xylem function and structure, to include other plant traits correlated with water stress tolerance, including specific leaf area (leaf area per leaf dry weight), stomatal response to water stress, and vegetative phenology (leaf longevity and abscission).

Community structure

In Chapters 4 and 5, plant structural and functional traits relating to water stress tolerance are used to predict species distributions within and between communities. In Chapter 4, the relationship between species traits and the phylogenetic relatedness of species are examined. The phylogenetic relationships among species have the potential to influence community assemblages and community structure. Additionally, plant functional traits may be associated with patterns of co-occurrence at local and regional scales. In Chapter 5, plant water status and soil moisture are used to predict community water use. This is then used to predict comparative invasibility among communities.

Convergence among Mediterranean-type climate regions.

In the last two chapters of this dissertation (Chapters 6 and 7), xylem tension, cavitation resistance, and xylem functional and anatomical traits are examined among several shrub species from the Mediterranean-type climate region of South Africa. This region is climatically similar to southern California, receiving primarily winter rain and experiencing a long, dry summer. In Chapter 6 (Jacobsen *et al.* 2007*a*), the negative tensions experience by 17 shrub species and the xylem anatomical and functional traits that are associated with experiencing more negative xylem tensions seasonally are examined. In Chapter 7, cavitation resistance of 15 South African shrub species is measured. These data are then analyzed in tandem with data from Chapters 2, 3, and 6 to examine how the water stress tolerating strategies of woody shrubs from South Africa and California compare. Are all woody arid shrubs utilizing a common water stress tolerating strategy or are there many different solutions to the mutual problem of seasonal water stress among aridland woody shrub species?

CHAPTER ONE

Jacobsen, A.L., Ewers, F.W., Pratt, R.B., Paddock, W.A., III & Davis, S.D. (2005) Do xylem fibers affect vessel cavitation resistance? *Plant Physiology,* **139**, 546-556.

DO XYLEM FIBERS AFFECT VESSEL CAVITATION RESISTANCE?

Abstract

Possible mechanical and hydraulic costs to increased cavitation resistance were examined among six co-occurring species of chaparral shrubs in southern California. We measured cavitation resistance (xylem pressure at 50% loss of hydraulic conductivity; P_{50}), seasonal low pressure potential (P_{min}), xylem conductive efficiency (specific conductivity; k_s), mechanical strength of stems (modulus of elasticity and modulus of rupture; MOE and MOR, respectively), and xylem density. At the cellular level, we measured vessel and fiber wall thickness and lumen diameter, transverse fiber wall and total lumen area, and estimated vessel implosion resistance using $(t/b)_h^2$ where "t" is the thickness of adjoining vessel walls and "b" is the vessel lumen diameter. Increased cavitation resistance was correlated with increased mechanical strength ($r^2 = 0.74$ and 0.76 for MOE and MOR, respectively), xylem density ($r^2 = 0.88$), and P_{min} ($r^2 = 0.96$). In contrast, cavitation resistance and P_{min} were not correlated with decreased k_s suggesting no tradeoff between these traits. At the cellular level, increased cavitation resistance was correlated with increased $(t/b)_h^2$ ($r^2 = 0.95$), increased transverse fiber wall area ($r^2 = 0.89$), and decreased fiber lumen area ($r^2 = 0.76$). The correlation between cavitation resistance and fiber wall area has not been previously shown and suggests a mechanical role for fibers in cavitation resistance. Fiber efficacy in prevention of vessel implosion, defined as inward bending or collapse of vessels, is discussed.

Introduction

Among vascular plants water is transported through xylem under negative pressure. Xylem must withstand both the mechanical stresses associated with negative pressure as well as the risk of air entering the hydraulic pathway. Failure to do so may lead to cavitation of water columns and blockage of water transport. Failure may occur when gas is pulled into water-filled xylem conduits from gas-filled cells or intercellular spaces through pores in the xylem pit membrane in a process referred to as air-seeding (Zimmermann 1983; Sperry & Tyree 1988; Baas *et al.* 2004). Failure may also occur when negative pressures overcome the ability of the xylem conduit walls to resist implosion (i.e. inward bending or collapse) (Carlquist 1975; Hacke *et al.* 2001*a*; Donaldson 2002; Cochard *et al.* 2004; Brodribb & Holbrook 2005). Implosion may trigger cavitation or, in leaves, may restrict hydraulic transport by reducing conduit diameter (Brodribb & Holbrook 2005). In addition to negative pressures, freezing can lead to failure when sap in the xylem freezes and the gas dissolved in the sap comes out of solution. This can lead to cavitation upon thawing if the bubbles do not go back into solution but instead expand (Yang & Tyree 1992). The result of cavitation is embolism or gas-blockage which reduces hydraulic transport and can result in reduced stomatal conductance (Pratt *et al.* 2005), reduced photosynthesis (Brodribb & Feild 2000), and dieback of branchlets (Rood *et al.* 2000; Davis *et al.* 2002).

Natural selection favors increased cavitation resistance among woody evergreen plants occurring in drought-prone environments; however, not all plants adapted to arid environments have high cavitation resistance (Pockman & Sperry 2000; Maherali *et al.* 2004). One explanation for this is that increased cavitation resistance may come at the cost of decreased xylem conductive efficiency, preventing plants from developing high levels of cavitation resistance. Additionally, a plant may have to develop thicker xylem walls and/or smaller conduit diameters to strengthen the resistance of a conduit to implosion under negative pressure (Hacke *et al.* 2001*a*). Such changes may increase xylem construction costs or decrease conductive efficiency.

Selection to resist freezing-induced cavitation is also linked to decreased xylem conductive efficiency. To resist freezing-induced cavitation a plant must have narrow vessel or tracheid diameters as resistance to freezing-induced cavitation is directly related to conduit diameter (Langan *et al.* 1997; Davis *et al.* 1999; Pittermann & Sperry 2003; Pratt *et al.* 2005). Narrowing of conduits to resist freezing-induced cavitation would lead to a decrease in hydraulic conductivity. Therefore, attempts to examine the relationship between resistance to water-stress induced cavitation and hydraulic efficiency may be confounded by selection to resist freezing-induced cavitation in environments that experience freezing temperatures.

In the present study, we examine the cost of increased cavitation resistance at a non-freezing site using a combined physiological and anatomical approach. We tested for several tradeoffs to increased cavitation resistance, including decreased conductive efficiency and increased xylem construction cost. Additionally, we expanded these traditional tradeoffs to include examination of a possible role for fibers in cavitation resistance. We sampled six species, examining cavitation resistance (xylem pressure at 50% loss of hydraulic conductivity; P_{50}), minimum seasonal pressure potential (P_{min}), xylem biomechanics (modulus of elasticity and modulus of rupture; MOE and MOR, respectively), xylem density, vessel and fiber anatomy, and xylem conductive efficiency (specific conductivity; k_s). These species correspond to three species pairs of chaparral shrubs from three angiosperm families all growing at the same coastal site where freezing temperatures and, presumably, freezing-induced cavitation do not occur. Using the species at this site allowed us to examine possible costs to water stress induced cavitation relatively independent of environmental differences including any limitations on vessel diameter resulting from selection to resist freezing-induced cavitation.

We hypothesized that increased mechanical strength (MOE and MOR) as well as xylem density would be correlated with increased cavitation resistance. We further hypothesized that the mechanical strength of stems would decrease with increasing water stress, since tension in the water column should exacerbate external mechanical stresses (Sperry & Hacke 2004). We hypothesized that increased cavitation resistance would come at the cost of decreased hydraulic efficiency. At the cellular level, we hypothesized that cavitation resistance would come at the cost of decreased vessel lumen diameter and increased thickness of xylem cell walls. Changes in either or both of these cellular dimensions would result in a greater thickness-to-diameter ratio ($(t/b)_h^2$) and would result in increased strength of cells against implosion under negative pressure (Hacke *et al.* 2001*a*).

Results

Mechanical properties of stems

Adenostoma fasciculatum, *A. sparsifolium*, and *Ceanothus megacarpus* showed great resistance to bending (high load required for displacement) followed by very sharp drop-offs, indicating sudden complete failure of stems (Figure 1.1 A and B). In contrast

Malosma laurina and *Rhus ovata* required less force for displacement and the resistance to displacement soon flattened out such that there was an extended period before complete failure; thus both partial and complete failure of stems occurred at very low load levels (Figure 1.1 C). *Ceanothus spinosus* had intermediate results compared to the other species (Figure 1.1 B).

The MOE and MOR were not different between our wet and dry treatments with two exceptions: the dry treatment did result in significantly greater MOR values in *C. megacarpus* and in significantly greater MOE in *C. spinosus* (Table 1.1). Stems of *C. spinosus* were dried down below the P_{min} measured in the field (-10.1 MPa compared to a P_{min} of -5.3 MPa); however, this value is not different from the P_{min} measured in previous years on this species ($P_{min} < -9$ MPa in 2002, data not shown) and is therefore still within the physiological range of this species. Modulus of rupture was positively correlated to MOE, both for the wet ($r^2 = 0.79$) and dry treatments ($r^2 = 0.87$; Figure 1.2). Linear regressions of MOE and MOR did not differ for wet and dry treatments in slope ($p = 0.72$) or intercept ($p = 0.84$; Figure 1.2); therefore, we used averaged results from the wet and dry treatments in our comparisons with other xylem parameters.

Cavitation resistance

The vulnerability curves are simplified down to one value, P_{50}, shown for each species in Table 1.1. The stems of *A. fasciculatum* (Figure 1.3 A) and *C. megacarpus* (Figure 1.3 B) were the most resistant to water stress induced cavitation, followed by *C. spinosus* and *A. sparsifolium*. The species most vulnerable to cavitation were *M. laurina* and *R. ovata* (Figure 1.3 C). *Malosma laurina* and *R. ovata* both displayed high levels of embolism at relatively high pressures (29 and 28% loss of hydraulic conductivity (PLC) at ≥-0.5 MPa) compared to the remaining four species (3.2 ± 1.6 PLC at ≥-0.5 MPa). This indicates that cavitation fatigue (sensu Hacke *et al.* 2001b) may have been present in *M. laurina* and *R. ovata*.

Stem traits

Stems that were more resistant to cavitation had stiffer (greater MOE) and stronger (greater MOR) stem tissue and experienced lower seasonal pressure potentials. Greater cavitation resistance was correlated to increased MOE ($r^2 = 0.74$; not shown), MOR ($r^2 = 0.76$; Figure 1.4 A), and xylem density ($r^2 = 0.88$; Figure 1.4 B). Resistance to cavitation (P_{50}) was positively correlated with P_{min} ($r^2 = 0.96$; Figure 1.4 C). Similarly, MOR was positively correlated with xylem density ($r^2 = 0.95$; Figure 1.4 D) and negatively correlated with P_{min} ($r^2 = 0.60$; Figure 1.4 E).

In contrast, xylem conductive efficiency (k_s) was not related to cavitation resistance or to measures of stem mechanical strength. Xylem conductive efficiency varied independently of P_{50} ($p = 0.15$), P_{min} ($p = 0.19$), MOE ($p = 0.20$), MOR ($p = 0.15$), and xylem density ($p = 0.11$; not shown).

Cellular traits

Percent transverse fiber wall area per total transverse xylem area ranged from 41.0% in *M. laurina* to 60.7% in *C. megacarpus*, with an across species mean of 53.3 ± 3.1%. Percent transverse vessel wall area per transverse xylem area occupied considerably less area and ranged from 3.5 to 5.5%, with an across species mean of 4.5 ± 0.3%.

At the cellular level, increased cavitation resistance and stem mechanical strength were associated with increased thickness of fiber cell walls. Resistance to cavitation (P_{50}) and MOR were correlated with increased strength against implosion of xylem vessel walls ($((t/b)_h^2$; $r^2 = 0.95$ and 0.85, respectively; Figure 1.5 A and D), increased percent transverse fiber wall area ($r^2 = 0.89$ and 0.71, respectively; Figure 1.5 B and E), decreased fiber lumen diameter ($r^2 = 0.84$ and 0.79, respectively; Figure 1.5 C and F), and decreased percent transverse lumen area ($r^2 = 0.76$ and 0.86, respectively; not shown). Modulus of rupture was also correlated with an increase in fiber wall thickness ($r^2 = 0.86$; not shown).

Increased vessel wall thickness was not correlated with increased cavitation resistance and mechanical strength. Vessel wall thickness was not correlated to P_{50} ($p = 0.56$), P_{min} ($p = 0.80$), MOE ($p = 0.37$), MOR (0.37), or xylem density ($p = 0.40$). Vessel wall thickness also was not correlated with fiber wall thickness ($p = 0.15$). Hydraulic vessel lumen diameter was correlated with xylem density ($r^2 = 0.67$), but was not correlated with cavitation resistance ($p = 0.07$; not shown).

Xylem conductive efficiency (k_s) was correlated with increased hydraulic vessel lumen diameter ($r^2 = 0.75$; not shown) and decreased fiber wall area ($r^2 = 0.75$; not shown). Xylem conductive efficiency was not correlated with total lumen area ($p = 0.06$; not shown).

DISCUSSION

Water stress induced cavitation in woody plants occurs when air is seeded into a functional conduit from an adjacent gas-filled cell or intercellular space (Jarbeau *et al.*

1995; Sperry *et al.* 1996; Tyree & Zimmermann 2002). Conduits may also cavitate following collapse under negative pressure. Conduit collapse may be reversible, depending on conduit structure and biomechanics (Cochard *et al.* 2004; Brodribb & Holbrook 2005). Although some have questioned the existence of these extreme negative pressures in the water transport system of woody plants (Zimmermann *et al.* 2004), the existence of tensions in the xylem is widely supported (see Angeles *et al.* 2004) and, presumably, plants have adapted to tolerate such stresses.

Hacke *et al.* (2001*a*) suggested that to resist collapse of conduits, plants that exhibit a high degree of cavitation resistance and that experience greater negative pressures display thicker vessel walls relative to their lumen diameter (i.e. higher $(t/b)_h^2$) and that such plants also have denser xylem. The link between vessel $(t/b)_h^2$ and xylem density is apparently due to correlated changes in vessel and fiber lumen to wall ratios (Hacke *et al.* 2001*a*; Sperry & Hacke 2004). Although data are lacking on the correlated nature of vessel and fiber lumen to wall ratios, its seems that the link between vessel $(t/b)_h^2$ and xylem density is dependent on this correlation since the density of xylem in angiosperms is primarily affected by the abundance and properties of fibers and only minimally affected by changes in vessel $(t/b)_h^2$. Our data supports the suggestion by Hacke *et al.* (2001*a*) that fiber and vessel properties are indeed correlated. Additionally, our results point to a possible role for fibers in increased vessel implosion resistance, an idea that was hypothesized, but not previously tested (Hacke *et al.* 2001*a*). Previous studies have not analyzed fiber anatomical properties independently and have relied on assumptions of fiber anatomical measures and mechanical properties. Here, we examine the role of changes in vessel and fiber anatomy and mechanical strength on cavitation resistance.

Vessel implosion resistance can be increased by a decrease in vessel lumen diameter, an increase in wall thickness, or both; however, the species included in this study did not appear to utilize all of these anatomical options. The variation in $(t/b)_h^2$ among species was largely due to changes in vessel lumen diameter and not to changes in vessel wall thickness. The range of vessel wall thicknesses among our sampled species was narrow (2.3 to 3.8 μm) and was not correlated with cavitation resistance (P_{50}) or minimum seasonal pressure potential (P_{min}). One interpretation would be that the sampled species were limited in their ability to alter their vessel wall thickness, limiting changes in vessel mechanics to changes in the lumen diameter. However, this would suggest that increased $(t/b)_h^2$ would correlate with decreased conduit efficiency, a tradeoff that was not observed. A second interpretation would be that other surrounding tissues, such as fibers, may act to strengthen vessel walls, somehow increasing resistance to cavitation without a necessary change in either vessel wall thickness or lumen diameter. Our data supports this second interpretation and suggests that viewing vessels as isolated pipes (i.e. $(t/b)_h^2$) may not fully describe their ability to resist mechanical stresses imposed by negative pressures. Indeed, strong correlations between fiber characters, cavitation resistance, and xylem density suggest fibers may be involved in cavitation resistance of woody plants. In the present study, fiber wall area was, on average, 53.3% of the total transverse xylem area, compared to less than 5% vessel wall area per transverse xylem area. Since more than ten times the biomass goes into fiber walls than vessel walls, the cost of fiber support for mechanical safety may be considerable in plants that experience very low xylem pressure potentials.

Direct evidence for fibers in preventing vessel collapse in stems is lacking; however, recent studies in leaves have demonstrated the importance of supportive tissue in the prevention of collapse. Under severe water stress, tracheids in conifer needles collapse preferentially when xylem conduits are adjacent non-strengthening parenchyma (Cochard *et al.* 2004), suggesting that the cells adjacent to a xylem conduit are important in preventing conduit collapse under negative pressure. The importance of the strength of the surrounding tissue is further supported by the finding that conifer stem tracheids, which are surrounded by a mechanically supportive tracheid matrix, did not exhibit collapse (Cochard *et al.* 2004). Brodribb and Holbrook (2005) similarly found that tracheids occurring outside leaf midveins exhibited collapse whereas tracheids within the more supported midveins did not display evidence of collapse. Vessel implosion has also been observed in stems of genetically modified angiosperms with weakened cell walls (Turner & Somerville 1997; Franke *et al.* 2002) demonstrating that mechanical strength of supportive tissue may be important in preventing implosion. The idea that the mechanical strength of stems is related to vessel implosion resistance contrasts with the idea that stem mechanical strength is mainly the result of selection for resistance to stem breakage due to wind or fruit-load (Wagner *et al.* 1998).

It has been hypothesized that stronger stems may be needed to resist whole stem bending which would exacerbate the internal stresses that occur during severe water stress (Sperry 2003; Sperry & Hacke 2004); however, our data do not support this. We found that stem mechanical strength did not vary between hydrated stems and stems dried down to their seasonal low pressure potential. Although *C. megacarpus* had greater MOR and *C. spinosus* had greater MOE in the dry treatment compared to the wet treatment, for five out of six species there was no difference in MOE or MOR with the treatments. This finding suggests that negative internal stresses within the stem do not affect whole stem bending strength. A caveat of these findings is that we do not know the xylem pressures while stems were being bent. Bending of vessels may have reduced vessel volume thereby reducing the negative pressures within the xylem. Regardless, the experiment did recreate the stresses that would occur with bending in intact hydrated and water-stressed stems in the field. A further criticism would be that we do not have evidence as to whether the fibers were embolized during the wet or dry treatments and, therefore, did not fully test the mechanism suggested by Sperry and Hacke (2004) of exacerbated bending stress with water stress. Thus, while we found no evidence that pressures are intensified in stems experiencing both water and bending stresses, this mechanism has not yet been fully tested.

It has also been hypothesized that stiffer and denser stems, which may be more resistant to vessel implosion, would be a mechanical liability because they are less flexible and more likely to break (Hacke *et al.* 2001*a*). Our data do not support this suggestion. For the species in this study, stems that were stiffer (i.e. less flexible; high MOE) and denser displayed the greatest strength (i.e. resistance to breakage; high MOR). This relationship did not vary with stem water stress suggesting that even under extreme water stress the stiffest stems remain the strongest and least likely to break.

Stem mechanical properties and cavitation resistance were unrelated to xylem conductive efficiency in the present study. This is consistent with previous studies that have surveyed many angiosperm families and found that there is only a weak tradeoff between hydraulic conductivity and cavitation resistance at best (Tyree *et al.* 1994; Maherali *et al.* 2004) and weak evidence for a tradeoff between conductivity and mechanical strength (Wagner *et al.* 1998). Tradeoffs may be present in the case of freezing-induced embolism where vessel diameter is directly related to susceptibility to cavitation (Cochard & Tyree 1990; Langan *et al.* 1997; Davis *et al.* 1999). The lack of a tradeoff between stem biomechanical traits and conductivity is perhaps because stem mechanics are largely dependent on fiber properties while conductivity is controlled by vessel properties. Xylem can be constructed in many different ways such that it can have a relatively high mechanical strength and simultaneously have high transport efficiency (Woodrum *et al.* 2003; Kern *et al.* 2005). In addition, certain factors impact mechanical properties of xylem but are independent of the number and diameter of vessels, including the amount of lignin in the fibers and the cellulose microfibril angle (Panshin & deZeeuw 1980; Niklas 1992).

Reinforcement of xylem tissue may represent a significant cost limiting increased cavitation resistance. Modulus of elasticity, MOR, and xylem density showed strong correlations with P_{50}, suggesting that stems that are mechanically stronger and denser are apparently able to withstand more negative pressures. This suggests that the fiber matrix may be important in increased resistance to cavitation. Partial implosion of vessel walls under negative pressure could lead to increased likelihood of air-seeding due to stretching or rupture of pit membranes (Zimmermann 1983; Sperry & Tyree 1988) or micro-fractures in other parts of the wall that could initiate cavitation (Pickard 1981) (Figure 1.6). The correlation between increased allocation to the fiber matrix and P_{50} support the suggestion by Niklas (1997) and Hacke *et al.* (2001*a*) that fibers play a key role in buttressing vessel walls against implosion under extreme negative pressure.

Pit membrane tears and microfracture of vessel walls may be more common than previously realized. Permanent implosion of stem tracheary elements has been observed only in plants with weakened cell walls that were deficient in lignin (Franke *et al.* 2002; Donaldson, 2002) or cellulose (Turner & Somerville 1997). The implosion observed by Cochard *et al.* (2004) and Brodribb and Holbrook (2005) was temporary and completely reversible, either with rehydration of the tissue or upon cavitation of the water columns, either of which would relieve tension on the walls. The reversibility of implosion means that implosion and resultant tears in the pit membrane or microfracture of the lignified cell walls might not be visible with conventional light microscopy and would be difficult to distinguish from artifacts with electron microscopy. However, some authors consider "checks" that are often seen in wood to be damage caused by negative pressure (Hunter 2001; Donaldson 2002).

For some species, once a tracheary element cavitates due to water stress it becomes more susceptible to cavitation following refilling and with subsequent water stress, i.e. a tracheary element is more susceptible to cavitation after a prior cavitation event. The phenomenon has been called "cavitation fatigue" (Hacke *et al.* 2001*b*). It has been suggested that cavitation fatigue may be the result of pit membrane damage or tearing (Hacke *et al.* 2001*b*). Consistent with our suggestion that the fiber matrix may play an important role in the prevention of implosion and possible permanent damage or weakening of vessels, we found evidence for fatigue in two species, ***M. laurina*** and ***R. ovata***. Both of these species have weak stems (low MOR), low transverse fiber wall area, and vessels that are predominantly surrounded by fibers. Additionally, the fiber walls are much thinner in these species (1.9 μm for both) compared to their vessel walls (3.8 and 3.4 μm, respectively), meaning that risk of implosion ($(t/b)_h^2$) may greatest between a vessel and its surrounding fibers rather than between two vessels. It is possible that the support of fibers helps to minimize cavitation fatigue; however, the mechanism for this remains to be elucidated.

Previous studies have found correlations between wood density and resistance to cavitation (Hacke *et al.* 2001a; Baas *et al.* 2004); however, no previous study has tested the relationship between stem mechanical strength and cavitation resistance. The correlations we found between wood anatomical traits and cavitation resistance suggest a greater role for fibers in imparting conductive safety than previously considered. While it is thought that resistance to cavitation is primarily a function of pore size in pit membranes (Jarbeau *et al.* 1995; Sperry *et al.* 1996), it may be that the mechanical properties of xylem fibers, as well as of other cell types associated with vessels including xylem parenchyma and, in some species, tracheids, are important in determining resistance to cavitation. Fibers may impact vessel implosion risk by reducing stresses that exacerbate pit membrane deflection or stresses that lead to vessel collapse and microfracture of the cell wall.

Materials and Methods

Study site

All plant material was collected at a site in the Santa Monica Mountains, 0.5 km south of Encinal Canyon Road, at an elevation of 480 m (34° 05' 27" N, 118° 50' 29" W). This site, described as "site 3" in Wagner *et al.* (1998), is a mixed stand of chaparral with a coastal exposure. Wildfires occurred at the site in 1956 and 1978, meaning that at the time of the study, in 2003, this was a mature chaparral stand with above ground shoots up to 26 years old. Measurements were made on six species of chaparral shrub, including: *Adenostoma fasciculatum* Hook. and Arn., *A. sparsifolium* Torrey, *Ceanothus megacarpus* Nutt., *C.spinosus* Nutt., *Malosma laurina* (Nutt.) Abrams (formerly *Rhus laurina*), and *Rhus ovata* S. Watson (nomenclature follows Hickman 1993).

Mechanical measurements

Twelve plants per species were marked and used for all measures. For comparison between stems dehydrated to their seasonal low water potentials and well hydrated stems, on each plant, two stems were identified that were very similar in size, appearance, exposure to the sun, and angle of inclination. The shoots, 1.5 to 2.0 m in length, were cut from the plant, immediately placed into plastic bags, and transported to the laboratory at Pepperdine University. In the laboratory, one shoot of each pair was re-cut at the base under water and the base was kept in water overnight with the aerial portions covered with a plastic bag. For the "wet" treatment twigs or leaves were sampled the next morning to measure the xylem pressure potential (P_x) using a pressure chamber (PMS, Corvalis, OR, USA) in order to confirm that the stems were rehydrated. The stems were then re-cut under water to a length of between 0.26 and 0.29 m and kept entirely submerged for another 24 hours. The "dry" treatment shoot of each pair was allowed to dehydrate in an air conditioned laboratory until their P_x values were close to the lowest pressures that the plants experience in the field. The shoots were then double bagged and allowed to equilibrate overnight. The next morning branchlets or leaves were sampled for P_x and the stems were cut to a length of slightly over 0.3 m. Stems of the wet and dry treatments were tightly wrapped in plastic and placed in separate bags before being shipped via overnight express to Michigan State University, where an Instron Universal Machine (model 4202, Instron Corporation, Canton, MA, USA) was used to measure MOE and MOR.

Stem segments were kept at approximately 10° C until MOE and MOR were measured. A four-point bending test with a compression load cell of 500 N was conducted as described by Woodrum *et al.* (2003). However, the stem segments used were longer (about 0.275 m) than were used in previous studies, so that most of the vessels would be intact during the bending experiments on wet versus dry treated stems. The span length (L), the distance between the two supported ends, was 0.21 m. The load was applied at two points along the span length with a crosshead speed of 20 mm/min and stems were stressed until the load reached a maximum value (F_{max}), determined as the maximum force obtained in the bending test prior to stem failure (Fig. 1). The distance between one supported end and the nearest loading point (*a* in the equations below) was 0.07 m. The elastic limit was always exceeded with the bending test, thus stems did not return to their original shape.

Flexural rigidity (EI) was calculated using slope (F/V) of the linear (elastic) portion of the curve (Fig. 1) and the equation $EI = (F/V)(a^2/12)(3L-4a)$ (modified from Gere and Timoshenko, 1997). Flexural rigidity was divided by I to determine MOE.

Modulus of rupture (MOR) was estimated from the equation: $MOR = (F_{max} * a * R_{major})/I$, modified from Ugural (1991), where F_{max} is the load at stem failure and R_{major} is the radius of the xylem. The second moment of area, I, was calculated as $\pi(R_{major}^4 - R_{minor}^4)/4$, with R_{minor} as the radius of the pith, based upon I of a hollow cylinder (Niklas 1992).

Adjacent stem segments were used to estimate xylem density (in kg m^{-3}) as the dry mass per saturated volume of xylem. For this measurement the pith and bark (including the phloem and vascular cambium) were removed from the xylem.

Hydraulic conductivity and cavitation resistance

Six plants per species were sampled for hydraulic conductivity (k_h), specific conductivity (k_h/sapwood area; k_s), and cavitation resistance of their stem xylem. One branch per plant, approximately 2 m in length, was excised, bagged in the field and taken to the laboratory. The branch was re-cut under water to obtain one "distal" stem segment 6 to 8 mm in diameter and 0.10 m in length for measurements of k_s and xylem anatomical features. An adjacent "proximal" segment was used for measurement of resistance to cavitation using the centrifuge technique.

Both the proximal and distal stems were connected to a tubing system and flushed with water that had been passed through a 0.1 μm filter and adjusted to a pH 2 with HCl in order to discourage microbial growth. The stems were flushed at a pressure of 100 kPa for one hour to remove gas emboli from the xylem vessels. The k_h (in m^4 MPa^{-1} s^{-1}) was then measured gravimetrically and the high pressure perfusion process repeated until a maximum value (k_{max}) was obtained for each segment (Sperry *et al.* 1988).

The distal segments were then attached to a tubing system that allowed uptake of a 0.1% (mass/volume) dye solution of crystal violet under a suction of 5-6 kPa for 25 min. The dye solution had been passed through a 0.1 μm filter. The midpoints of the distal segments were transversely sectioned at a thickness of 40 μm with a sliding microtome. The active sapwood area, indicated by the dye, was measured with a light microscope (Nikon microscope, Model Microphot-FX, Japan and Spot RT Color camera, Diagnostic Instruments, USA) and analyzed using image analysis software (Image v.1.61, National Institute of Health). The k_s value (in $m^2 MPa^{-1} s^{-1}$) was then calculated as k_{max}/active sapwood area.

The proximal stem segments, following determination of their k_{max} were spun in a centrifuge (RC5G Plus, Sorvall, Kendro Laboratory Products, Asheville, NC, USA), using a modified rotor to accommodate stem segments. Stems were spun at a prescribed RPM in order to generate a known negative pressure on the water column in the xylem vessels (Alder *et al.* 1997). The proximal segments were 0.271 m long (for use with a large centrifuge rotor) in the case of *C. megacarpus* and *A. fasciculatum* and 0.14 m long (for use with a smaller rotor) in the case of the other four species. The larger rotor was needed to create greater centrifugal force for the species that, based upon previous studies, required greater tensions (measured as lower P_x in previous studies) to induce embolism (Kolb & Davis 1994; Redtfeldt & Davis 1996; Davis *et al.* 1999). Vulnerability curves were constructed by plotting decreasing values of xylem pressure potential versus the percent loss in hydraulic conductivity (PLC). Two species, *M. laurina* and *R. ovata*, demonstrated evidence of cavitation fatigue (Hacke *et al.* 2001*b*). For these species, a correction for fatigue was done by calculating PLC using the k_h measure from a centrifuge spin at $\geq$-0.5 MPa instead of k_{max}. For each stem the PLC values were fitted with a polynomial model, which was used to predict the pressure potential at 50 PLC (P_{50}). The mean P_{50} value was thus determined for each species. While this is a relatively arbitrary value, it is one that is widely used and can be objectively applied to a wide-range of vulnerability curves.

Minimum field pressure potentials

The lowest xylem pressure potentials (P_{min}) occurred following the summer drought but before the first rains in autumn (Kolb & Davis 1994; Redtfeldt & Davis 1996; Davis *et al.* 1999). On October 13, 2004 we sampled six of the marked plants of each species for midday xylem pressure potential, measured from branchlets with the pressure chamber technique (Scholander *et al.* 1965). Since the first rain of the fall season fell on October 17, 2004, ending a greater than 7 month period with no rain, we assumed the October 13th measurements were representative of the lowest pressure potentials that the plants would experience in 2004.

Xylem anatomical measures

Images were taken of wedge-shaped sectors, using vascular rays as the borders, to sample for vessel and fiber features. Vessel lumen diameter (d in µm), fiber lumen diameter, fiber wall thickness, total transverse lumen area (vessel + fiber lumen area/sapwood area), and transverse fiber wall area/sapwood area were measured with these images. All of the vessels and fibers in sectors were measured until a sample size of 200 vessels and 100 fibers was obtained for a stem. The hydraulic vessel diameter (d_h) was calculated from the formula $d_h = (\Sigma d^5)/(\Sigma d^4)$, based upon all the sampled vessels in a stem. The vessel implosion resistance ($(t/b)_h^2$) (Hacke *et al.* 2001a) was determined for those vessels, within the sampled 200 vessels per stem, that formed pairs in which one or both vessels fell within ±5 µm of the calculated d_h, with "t" as the thickness of adjoining vessel walls and "b" as the lumen diameter of the vessel.

Data analysis

Modulus of elasticity and MOR of wet and dry treatments were compared within a species using t-tests. An ANCOVA was used to compare the relationship between MOE and MOR for wet and dry treatments. Parameters were compared across species using an ANOVA followed by a Fisher's LSD post-hoc analysis. Linear regression analysis was used to examine the correlations between parameters as predicted in our *a priori* hypotheses (StatView, SAS Institute Inc., Cary, NC, USA). We used an α of 0.05 to determine statistical significance.

Table 1.1. Xylem functional properties for six chaparral shrub species. Xylem specific conductivity (K_s), xylem density, stem modulus of rupture (MOR), modulus of elasciticity (MOE), xylem pressure potential (P_x) of the dry and wet treatments and at 50% loss of hydraulic conductivity (P_{50}), and the 2004 seasonal low xylem pressure potential (P_{min}) measured in six co-occurring chaparral species. All values are means ± 1 SE and the sample sizes are given in column "n." An *asterisk* indicates significant different ($P < 0.05$) between fully saturated "wet" stems and "dry" stems dehydrated to approximate minimum water potentials experienced in the field. Letters indicate significant difference between species for a given parameter.

Species		***Adenostoma fasciculatum***	***A.sparsifolium***	***Ceanothus megacarpus***
Family	n	Rosaceae	Rosaceae	Rhamnaceae
K_s (10^{-3} m^2 MPa^{-1} s^{-1})	6	0.88 ± 0.01 a	1.60 ± 0.12 a	1.54 ± 0.12 a
Xylem Density (kg m^{-3})	12	891 ± 65.0 a	684 ± 26.4 bc	779 ± 34.6 b
MOR (N mm^{-2})				
dry	12	235 ± 7.5 a	175 ± 7.8 b	212 ± 8.3 c *
wet	12	237 ± 11.2 a	178 ± 5.2 b	164 ± 8.1 b
MOE (N mm^{-2})				
dry	12	10794 ± 505 ac	7104 ± 498 b	8617 ± 955 bc
wet	12	10804 ± 707 a	7353 ± 608 bc	7722 ± 1526 b
P_x (MPa)				
dry	12	-8.13 ± 0.39 a *	-4.45 ± 0.20 b *	-10.10 ± 0.31 c *
wet	3-12	-0.18 ± 0.06 a	-0.14 ± 0.03 a	-0.26 ± 0.05 ab
P_{50}	6	-8.23 ± 0.19 a	-5.09 ± 0.34 b	-9.30 ± 0.66 c
P_{min}	6	-6.53 ± 0.15 a	-4.06 ± 0.18 b	-7.99 ± 0.44 c

Table 1.1 (cont'd).

C. spinosus	***Malosma laurina***	***Rhus ovata***
Rhamnaceae	Anacardiaceae	Anacardiaceae
1.82 ± 0.29 a	6.46 ± 1.36 b	1.95 ± 0.15 a
659 ± 27.4 c	489 ± 6.2 d	529 ± 21.1 d
152 ± 5.3 d	114 ± 4.7 e	112 ± 6.1 e
132 ± 8.7 c	112 ± 4.2 c	130 ± 10.0 c
9505 ± 369 c *	5392 ± 265 d	4661 ± 349 d
6689 ± 417 c	5450 ± 339 c	5349 ± 620 c
-10.05 ± 0.62 c *	-2.53 ± 0.96 d *	-2.85 ± 0.50 d *
-0.16 ± 0.02 a	-0.43 ± 0.09 b	-0.43 ± 0.10 b
-5.38 ± 0.46 b	-0.98 ± 0.31 d	-1.23 ± 0.08 d
-5.34 ± 0.31 d	-2.34 ± 0.09 e	-2.65 ± 0.10 e

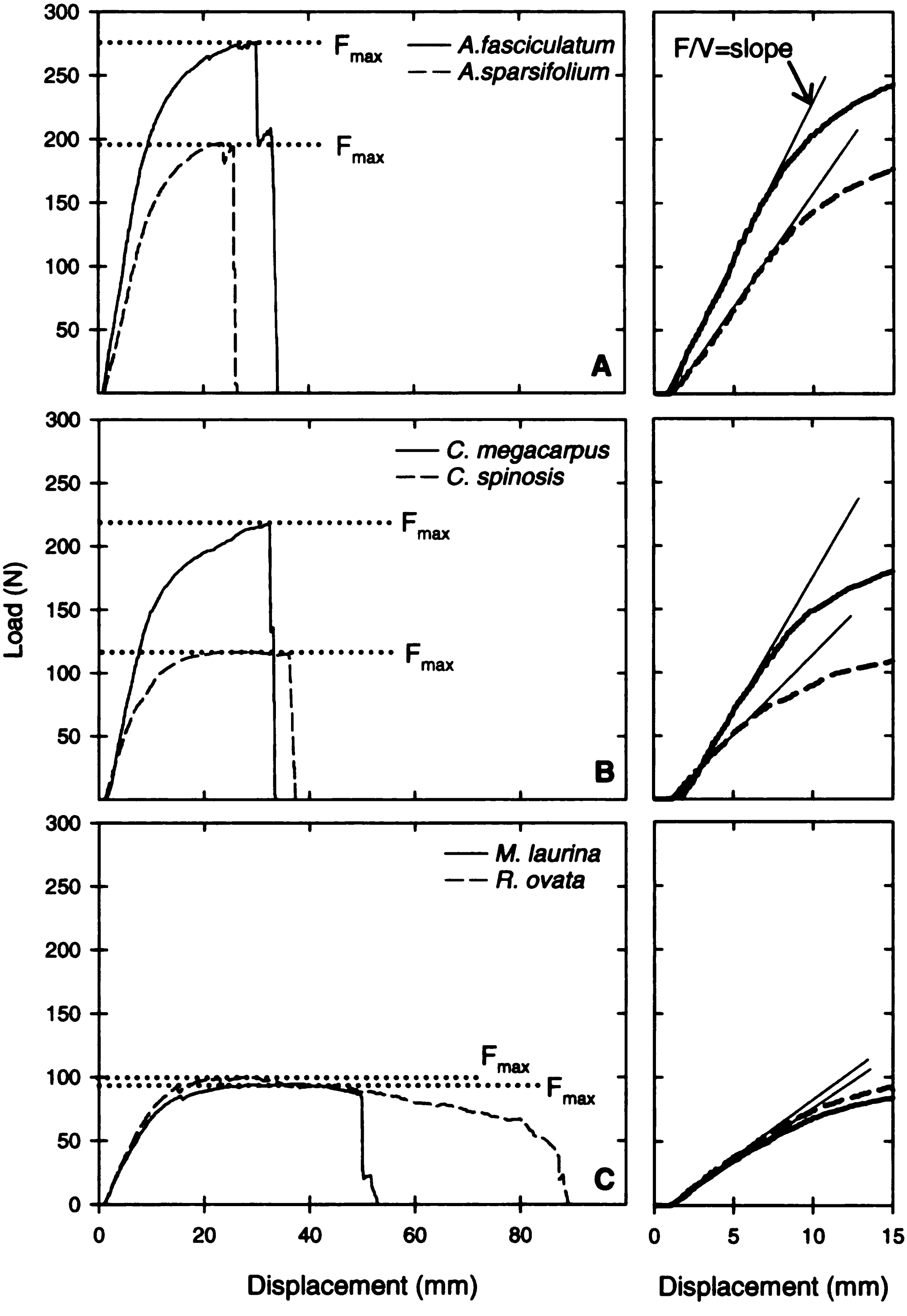

Figure 1.1. Load versus displacement in four-point bending tests for stems of six species of chaparral shrubs. The graphs on the left show the entire test for representative stems, the graphs on the right are a close-up of the earlier part of the bending tests. The slope of the initial, linear portion of the function (F/V) is used to calculate flexural stiffness and modulus of elasticity (MOE), whereas the maximum load (F_{max}) is used to calculate the modulus of rupture (MOR).

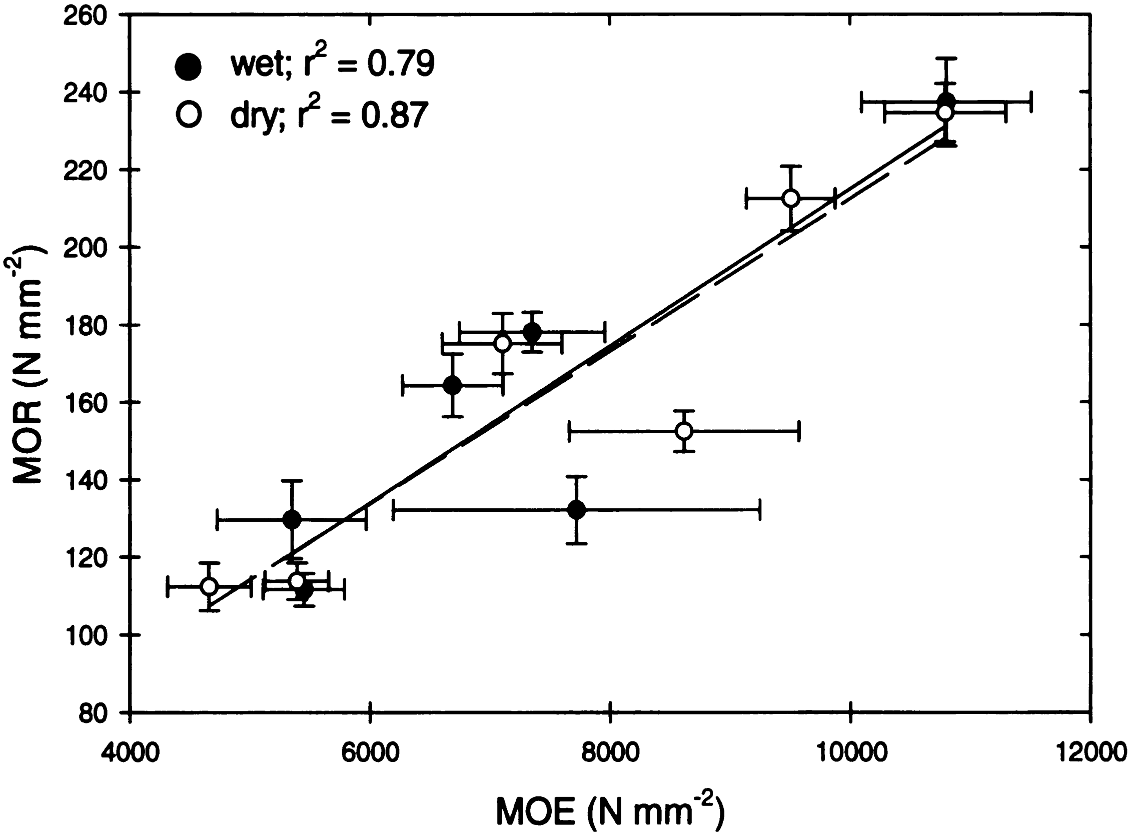

Figure 1.2. Modulus of rupture (MOR) versus modulus of elasticity (MOE) for wet and dry treatments of stems of six species of chaparral shrubs (n = 12 plants per treatment per species).

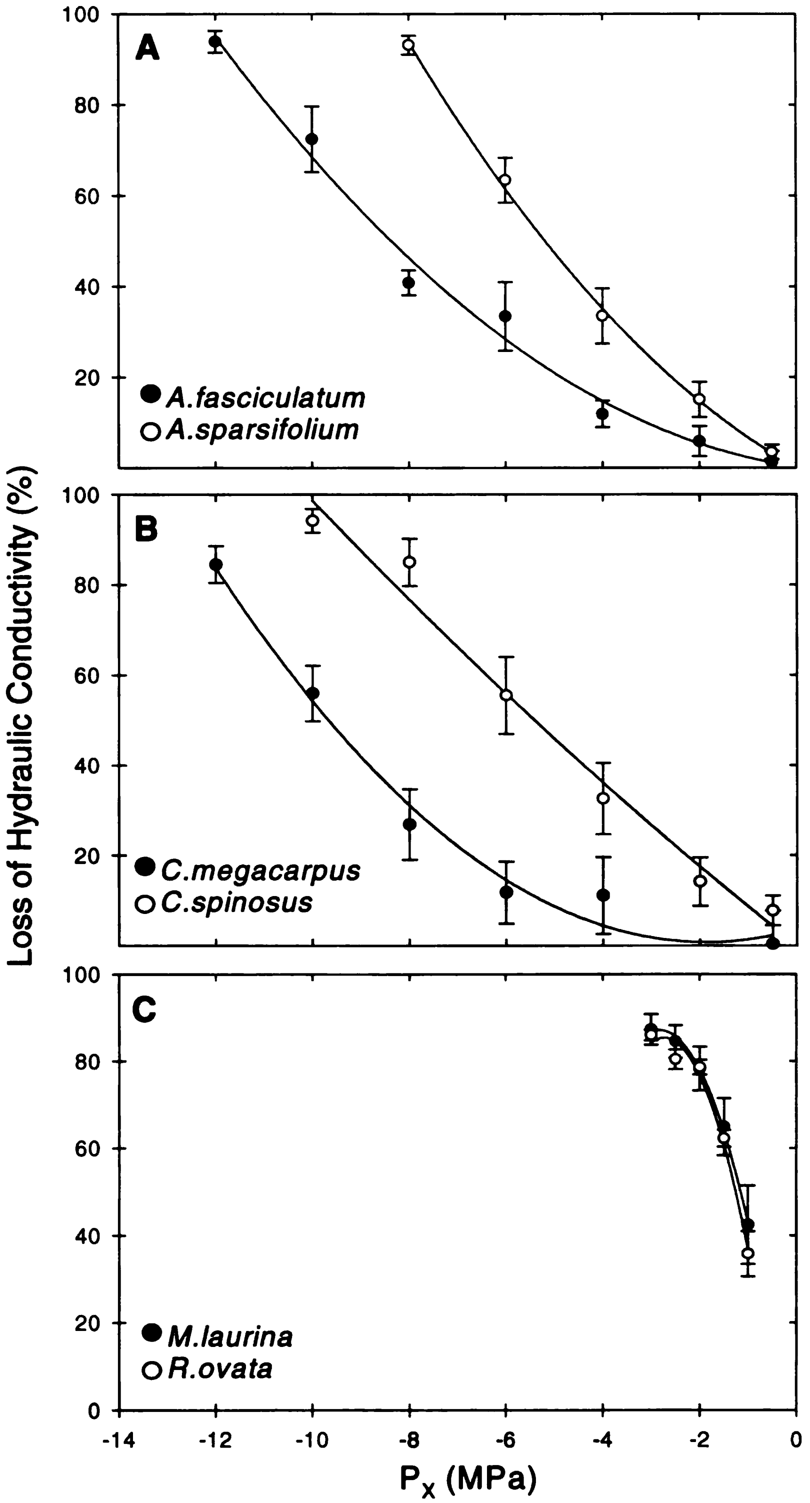

Figure 1.3. Percent loss of hydraulic conductivity of stems as a function of xylem pressure potential (P_x). Curves were fit with a second order polynomial ($r^2 \geq 0.99$ and $p \leq 0.01$, for all curves). Means ± 1 SE are shown with n = 6 plants per species. The graphs were used to calculate for each species the xylem pressure at which there was 50% loss of conductivity (P_{50} in Figures 1.4 and 1.5).

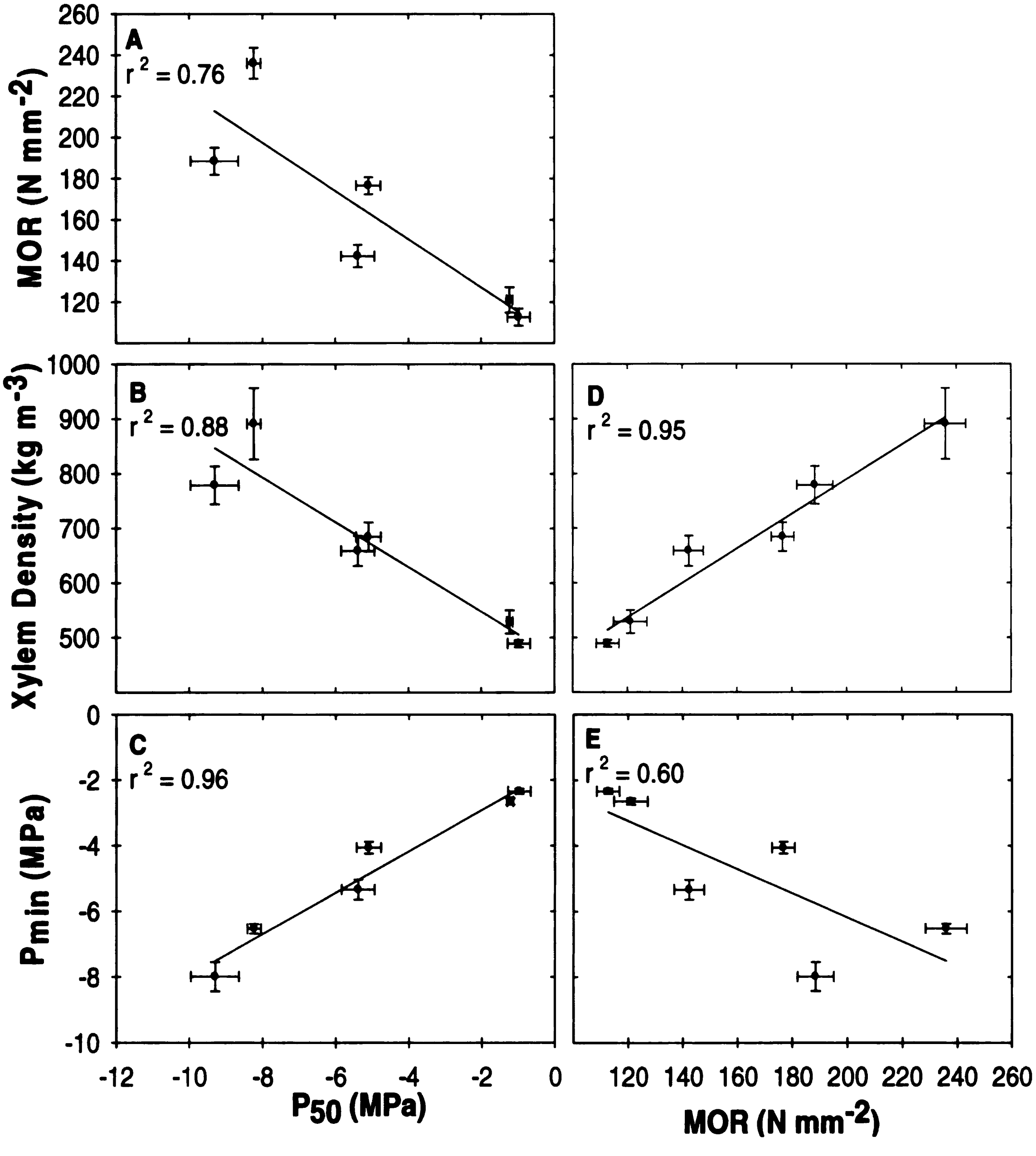

Figure 1.4. A. Modulus of rupture (MOR), B. xylem density, and C. minimum seasonal pressure potential (P_{min}) as functions of xylem pressure at 50% loss of conductivity (P_{50}) and D. xylem density and E. P_{min} as functions of MOR for six species of chaparral shrubs. Means ± 1 SE are shown and n = 12 plants per species, except for P_{50} where *n* = 6 plants per species.

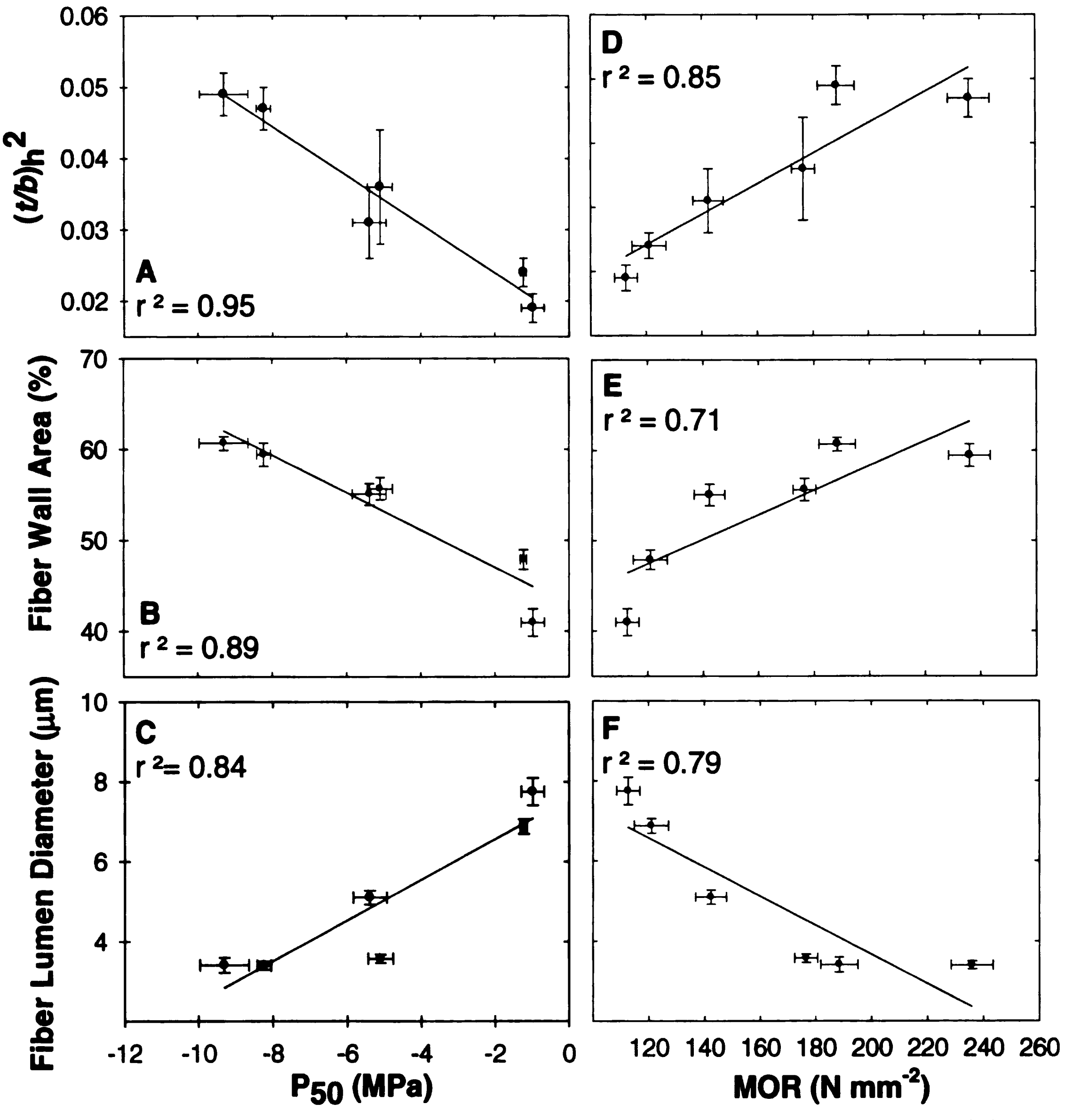

Figure 1.5. Xylem anatomical measures as functions of the xylem pressure at which there is a 50% loss of conductivity (P_{50}) and modulus of rupture (MOR) including estimated vessel implosion resistance ($(t/b)_h^2$) (A and D), percent fiber wall area (B and E), and fiber lumen diameter (C and F) in six species of chaparral shrubs. Means ± 1 SE are shown and n = 6 plants per species for P_{50} and *n* = 12 for MOR.

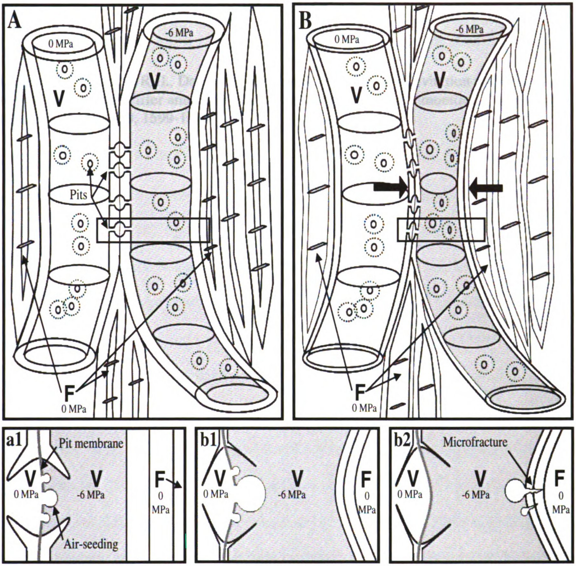

Figure 1.6. Some possible causes of embolism in a woody angiosperm stem. Depicted in the diagram is a segment of an embolized vessel represented by several vessel elements (V; 0 MPa), adjacent a functional water-filled vessel (V; -6 MPa) and surrounded by a matrix of fibers (F). In this diagram we have assumed that the fibers are embolized (0 MPa). The lower panels represent more detailed diagrams of an intervessel pit, and are close-ups of the rectangular areas indicated in panels A and B. With thick fiber and vessel walls (A), membrane deflection may occur, but it is not exacerbated by wall-buckling, limiting possible causes of hydraulic failure to air-seeding at the pit pore or membrane rupture (a1). With thin vessel and fiber cell walls (B), there is partial implosion of the water-filled vessel (indicated by thick arrows). With partial implosion there is stretching of the pit membrane with water stress, stretching the pores in the pit membrane and increasing the likelihood that air-seeding will occur either at the pit pore or following membrane rupture (b1). Additionally, implosion of vessel walls could lead to microfractures, another source of nucleation which would lead to cavitation of the water column (b2).

CHAPTER TWO

Jacobsen, A.L., Pratt, R.B., Davis, S.D. & Ewers, F.W. (2007) Cavitation resistance and seasonal hydraulics differ among three arid Californian plant communities. *Plant, Cell and Environment,* **30**, 1599-1609.

CAVITATION RESISTANCE AND SEASONAL HYDRAULICS DIFFER AMONG THREE ARID CALIFORNIAN PLANT COMMUNITIES

Abstract

Vulnerability to water stress induced cavitation was measured on 27 woody shrub species from three arid plant communities, including chaparral, coastal sage, and Mojave Desert scrub. Dry season native embolism and predawn water potential and both wet and dry season xylem specific hydraulic conductivity (K_s) were measured. Cavitation resistance, estimated as water potential at 50% loss in conductivity (Ψ_{50}), was measured on all species during the wet season and on a subset of species during the dry season. Cavitation resistance varied with sampling season, with 8 of 13 sampled species displaying significant seasonal shifts. Native embolism and water potential were useful in identification of species displaying seasonal shifts. The K_s was not different among sites or seasons. The Ψ_{50} varied among species and communities. Within communities, interspecific variation may be partially explained by differences in rooting depth or leaf habit (evergreen, semi-deciduous, deciduous). Communities diverged in their Ψ_{50} with chaparral species displaying the greatest cavitation resistance regardless of sampling season. The greater cavitation resistance of chaparral species is surprising considering the greater aridity of the Mojave Desert site. Adaptation to arid environments is due to many plant traits and aridity does not necessarily lead to convergence in cavitation resistance.

Introduction

Woody plant species from arid communities are more resistant to water stress induced cavitation than species from more mesic regions when compared across broad spatial scales (Brodribb & Hill 1999, Maherali, Pockman & Jackson 2004). This same pattern is present across smaller scales, such as the transition from riparian to upland communities in the Sonoran Desert (Pockman & Sperry 2000). In addition to moisture availability, growth form and leaf habit may also influence cavitation resistance, with shrubs having greater resistance to cavitation than trees (Maherali *et al.* 2004) and a trend for evergreen shrubs to have greater resistance to cavitation than deciduous shrubs (Martínez-Vilalta *et al.* 2002; Maherali *et al.* 2004). Aridland shrub communities, particularly those with evergreen species, may be convergent in containing species that are highly cavitation resistant (water potential at 50% loss in hydraulic conducticity [Ψ_{50}] < -4 MPa).

There have been several studies examining cavitation resistance of species within arid plant communities (*c.f.* Sobrado 1997; Hacke, Sperry & Pittermann 2000; Pockman & Sperry 2000; Martínez-Vilalta *et al.* 2002; Sperry & Hacke 200; Jacobsen *et al.* 2007*b*); however, there have been far fewer studies comparing species across different types of arid communities. These studies have often been limited to few species (Kolb & Davis 1994, however see Pockman & Sperry 2000) or investigations of a few widely distributed generalist species whose ranges overlap different communities (Mencuccini & Comstock 1997; Kolb & Sperry 1999*b*; Stout & Sala 2003). While a recent meta-analysis compared resistance to cavitation across expansive vegetation types (Maherali *et al.* 2004), such broad studies can be problematical because they do not control for data gathered across different seasons and years and the utilization of different methods.

There have been few, if any, comparisons among arid plant communities utilizing common methods and taking into account seasonal variation.

Vulnerability of stems to cavitation has been shown to vary greatly over the course of the growing season (from a Ψ_{50} of approximately -1.6 to -4.5 MPa over three months for *Artemisia tridentata*; Kolb & Sperry 1999a) as well as following drought or freezing stress (Hacke *et al.* 2001 and Sperry *et al.* 1994, respectively). Such shifts can lead to significant changes in the vulnerability of stems to cavitation seasonally and, if widespread, may have a great effect on species and community comparisons. The season in which species are sampled is rarely reported; however, for studies that do report sampling season, there is considerable variation (*c.f.* Sobrado 1997; Tyree, Patiño & Becker 1998; Kolb & Sperry 1999b; Hacke *et al.* 2000; Martínez-Vilalta *et al.* 2002; Stout & Sala 2003; Ewers *et al.* 2004; Jacobsen *et al.* 2007*b*). While techniques have been developed for correcting for freezing or water stress induced cavitation fatigue, such as calculating loss in conductivity relative to the conductivity after application of a modest negative pressure (> -0.5 MPa; Hacke *et al.* 2000; Sperry & Hacke 2002; Maherali *et al.* 2006), it is possible that shifts occurring during the growing season may be harder to standardize.

In the current study, we examined, in the same year and season, resistance to water stress induced cavitation (estimated by Ψ_{50} and Ψ_{75}) among three arid plant communities of southern California, including chaparral, coastal sage scrub, and Mojave Desert scrub communities. Vulnerability to cavitation curves were determined for 27 shrub species, including 12 for which no vulnerability curves have previously been published. In addition to vulnerability curves, native percent loss in hydraulic conductivity (*i.e.* native embolism) and predawn water potential were measured on plants during the dry season and xylem specific conductivity (K_s) was measured in both the wet and dry seasons. In order to evaluate the possibility of seasonal shifts in cavitation resistance among these species, which may complicate comparisons with previously published curves, vulnerability to cavitation curves were measured during both the wet and dry seasons on a subset of these species.

We predicted that among these three arid communities, species would show similar mean xylem resistance to water stress induced cavitation and a similar range in values. This is consistent with findings of similar ranges and means in Ψ_{50} among woody species from Mediterranean-type climate regions compared to deserts in a recent meta-analysis (Maherali *et al.* 2004). Additionally, this is consistent with predictions of plant functional convergence in response to similar environments (Meinzer 2003).

Methods

Sites and Species

Three diverse aridland plant communities were selected based on their high prevalence of woody shrub species. All sites were located in the winter rainfall-summer dry area of southern California, USA. The chaparral site was located in Cold Creek Canyon Preserve in the Santa Monica Mountains. The coastal sage scrub site was located on the campus of Pepperdine University in an ecological preserve also located in the Santa Monica Mountains. These two sites experienced similar precipitation over the course of the study (Feb 2006-Feb 2007: 433 and 406 mm, respectively) and similar cold season mean temperatures (10°C compared to 9°C at the chaparral site) but the coastal sage scrub site had slightly higher mean summer temperatures (26°C compared to 23°C at the chaparral site) and a narrower daily temperature range (approximately 10°C compared to 16°C for the coastal sage and chaparral sites, respectively). The Mojave Desert site was located in Red Rock Canyon State Park and experienced less annual precipitation for the year sampled than the other two sites (138 mm). This site had similar summer mean temperatures as the coastal sage scrub (26°C) site but had cooler mean winter temperatures than either of the other two sites (4°C).

All of the woody shrub species with unbranched, straight stems longer than 14 cm that had at least 12 individuals present at a site were included in the present study (Table 2.1) with two exceptions. *Arctostaphylos glauca* was excluded from the chaparral site because it is locally rare and we did not have permission to sample it and *Gutierrezia microcephala* was excluded from the Mojave Desert site because we were unable to measure hydraulic flow due to vessel blockage in this species. The remaining species included 10 chaparral species, 9 coastal sage scrub species, and 9 Mojave Desert scrub species. These species represent 15 families and several leaf habits (Table 2.1). Nomenclature follows Hickman (1993). One species, *Malosma laurina*, occurred at both the chaparral and coastal sage scrub sites and was sampled at both making the total number of sampled species 27.

Soil texture, which can influence xylem cavitation resistance (Sperry and Hacke 2002), was measured beneath one individual of each species at each site (*n* = 9-10 per site). Soil was collected from surface level to a depth of 30 cm and texture determined using the hydrometer method (Soil hydrometer, VWR Scientific, West Chester, PA, USA) described in Sheldrick and Wang (1993).

Wet Season Vulnerability to Cavitation

Wet season vulnerability to cavitation was measured from February through early July 2006 on at least 6 individuals per species. The last rainfall of the wet season at all sites occurred May 18-23 and water potentials of plants did not begin to decline until late July. Stems were trimmed under water in the field to a length of approximately 30 cm, sealed in plastic bags with a moist paper towel, placed in a cooler on ice, and transported to the laboratory at California State University, Bakersfield (CSUB) where they were refrigerated until measured (within 4 days of field collection). Stems were trimmed under water from both ends until a segment 4-8 mm in diameter and 14 cm in length was obtained, except for stems of *Adenostoma fasciculatum*, *Ceanothus cuneatus*, *C. megacarpus*, and *Larrea tridentata*. For these species, stem segments at least 50 cm long where collected in the field. These stems were then trimmed under water to 27 cm in length in the lab. The longer stem sizes were needed for use in a larger rotor which could generate more negative pressures since prior studies indicated this may be needed for these species (Pockman & Sperry 2000; Jacobsen *et al.* 2007b).

Vulnerability to cavitation was determined for stem segments as described in Jacobsen *et al.* 2005. In brief, stems were connected to a tubing system and flushed for 1 h at 100 kPa and the maximum hydraulic conductivity (K_{hmax}) of stems was measured gravimetrically (Sperry, Donnelly & Tyree 1988) using an analytical balance (CP124S, Sartorius, Goettingen, Germany). Following determination of their K_{hmax}, stems were spun in a centrifuge (Sorvall RC-5B Refridgerated Superspeed Centrifuge or RC-5C, Thermo Fisher Scientific, Waltham, MA, USA), using either a small (for 14 cm stem segments) or a large (for 27 cm stem segments) custom built rotor (Alder *et al.* 1997). Vulnerability to cavitation curves were constructed by plotting the water potential (generated using the centrifuge) versus the percent loss in hydraulic conductivity (PLC). For each stem, curves were fit with a second order polynomial model (Jacobsen *et al.* 2007b).

Additionally, PLCs were calculated and curves were generated using the K_h from an initial spin of -0.25 to -0.5 MPa in place of the K_{hmax} in order to correct for cavitation fatigue of the xylem (Hacke *et al.* 2000; Sperry and Hacke 2002; Maherali *et al.* 2006). This is done since xylem conduits that were previously embolized or damaged may become conductive following flushing resulting in an elevated K_{hmax}. Using K_h following a relatively mild pressure (>-0.5 MPa) embolizes these non-functional conduits while leaving functional conduits intact thus yielding a more realistic K_{hmax}. These corrected curves were then used to predict the water potential at 50% and 75% loss in hydraulic conductivity (Ψ_{50} and Ψ_{75}, respectively) for each stem and these values were averaged to get a species mean (n = 6-12 per species with a greater number of samples for those species for which no prior data existed on vulnerability to cavitation).

The xylem specific hydraulic conductivity (K_s) of stems was determined using the methods described in Jacobsen *et al.* (2007a). Stained active xylem area was determined using a digital camera and image analysis software (Olympus SP-500UZ, Olympus Imaging Corp., Center Valley, Pennsylvania, USA and Scion Image v. Beta 4.0.3, Scion Corp., Frederick, Maryland, USA). The whole xylem area in cross-section (minus the pith) was also determined. The K_{hmax} was then divided by either the active (stained) xylem area or the whole xylem area to obtain two different measures of xylem specific conductivity (K_s).

Dry Season Predawn Water Potential and Native Percent Loss in Hydraulic Conductivity

In September 2006, branchlets from six individuals of each of four species, *A. polycarpa*, *H. salsola*, *I. arborea*, and *L. tridentata*, were collected predawn and water potential was immediately determined in the field using a pressure chamber (Model 2000 Pressure Chamber Instrument, PMS Instrument Company, Albany, OR, USA). A second set of branchlets were collected at the same time from the same individuals, double bagged, placed in a cooler on ice, and transported to CSUB where they were rapidly processed in an air-conditioned lab using the same pressure chamber. Additionally, stem segments from the same individuals were collected predawn. Leaves were removed from these branches prior to the branches being removed from the plant, stems were then double bagged, placed in a cooler on ice, and transported to CSUB where xylem segments of approximately 4 mm were rapidly excised and placed into calibrated psychrometers (PST-55-30-SF, Wescor Inc., Logan, UT, USA) connected to a microvolt meter (HR-33T Dew Point Microvoltmeter, Wescor Inc., Logan, UT, USA) for determination of the stem xylem water potential. For these four species, there was no significant difference between water potential measured on branchlets in the field or laboratory ($P > 0.05$ for all); therefore, for the remaining 23 species branchlets were transported to the lab at CSUB (for the Mojave Desert species) or Pepperdine University (for the chaparral and coastal sage scrub species) for measures of branchlet water potential.

Predawn water potential was measured during the dry season (measurements made 30 August 2006 through 6 September 2006). Six individuals were measured per species. On the same mornings that predawn branchlet water potential was determined, stems approximately 0.5 m in length where collected from the same individuals to determine native embolism. Stems were cut from the plant while underwater to prevent the introduction of air into the xylem. The end of each stem was covered with a small piece of moist paper towel and sealed with Parafilm Laboratory Film and the whole branch was double bagged in plastic bags containing moist paper towels. Branches were rapidly transported to the lab at CSUB or Pepperdine University and measured within 5 h of collection. Stems were trimmed underwater from both ends until an unbranched straight stem segment 10 cm in length and 4-8 mm in diameter was obtained. These stems were connected to a tubing manifold and the native K_h determined gravimetrically. Stems were then flushed following the procedure describe previously and K_{hmax} determined. Using these values, the native PLC of stems was determined. Native PLC was not measured on *Malacothamnus fasciculatus* due to mucilaginous clogging of stems. Stems were then sectioned at their midpoint and whole xylem area in cross-section (minus the pith) determined. The K_{hmax} was then divided by the whole xylem area to obtain the xylem specific conductivity (K_s) for the dry season.

Dry Season Vulnerability to Cavitation

Four or five species were selected at each site for determination of dry season vulnerability to cavitation. We attempted to measure the species with the most negative wet season Ψ_{50} in order to determine the species with greatest cavitation resistance at each site and also to sample phylogenetically diverse species. Dry season vulnerability curves were constructed using the same methods as described for the wet season vulnerability curves. Dry season curves were measured during November and December 2006 when plants were at or near their annual minimum water potential (Jacobsen, *unpublished data*). Dry season measures were halted after the onset of significant winter rain in mid-December and for this reason we were unable to measure dry season curves on additional species.

Statistical Analyses

Statistical analyses were preformed using Minitab (Release 14.12.0, Minitab Inc., State College, PA, USA) and/or Statview (v. 5.0.1, SAS Institute Inc., Cary, NC, USA). Alpha was set at 0.05 for all comparisons. Water potential as measured on branchlets using the pressure chamber in the lab or field or on stem xylem using psychrometers where compared within species using ANOVAs followed by a Fisher's PLSD post-hoc analysis when appropriate. Interspecific differences in Ψ_{50} were analyzed within site using ANOVAs, and ANOVAs were also used to analyze across site differences in Ψ_{50}, Ψ_{min}, and K_s. For comparisons of Ψ_{50} and Ψ_{75} among species with different leaf habits, species were grouped into three categories, evergreen, semi-deciduous, or deciduous, and ANOVAs were used.

Non-fatigue corrected vulnerability to cavitation curves were used to predict the expected PLC at the predawn water potential for comparison with the dry season field measured native PLC. For comparison of the measured native PLC with the wet and/or dry season calculated PLC, *t*-tests were used. For seasonal comparisons of wet and dry season Ψ_{50} and Ψ_{75} within species, *t*-tests were used and species were considered to have a significant seasonal shift in their vulnerability to cavitation if either of those parameters differed significantly. For comparisons within individual species of wet season stained K_s, wet season K_s, and dry season K_s, ANOVAs were used.

Results

The 27 species showed a range of cavitation resistances. This is indicated by the variation in the size and magnitude of their wet season vulnerability to cavitation curves, with curves ranging in shape from convex to linear to concave (Figures 2.1, 2.2, & 2.3). In regression analyses of these vulnerability to cavitation curves, water potential described more than 82% of the variation in percent loss in hydraulic conductivity (PLC) in all cases (i.e. $r^2 > 0.82$), and often much more (most $r^2 > 0.95$).

Soil texture was not different among sites, with no significant differences in the sand, clay, or silt fractions among sites ($P > 0.05$ for all). All soil samples fell within the Sand to Loamy Sand categories.

Wet season vulnerability to cavitation (estimated by the mean water potential at 50% loss in hydraulic conductivity; Ψ_{50}) was significantly different among the three arid communities ($P = 0.003$). The Ψ_{50} ranged from -0.5 (*Malosma laurina*) to -9.5 MPa (*Adenostoma fasciculatum*) (Figure 2.4). The chaparral species were significantly more cavitation resistant (lower Ψ_{50}) than the coastal sage scrub ($P = 0.003$) and the Mojave Desert scrub species ($P = 0.003$) with 7 out of 10 chaparral species displaying greater resistance than the most resistant species of either of the other two communities. The Mojave Desert and coastal sage scrub species were not different from one another ($P =$

0.909) (Figure 2.4 B inset). Water potential at 75% loss in hydraulic conductivity followed the same pattern as Ψ_{50} (not shown) and non-fatigue corrected values were positively correlated with fatigue corrected values ($P < 0.001$; not shown). *Malosma laurina* was the only species that occurred at more than one site, in both the chaparral and coastal sage scrub, and its Ψ_{50} and Ψ_{75} did not vary between sites ($P = 0.471$ and 0.652 for Ψ_{50} and Ψ_{75}, respectively).

Species displaying different leaf habits (*i.e.* species with evergreen, semi-deciduous, or deciduous leaves) did not significantly differ in either Ψ_{50} or Ψ_{75} ($P =$ 0.186 and 0.188, respectively; see Table 2.1 for leaf habits of individual species). However, there was a trend for evergreen species to be more resistant to cavitation. Evergreen species had a mean Ψ_{50} of -3.1 ± 0.6 MPa compared to -1.8 ± 0.2 and -1.4 ± 0.5 MPa for the semi-deciduous and deciduous species, respectively.

Mean native percent loss in hydraulic conductivity (native PLC) measured in September was near 50% for all communities and was not different among communities (Figures 2.1, 2.2, & 2.3; $P = 0.417$). Mean native PLC for the chaparral was 41.8 ± 7.6% versus 49.0 ± 6.7% for the coastal sage scrub and 55.6 ± 7.7% for the Mojave Desert scrub. Among individual species, native PLC ranged from 10.2% in *Hazardia squarrosa* to 91.9% in *Quercus berberidifolia.* Native PLC significantly differed from the PLC predicted by wet season vulnerability curves and predawn branchlet water potential in 14 out of 26 species (Figures 2.1, 2.2, & 2.3; $P > 0.05$). Note, only 26 species were included in this analysis because we were unable to measure native PLC on *M. fasciculatus* (see Methods).

Predawn branchlet water potential (Ψ_{pd}) measured in September during the collection of native PLC values was not different among the three communities ($P = 0.151$). The mean $\Psi_{pd} \pm 1$ SE was -3.32 ± 0.59 MPa for the chaparral, -2.42 ± 0.26 MPa for the coastal sage scrub, and -4.06 ± 0.70 MPa for the Mojave Desert scrub (grey bars in Figures 2.1, 2.2, & 2.3).

Xylem specific conductivity (K_s) did not differ among the three communities (Figure 2.5 C inset; $P = 0.107$). None of the measured species experienced a significant change in xylem specific conductivity from the wet season to the dry season (grey versus open bars in Figure 2.5). For several species, not all growth rings in the xylem area were active as indicated by a significant difference between the stained area wet season K_s versus the whole xylem area wet season K_s (black versus grey bars in Figure 2.5).

For four species in which water potentials were measured on branchlets in the field and laboratory, there was no significant difference between these water potentials ($P > 0.05$ for all; data not shown). For three out of four species, these branchlet water potentials were also not different from stem xylem water potentials as measured with psychrometers ($P > 0.05$; data not shown); however, for one species, *Larrea tridentata*, the stem xylem water potential measured by psychrometers was significantly more hydrated than branchlet water potential measured using a pressure chamber ($P < 0.001$ for stem water potential compared to both the lab and field measured branchlet water potentials; data not shown).

Among the 13 species for which dry season vulnerability to cavitation curves were measured, there was great variability in seasonal response when compared to wet season vulnerability curves. In some species the dry season curve was more vulnerable

(Figure 2.1 A & Figure 2.3 G), in others there was no significant difference (Figure 2.1 D & H, Figure 2.2 A, Figure 2.3 D & E), and in others the dry season curve was more resistant (Figure 2.1 F, Figure 2.2 D, H, & I, & Figure 2.3 C & F). Of the 13 species for which dry season vulnerability to cavitation curves were measured, 5 had predicted PLC that was significantly different from the measured native PLC (Figures 2.1, 2.2, & 2.3; $P > 0.05$). However, if the stem xylem water potential for *Larrea tridentata* is used instead of the branchlet value, both the dry season and wet season curves predict PLC for *Larrea* (open bar Figure 2.3 G) reducing this to 4 of 13 species that have predicted PLC that are different from the measured native PLC.

Dry season Ψ_{50} and Ψ_{75} values differed significantly from wet season values in eight of 13 species (Figure 2.6). In most of these cases, the dry season value was lower than the wet season value, indicating a shift toward being more resistant to cavitation during the dry season. However, in two cases the dry season value was higher than the wet season value (*Adenostoma fasciculatum* and *Larrea tridentata*). Wet season and dry season Ψ_{50} were significantly correlated ($P < 0.001$).

Discussion

Non-convergence of cavitation resistance

Across three arid plant communities, shrub species appear to diverge in their cavitation resistance in spite of similar growth form and environment. Among 27 woody shrub species from three arid shrub plant communities there is considerable variability in vulnerability to water stress induced cavitation. Within communities, this variability may be due to differences in rooting depth or heterogeneity of soil moisture availability within sites (Jacobsen *et al.* 2005; Jacobsen *et al.* 2007b). While soil texture of shallow soils was similar among sites and did not vary greatly within sites, deeper soil layers may exhibit greater heterogeneity (c.f. Davis & Mooney 1985). Differences in rooting depths and physiological traits among species are likely primary determinants of variability in vulnerability to cavitation. Indeed, a previous investigation found a similar range in cavitation resistance (Ψ_{50}) as found in the current study when comparing 6 co-occurring species of even-aged chaparral shrubs growing at the same microsite (Jacobsen *et al.* 2005).

While all three communities include species that are relatively vulnerable, the chaparral has many more species that are highly resistant to cavitation, including 7 species with Ψ_{50} lower than -4 MPa. These species are more resistant than any species measured in either the coastal sage or Mojave Desert scrub. Indeed, chaparral species are, on average, more resistant to water stress induced cavitation than species from the other two arid communities. This contrasts with findings of similar ranges and means in Ψ_{50} among woody species from deserts and winter rainfall regions when compared across a much broader scale (Maherali *et al.* 2004). Thus, species in these communities do not converge to a common level of xylem cavitation resistance as predicted. Additionally, the greater resistance of chaparral species to water stress induced cavitation is surprising considering the greater aridity of the Mojave Desert site (Maherali *et al.* 2004). This highlights that habitat aridity is not necessarily a good predictor of species resistance to water stress induced cavitation across arid ecosystems.

Species in the coastal sage scrub and Mojave Desert scrub may have traits allowing them to mitigate extreme water stress in xylem tissues. This is supported by similar levels of native embolism among species in all three communities of approximately 50% loss in hydraulic conductivity during the dry season. This suggests that species are similarly avoiding high levels of xylem cavitation in spite of differences in cavitation resistance. Variation in leaf habit or rooting depth among the sampled species may partially explain the different water stress tolerating strategies among species, both within communities as well as between them. For instance, while the chaparral retain a near complete canopy throughout the dry season, many of the shrub species of the coastal sage and Mojave Desert are semi-deciduous or facultatively drought deciduous and adjust their canopy size during the dry season. Additionally, chaparral species tend to be larger and have fuller canopies than species occurring in the other two communities (Jacobsen, ***unpublished data***).

Seasonal shifts in cavitation resistance

Vulnerability to water stress induced cavitation significantly varied depending on the season in which stems were measured. In many cases, non-fatigue corrected wet season vulnerability curves were predictive of dry season native PLC. Native PLC values agreed with the percent loss in hydraulic conductivity predicted by 13 out of 26 wet season curves suggesting that seasonal shifts are not likely in these species. However, in 13 other species, native PLC was significantly different than that predicted by wet season vulnerability to cavitation curves. This suggested that shifts were likely or, alternatively, that branchlet water potential was in disequilibrium with stem xylem water potential in these other species. Indeed, of the 13 species for which vulnerability to cavitation curves were measured in both the wet and dry seasons, 8 species displayed significant seasonal shifts. Most species shifted to become more resistant later in the season as was observed previously in *Artemisia tridentata* (Kolb & Sperry 1999*a*), whereas a few species became more vulnerable during the dry season. Species in the present study experienced as much as a twofold change in vulnerability to cavitation from the wet season to the dry season and seasonal shifts appear to be relatively common among shrub species in all three communities.

For a few species, *Ceanothus cuneatus*, *Malosma laurina*, *Hazardia squarrosa*, and *Larrea tridentata*, dry season vulnerability to cavitation curves predicted PLC values significantly higher than the measured dry season native PLC. This pattern has been reported previously for tropical shrubs and trees (Lopez *et al.* 2005) and may be due to branchlet water potentials that are more negative than the pressure experienced by the stem xylem on which native PLC is measured. This leads to artificially high predictions of PLC similar to the pattern seen in these species. Nighttime transpiration and solutes in the xylem sap have been found to partially explain this disequilibrium in arid plants (Donovan *et al.* 1999; Donovan, Linton & Richards 2001; Donovan, Richards & Linton 2003); however, solutes in the xylem sap do not seem likely for the species in the current study because we did not find significant xylem sap osmotic potential in individuals in which this was examined (Jacobsen, *unpublished data*). Evidence of predawn disequilibrium between stem xylem and branchlets was found in one species. For *L. tridentata*, native PLC was not different from that predicted by either wet or dry curves if the water potential of stem xylem measured by psychrometers was used instead of branchlet water potential measured by pressure chamber (Figure 2.3 G). For the 5 species in the present study for which native PLC differed significantly from predictions based on wet season vulnerability curves but for which dry season vulnerability curves were not measured, seasonal shifts and/or disequilibrium are likely.

Shifts in vulnerability to cavitation may explain some of the variability among published curves. Vulnerability to cavitation of chaparral species, both from the wet and dry seasons are similar in range to those previously reported for these species (*c.f.* Jacobsen *et al.* 2005; Jacobsen *et al.* 2007*b*; Pratt *et al.* 2007). There have been relatively few vulnerability curves published for coastal sage scrub species, but for the species for which there are published curves, *Malosma laurina* and *Salvia mellifera*, our results are within the same range of published values (Kolb & Davis 1994; Langan, Ewers & Davis 1997; Jacobsen *et al.* 2005; Jacobsen *et al.* 2007*b*). For the Mojave Desert species, the curves for *Larrea tridentata* and *Hymenoclea salsola* are similar and within the range of other published curves for these species (Pockman & Sperry 2000 and Mencuccini & Comstock 1997, respectively). For the other two species for which curves have been published, *Ambrosia dumosa* and *Atriplex canescens*, the curves in the present study were more vulnerable than those reported elsewhere (Mencuccini & Comstock 1997 and Hacke *et al.* 2000, respectively). This may have resulted because the curves in the present study for these species were wet season curves. A shift in *A. canescens* of similar magnitude to that found in the closely related species *A. polycarpa* would produce a dry season curve similar to that reported by Hacke *et al.* (2000).

Influence of seasonal shifts on interspecific and community comparisons

Wet season cavitation resistance was correlated to dry season cavitation resistance in the present study and similar differences were found among communities when data were compared within a season (*i.e.* mean Ψ_{50} is approximately 2 MPa lower in the chaparral compared to the other two communities when either wet or dry season values are compared). However, results could be altered significantly if Ψ_{50} values from different seasons were used to compare communities. This suggests that care must be taken in comparison of vulnerability curves completed during different seasons or for which no sampling season is reported. This may complicate attempts to use published values in broad scale meta-analyses. Measures of native embolism and water potential may be useful in identifying species in which seasonal shifts are likely or to validate existing curves.

Physiology of seasonal shifts

Seasonal shifts in vulnerability to water stress induced cavitation are likely of physiological significance and may be due to maturation of xylem as suggested by Kolb & Sperry (1999*a*) or to changes in pit membranes or mechanical strength. Several of the species that experienced significant shifts in xylem vulnerability to cavitation in the present study also have a large portion of inactive xylem as indicated by the significant difference between stained xylem specific hydraulic conductivity (K_s) and whole xylem K_s in these species, including *Ceanothus megacarpus*, *Ambrosia dumosa*, and *Isomeris arborea*. This suggests that vulnerability to cavitation curves in these species largely measure the vulnerability of newer xylem and may therefore be sensitive to maturation of the xylem over the course of the growing season. It is unlikely that the shifts seen in the present study are the result of loss of function or sealing off of old wood or early wood over the course of the growing season as is seen in ring-porous species because these would likely affect K_s or would result in high levels of cavitation fatigue, neither of which were observed. Additionally, such shifts could be explained by seasonal hydrogel or other pit membrane changes (Gascó *et al.* 2006; Gascó *et al.* 2007) although such changes would also likely have resulted in significant differences in K_s seasonally.

Seasonal changes in xylem mechanical strength may influence xylem cavitation without affecting seasonal hydraulic conductivity. Young xylem may not contain enough mature and mechanically robust cells to buttress newly functional vessels against implosion when exposed to high tensions (Jacobsen *et al.* 2005). Indeed, seasonal changes in mechanical strength against stem breakage have been found among several species of Rhamnaceae shrubs of the California chaparral, including the four *Ceanothus* species included in the present study (Pratt, *unpublished data*). These species experienced significant increases in modulus of rupture (MOR) from the wet season to the dry season. Seasonal changes in xylem biomechanics may have important implications for dry season acclimation of woody shrubs, perhaps through increased resistance to implosion (Jacobsen *et al.* 2005). This suggests that care should be taken in interpreting vulnerability curves collected on non-hardened xylem which may be not be indicative of dry season values. Thus, dry season vulnerability to cavitation curves may be preferable in determination of the resistance to cavitation of the hardened xylem that actually tolerates low seasonal water potentials. Additionally, while dry season curves are likely to have greater levels of xylem fatigue compared to wet season curves, a standard fatigue correction can be applied to curves minimizing the influence of fatigue on comparisons (Hacke *et al.* 2000; Sperry & Hacke 2002; Maherali *et al.* 2006).

In the present study, the greater resistance to cavitation of chaparral shrub species compared to coastal sage or Mojave Desert shrub species is similar regardless of whether wet or dry season curves are examined. Curves for all 27 species were completed during the wet season providing a more comprehensive comparison of these communities and similar differences in cavitation resistance were also found when the fewer dry season curves were compared. To conclude, in spite of environmental similarities and the common stress of summer drought, species from these three communities have diverged in their xylem physiology with chaparral species displaying the greatest resistance to water stress induced xylem cavitation regardless of sampling season. Among these communities, species are likely utilizing differing suites of whole plant traits in order to persist in these arid environments.

Table 2.1. **Vegetation community, location, and sampled species within each community** along with families, codes, and leaf habits.

Vegetation type & Location	Species	Family	Species Code	Leaf Habit[1]
Chaparral--Cold Creek Canyon Preserve, Santa Monica Mountains, CA, USA (34.5 N 118.4 W)				
	Adenostoma fasciculatum Hook. & Arn.	Rosaceae	Af	E
	Adenostoma sparsifolium Torrey	Rosaceae	As	E
	Arctostaphylos glandulosa Eastw.	Ericaceae	Ag	E
	Ceanothus cuneatus (Hook.) Nutt.	Rhamnaceae	Cc	E
	Ceanothus megacarpus Nutt.	Rhamnaceae	Cm	E
	Ceanothus oliganthus Nutt.	Rhamnaceae	Co	E
	Ceanothus spinosus Nutt.	Rhamnaceae	Cs	E
	Malosma laurina (Nutt.) Abrams[2]	Anacardiaceae	Ml	E
	Quercus berberidifolia Liebm.[3]	Fagaceae	Qb	E,FDD
	Rhus ovata S. Watson	Anacardiaceae	Ro	E
Coastal Sage Scrub--Preserve located on Pepperdine University's campus, Malibu, CA, USA (34.2 N 118.4 W)				
	Artemisia californica Less.	Asteraceae	Ac	SD,FDD
	Encelia californica Nutt.	Asteraceae	Eca	D
	Eriogonum cinereum Benth.	Polygonaceae	Eci	E
	Hazardia squarrosa (Hook. & Arn.) E. Greene	Asteraceae	Hs	E
	Lotus scoparius (Nutt.) Ottley	Fabaceae	Ls	D,PS
	Malacothamnus fasciculatus (Torrey & A. Gray) E. Greene	Malvaceae	Mf	SD,FDD
	Malosma laurina (Nutt.) Abrams[2]	Anacardiaceae	Ml	E
	Salvia leucophylla E. Greene	Lamiaceae	Sl	SD,FDD
	Salvia mellifera E. Greene	Lamiaceae	Sm	SD,FDD
Mojave Desert Scrub--Red Rock Canyon State Park, CA, USA (35.2 N 117.6 W)				
	Ambrosia dumosa (A. Gray) Payne	Asteraceae	Ad	SD,DD
	Atriplex canescens (Pursh) Nutt.	Chenopodiaceae	Ac	E
	Atriplex polycarpa (Torrey) S. Watson	Chenopodiaceae	Ap	E
	Coleogyne ramosissima Torrey	Rosaceae	Cr	E,FDD
	Hymenoclea salsola A. Gray	Asteraceae	Hs	SD,FDD,PS
	Isomeris arborea Nutt.	Capparaceae	Ia	SD,FDD,PS
	Larrea tridentata (DC.) Cov.	Zygophyllaceae	Lt	E
	Lepidospartum squamatum (A. Gray) A. Gray[4]	Asteraceae	Ls	E
	Lycium andersonii A. Gray	Solanaceae	La	D

[1] E (evergreen), SD (semi-deciduous), FDD (facultatively drought deciduous), D (deciduous), PS (photosynthetic stems)

[2] Formerly *Rhus laurina*; this species was present at both the chaparral and coastal sage scrub sites

[3] May be facultatively drought deciduous during extreme drought events (Pratt and Davis, *unpublished data*) but was not during the studied time period in the present study

[4] Scale-like leaves

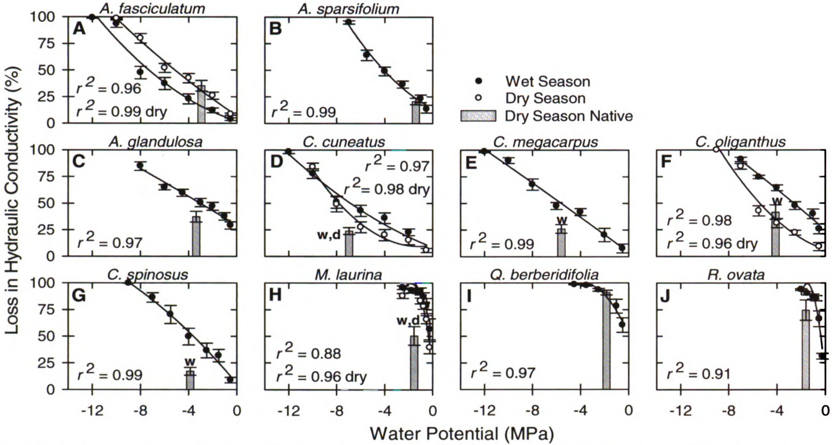

Figure 2.1. Vulnerability to cavitation curves measured on 10 chaparral shrub species during the wet season (Feb-May 2006; closed circles, mean ± 1 SE; $n = 6\text{-}12$) and for 4 species measured during the dry season (Aug-Dec 2006; open circles, mean ± 1 SE; $n = 6$). Curves shown are uncorrected for xylem fatigue (see Methods). Native percentage loss in conductivity (PLC) and predawn water potential from the dry season are also shown (grey bars, mean ± 1 SE; $n = 6$). A "w" indicates that native PLC are significantly different from those predicted by wet season vulnerability curves and a "d" indicates native values are significantly different from those predicted by dry season vulnerability curves.

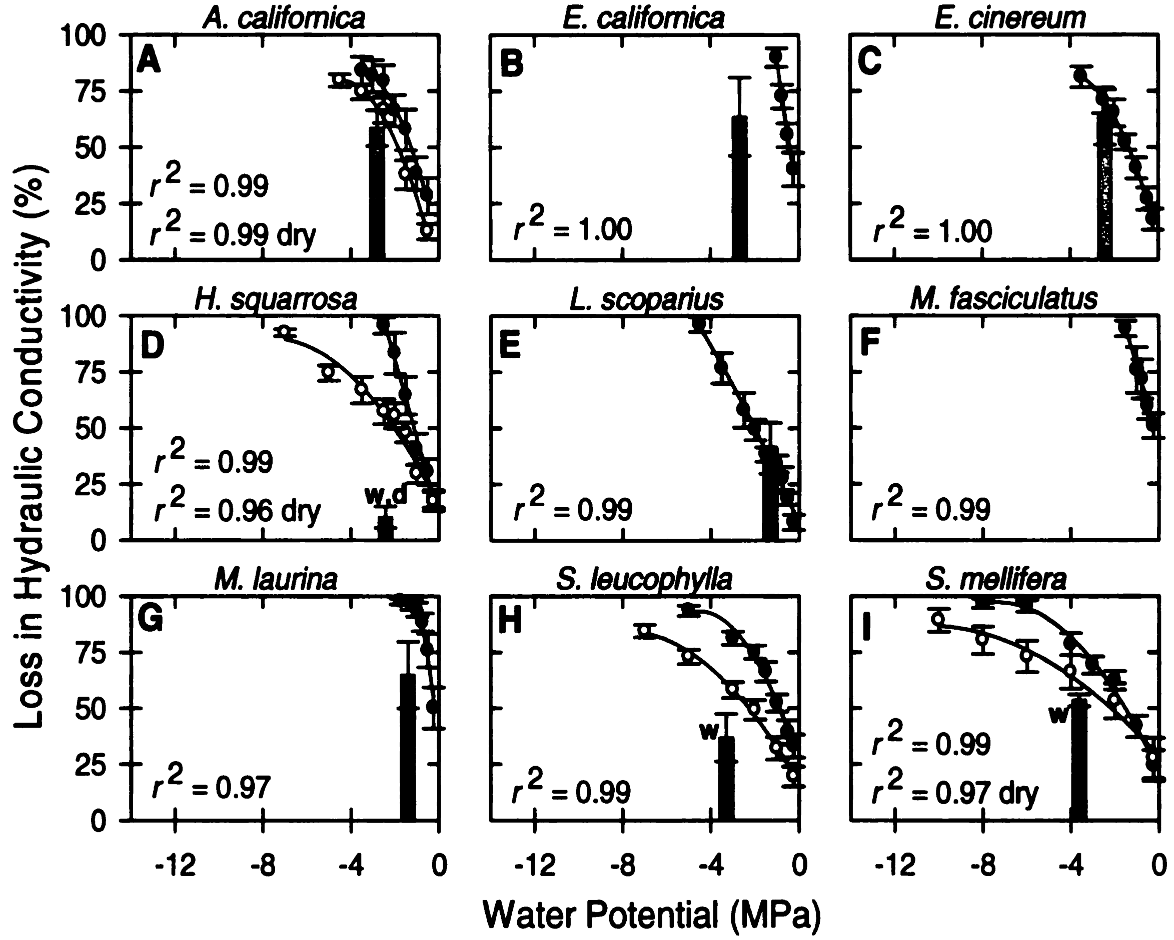

Figure 2.2. Vulnerability to cavitation curves measured on 9 coastal sage scrub species during the wet season (Feb-May 2006; closed circles, mean ± 1 SE; $n = 6\text{-}12$) and for 4 species measured during the dry season (Aug-Dec 2006; open circles, mean ± 1 SE; $n = 6$). Curves shown are uncorrected for xylem fatigue (see Methods). Native percent loss in conductivity (PLC) and predawn water potential from the dry season are also shown (grey bars, mean ± 1 SE; $n = 6$). A "w" indicates that native PLC are significantly different from those predicted by wet season vulnerability curves and a "d" indicates native values are significantly different from those predicted by dry season vulnerability curves.

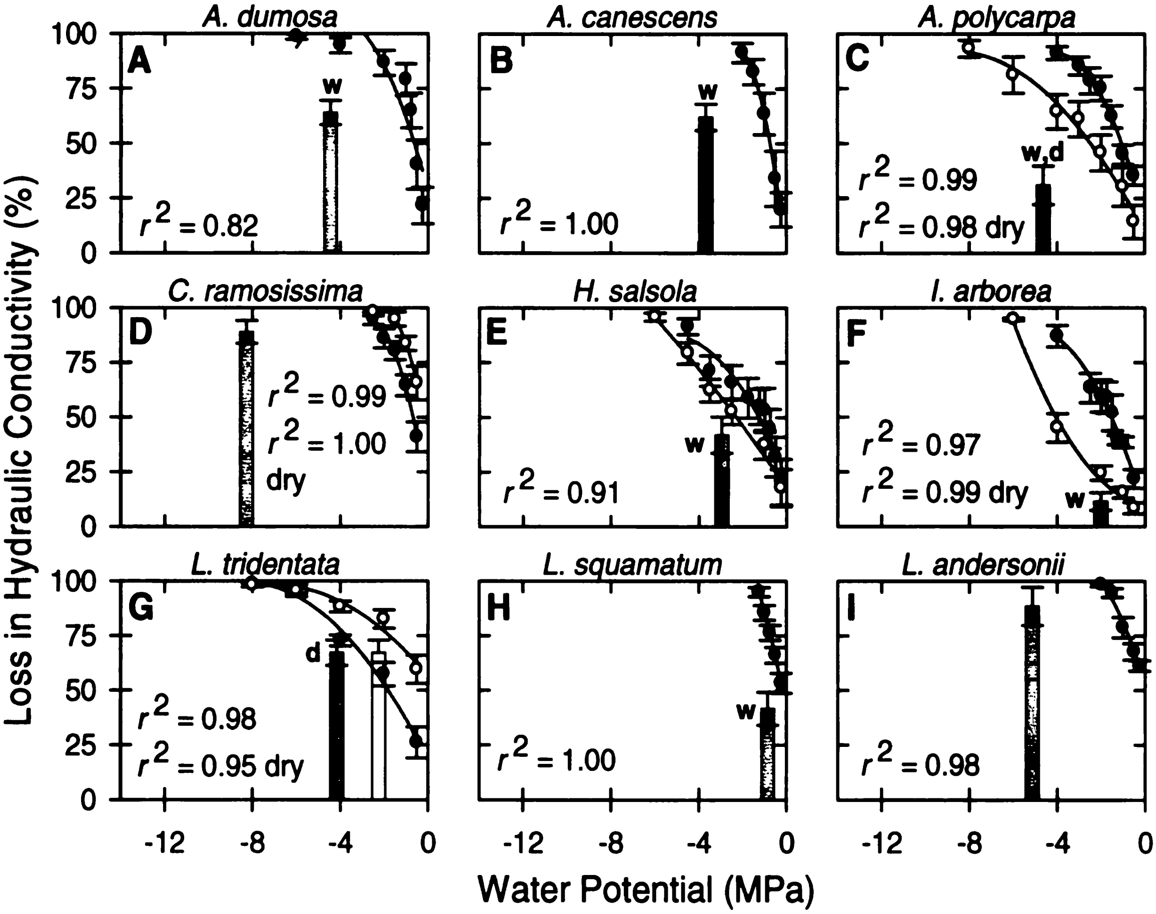

Figure 2.3. Vulnerability to cavitation curves measured on 9 Mojave Desert scrub species during the wet season (Feb-May 2006; closed circles, mean ± 1 SE; n = 6-12) and for 5 species measured during the dry season (Aug-Dec 2006; open circles, mean ± 1 SE; n = 6). Curves shown are uncorrected for xylem fatigue (see Methods). Native percent loss in conductivity (PLC) and predawn water potential from the dry season are also shown (grey bars, mean ± 1 SE; for predawn water potential as measured with psychrometers, white bar ± 1 SE; n = 6). A "w" indicates that native PLC are significantly different from those predicted by wet season vulnerability curves and a "d" indicates native values are significantly different from those predicted by dry season vulnerability curves.

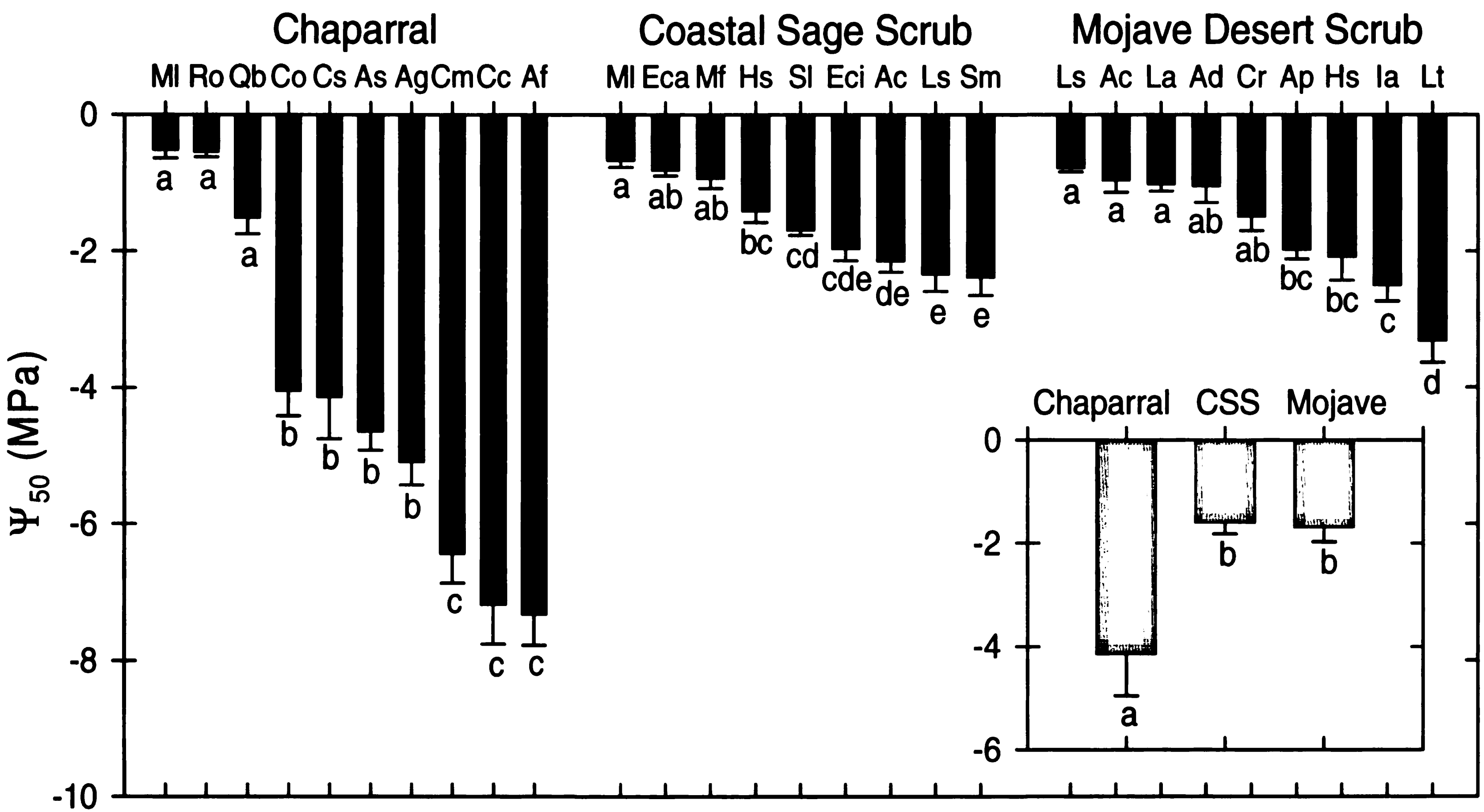

Figure 2.4. Water potential at 50% loss in hydraulic conductivity (Ψ_{50}) for 27 species from three arid plant communities (black bars, mean ± 1 SE; *n* = 6-12) corrected for fatigue (see Methods). Different letters indicate significant differences between species within a community. See Table 2.1 for species codes. Inset shows the mean Ψ_{50} for each community (grey bars ± 1 SE) and unique letters indicate significant differences among communities.

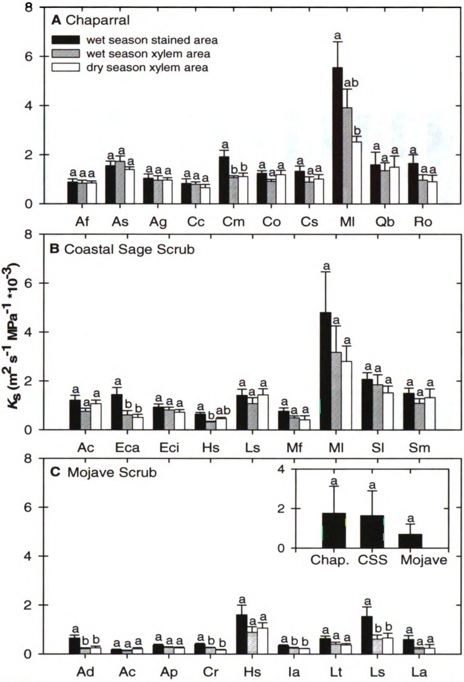

Figure 2.5. Xylem specific conductivity (K_s) for 27 species from three different arid plant communities as determined during the wet season (black and grey bars, mean ± 1 SE; n = 6-12) and dry season (open bars, mean ± 1 SE; n = 6). See Table 2.1 for species codes. Wet season K_s was determined by dividing the maximum hydraulic conductivity (K_{hmax}) by either the stained active area (black bars) or the whole xylem area (grey bars). Dry season K_s was determined by dividing K_{hmax} by the whole xylem area only (open bars). Unique letters indicated significant differences between measures within a species. Inset in C shows the mean wet season stained K_s for each community (black bars, mean ± 1 SE) and letters indicate that there was not a significant difference in K_s among communities.

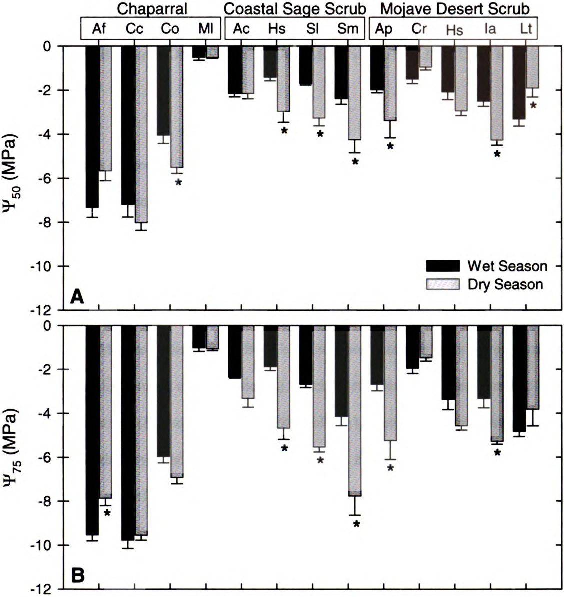

Figure 2.6. Mean water potential at 50% loss in hydraulic conductivity (Ψ_{50}) (A) and Ψ_{75} (B) for 13 species from three arid plant communities (black and grey bars ± 1 SE) corrected for fatigue (see Methods for details). See Table 2.1 for species codes. Species means were determined from vulnerability to cavitation curves measured during the wet season (black bars) and dry season (grey bars). See Figures 2.1-2.3 for vulnerability to cavitation curves. An asterisk beneath the dry season bar indicates that the dry season and wet season Ψ_{50} or Ψ_{75} values were significantly different for that species.

CHAPTER THREE

COMPARATIVE COMMUNITY PHYSIOLOGY: NON-CONVERGENCE IN WATER RELATIONS AMONG THREE SEMI-ARID SHRUB COMMUNITIES

Abstract

Plant adaptations to the environment are limited and plants in similar environments may display similar functional and physiological traits, a pattern termed functional convergence. Evidence was examined for functional convergence among 28 evergreen woody shrubs occurring in three different plant communities in the semi-arid winter rainfall region of southern California. These communities included chaparral, coastal sage scrub, and Mojave Desert scrub. Both leaf and water relations traits were examined, including seasonal stomatal conductance (g_s), specific leaf area (SLA), leaf specific conductivity of stems (K_l), seasonal water potential (Ψ_w), stem cavitation resistance (Ψ_{50}), and stem xylem density. These communities displayed a similar level of water stress, with a community level average minimum seasonal water potential (Ψ_{wmin}) of approximately -4.6 MPa which agrees with average Ψ_{wmin} reported for other arid communities. Pooled across sites, there was a strong correlation between Ψ_{wmin} and xylem density suggesting that these traits are broadly related and predictive of one another. In contrast, the relationship between Ψ_{wmin} and Ψ_{50} appears to be community specific and may be related to differential ability of species to tolerate or avoid high seasonal levels of embolism. Specific leaf area differed among communities with the coastal sage scrub community displaying greater SLA than the other two communities.

Specific leaf area was not a good predictor of water relations traits within or across communities. The relationship between g_s and Ψ_w also appears to be community specific and likely reflects differential water use by shrubs in these communities, with coastal sage scrub species adapted to utilizing shallow pulses of water, Mojave Desert scrub species relying on deeper water reserves, and chaparral species utilizing both shallow and deep moisture reserves. The comparative community physiology approach used here may provide a useful tool to test hypotheses of functional convergence across structurally similar semi-arid communities.

Introduction

Plant functional convergence studies both at the global and community levels have suggested that plants may be limited in their physiological solutions to environmental stresses and that plants in similar environments may therefore display similar functional and physiological traits (Reich *et al.* 1997; Paruelo *et al.* 1998; Meinzer 2003; Reich *et al.* 2003; Bucci *et al.* 2004; Swenson & Enquist 2007). Such findings suggest that there is potential to predict plant traits and related ecological processes across broad regions based on environmental conditions. Additionally, such correlations may be useful in predicting plant traits in regions that have been poorly studied. Many studies examining plant functional traits have examined plant form (Paruelo *et al.* 1998), xylem density (Swenson & Enquist 2007), water relations (Ackerly 2004; Bucci *et al.* 2004; Bhaskar & Ackerly 2006), and leaf traits (Reich *et al.* 1997; Ackerly 2004) as traits associated with specific aspects of plant functional variation.

Across broad scales, plant form appears convergent leading to the recognition of vegetatively similar biomes. A classic example of this is convergence to a shrub dominated landscape in Mediterranean-type climate regions (Aschmann 1973; Paruelo *et al.* 1998). These plant communities from around the world have often been compared to one another, although not in terms of the detailed physiological parameters used in the present study (*e.g.* Aschmann 1973; di Castri & Mooney 1973; Parsons & Moldenke 1975; Cody & Mooney 1978; Cowling & Campbell 1980; Kruger *et al.* 1983). While there appears to be great similarity in plant form among species occurring in these regions (*i.e.* shrubs with evergreen, tough leathery leaves), it is not clear that this morphological similarity corresponds with similar functional traits among species.

We examined evidence for functional convergence among the evergreen woody shrubs occurring in three semi-arid plant communities in the winter rainfall region of southern California. These communities included chaparral, coastal sage scrub, and Mojave Desert scrub. In the present study we included leaf, stem, and water relations traits. Leaf level measurements included specific leaf area (SLA). Stem level measurements included leaf and xylem specific conductivity (K_l and K_s, respectively), xylem resistance to cavitation (Ψ_{50}), and xylem density (K_s and Ψ_{50} were previously reported by Jacobsen *et al.* (2007*c*)). To characterize plant water relations, seasonal measures were made of stomatal conductance (g_s) and leaf branchlet water potential (Ψ_w) for 12 to 14 months. Additionally, temperature and precipitation were monitored at each site.

We predicted that the species occurring in these communities would display similar water relations and functional traits in response to their common arid winter rainfall environment. We predicted that, among sites, species would experience dry season water potentials (Ψ_{wmin}) similar to their xylem cavitation resistance (Ψ_{50}) that have already been reported (see Jacobsen *et al.* 2007*c*). This would be consistent with the 1:1 relationship between Ψ_{wmin} and Ψ_{50} reported for arid communities, including 26 species of chaparral (Jacobsen *et al.* 2007*b*; Pratt *et al.* 2007*a*) and six shrubs from the Great Basin Desert (Hacke *et al.* 2000). Based upon previously reported Ψ_{50}, a 1:1 relationship would result in differential Ψ_{wmin} among the sites such that the chaparral species would experience the lowest Ψ_{wmin} and species in the other two sites would experience less negative Ψ_{wmin} relative to the chaparral.

We predicted g_s would decline with declining Ψ_w during the course of the dry season when plant water status declined and the vapor pressure deficit increased (Poole & Miller 1975; Davis & Mooney 1985; Schulze 1986; Thomas & Eamus 1999; Brodribb & Holbrook 2003; Barradas *et al.* 2004; Brodribb & Holbrook 2004; Ladjal *et al.* 2005; Galmés *et al.* 2007). Such a stomatal response is presumably necessary in order for plants to avoid extreme negative pressures and runaway cavitation during the dry season especially among evergreen plants (Cochard *et al.* 2002; Sperry *et al.* 2002). Additionally, we predicted that chaparral species would have a shallower slope in their relationship between Ψ_w and g_s (*i.e.* delayed stomatal closure) consistent with their more cavitation resistant xylem (Ladjal *et al.* 2005; Jacobsen *et al.* 2007*c*).

Relationships among traits were also examined in order to determine the predictability of functional traits using traits that are commonly used as indices of plant functional variation. We predicted that xylem density would be correlated with Ψ_{wmin} (Jacobsen *et al.* 2007*a*; Jacobsen *et al.* 2007*b*), Ψ_{50} (Hacke *et al.* 2000; Jacobsen *et al.*

2007*b*), and g_s (Bucci *et al.* 2004). We predicted that SLA would be correlated with Ψ_{wmin} and Ψ_{50} since SLA is associated with increased aridity tolerance (Wright *et al.* 2002). Lastly, we predicted that SLA, K_s, and K_l would be associated with g_s (Sperry 2000; Gulías *et al.* 2003; Santiago *et al.* 2005; Poorter & Bongers 2006). Correlations between these traits were also analyzed using phylogenetic independent contrasts in order to account for the phylogenetic relationships among species in analyses (Felsenstein 1985).

Materials and Methods

Sites and Species

Three diverse aridland plant communities were selected based on their high diversity of woody shrub species. All sites were located in the winter rainfall-summer dry area of southern California, USA. The chaparral site was located in Cold Creek Canyon Preserve in the Santa Monica Mountains, the coastal sage scrub site was located on the campus of Pepperdine University also located in the Santa Monica Mountains, and the Mojave Desert site was located in Red Rock Canyon State Park (see Chapter 2; Jacobsen *et al.* 2007*c* for site descriptions).

All of the shrub species that occurred in sufficient numbers and for which we were able to obtain permission to sample were studied at each of these sites. This included 10 chaparral species, 9 coastal sage scrub species, and 10 Mojave Desert species (see Table 3.1). Most of these species are evergreen (see Jacobsen *et al.* 2007*c*). At each site, six individuals of each species were tagged at the beginning of the study. All measurements throughout the course of this study were conducted on these same individuals.

Xylem specific conductivity (K_s) and xylem vulnerability to cavitation (Ψ_{50}) were also measured on these same individuals or individuals of the same species located in close proximity to the sampled individuals. These data were published as part of a separate study (Jacobsen *et al.* 2007*c*).

Weather

Temperature and precipitation were monitored at sites via Remote Automated Weather Stations (RAWS) run by the Western Regional Climate Center for which temperature and precipitation at weather stations is monitored hourly and available online (http://www.wrcc.dri.edu/wraws/). Conditions at the chaparral site were approximated using the Malibu Canyon California RAWS, at the coastal sage scrub site using the Malibu Hills California RAWS, and for the Mojave Desert scrub site using the Jawbone California RAWS.

Seasonal water potential

Water potential (Ψ_w) was measured on all species approximately monthly beginning in February 2006 and continuing through April 2007. Leaves or branchlets were removed from plants at midday, double-bagged, and placed in a cooler on ice. Samples were then rapidly transported to an air-conditioned laboratory at Pepperdine University, Malibu, CA, USA for the chaparral and coastal sage scrub species or California State University, Bakersfield, CA, USA (CSUB) for the Mojave Desert scrub species. Samples were rapidly processed using a pressure chamber (Model 2000 Pressure Chamber Instrument, PMS Instruments, Corvalis, Oregon, USA). A previous study concluded that laboratory measured Ψ_w samples were no different from those measured immediately in the field (Jacobsen *et al.* 2007*c*).

Seasonal stomatal conductance

Stomatal conductance (g_S) was measured on all species approximately monthly beginning in April 2006 and continuing through April 2007. Stomatal conductance was measured on a leaf or branchlet of six individuals per species at each sampling time using a steady state porometer (LI-COR, Inc., LI-1600 Steady State Porometer with a 1600-07 Cylindrical Chamber, Lincoln, Nebraska, USA). For each sampling time, all species within a site were measured within a single day beginning around 10:00 am and ending around noon. Leaf and air temperatures were periodically checked using an infrared thermometer for leaf temperatures (Fluke Corporation, Fluke 572 CF IR thermometer, Germany) and a thermocouple for air temperatures (Control Company, 4015 Traceable Total Range Thermometer with a beaded Type-K thermocouple probe, Friendswood, TX, USA). Sampled leaves or branchlets were collected and the sampled leaf area determined using a leaf area meter (LI-3100, LI-COR, Inc., Lincoln, Nebraska, USA). Stomatal conductances of species where then corrected using the area of the leaves containing stomata (*i.e.* species with stomata on only one side of the leaf where corrected using the one-sided leaf area, species with amphistomatic leaves were corrected using the two-sided leaf area, and species with needle like leaves were corrected by calculating the surface area of the leaf based on cross sectional measures of leaf geometry).

Specific Leaf Area

Specific leaf area (SLA) was measured on fully mature leaves at the start of the summer dry period. Branchlets were collected from individuals in the field, placed in plastic bags, and transported to the laboratory at CSUB. Individual leaves were then taken from each branchlet with a total of 10 leaves from 10 individuals for each species and their area was measured using a leaf area meter. Leaves were then placed in a drying oven at 60°C for four days and their dry mass was determined using an electronic balance (CP124S, Sartorius, Goettingen, Germany). Specific leaf area was calculated as the leaf area per dry leaf mass (mm^2 mg^{-1}). The SLA was not determined for *Lepidospartum squamatum* separating its small adherent scale-like leaves from the stem.

Leaf Specific Hydraulic Conductivity

Leaf specific hydraulic conductivity was measured during the dry season in August and September 2006 on 24 of the 28 species included in the present study. The remaining 4 species had very small, scale-like, or adherent leaves which prevent accurate determination of leaf area. Stems approximately 0.5 m in length were collected predawn. Stems were cut from the plant while underwater, the end of each stem was covered with a small piece of moist paper towel and sealed with Parafilm Laboratory Film, and the whole branch was double bagged in plastic bags containing moist paper towels. Branches were rapidly transported to the lab and native conductivity was measured on a stem segment 10 cm in length and 4-8 mm in diameter within 5 h of collection following the methods in Jacobsen *et al.* (2007*c*). All leaves distal to the measured stem were collected and their leaf area determined using a leaf area meter. The native conductivity was then divided by this leaf area to determine the dry season leaf specific xylem conductivity (K_l).

Xylem Density

Xylem density was determined as the dry mass per water saturated volume. Stem segments approximately 5 cm long and 6-8 mm in diameter were collected adjacent to those used for determination of vulnerability to xylem cavitation in a prior study (Jacobsen *et al.* 2007*c*). Stem segments were split longitudinally and their pith and bark removed. Xylem segments were then soaked in degassed water adjusted to a pH of 2 overnight and saturated volume was determined using the methods of Hacke *et al.* (2000). Samples were then placed in a drying oven at 60°C for four days and their dry mass determined.

Data analysis

Statistical analyses were preformed using Minitab (Release 14.12.0, Minitab Inc., State College, PA, USA) and/or Statview (v. 5.0.1, SAS Institute Inc., Cary, NC, USA). Alpha was set at 0.05 for all comparisons. Differences in minimum and maximum seasonal Ψ_w and minimum and maximum g_s were analyzed among species at a single site and among sites using ANOVAs followed by Bonferroni/Dunn post-hoc analyses when appropriate. Seasonal changes in Ψ_w and g_s were analyzed using repeated measures ANOVAs followed by Bonferroni/Dunn post-hoc analyses when appropriate.

Linear regression models were used to analyze the within and among species relationships between g_s and Ψ_w and ANOVAs were used to examine differences in the slopes and intercepts of these regressions among species and sites. Linear regressions were also used to examine the relationship between these seasonal measures and SLA, K_l, K_s, Ψ_{50}, and xylem density.

A principle components analysis was used to examine differences among the suites of xylem traits used by species and communities. Minimum seasonal water potential (Ψ_{wmin}), Ψ_{50}, K_s, and xylem density were included in this analysis. A MANOVA was used to determine if communities differed in the relationships among these multiple traits.

The phylogeny used for phylogenetic independent contrast (PIC) calculations was constructed based on the relationships among families and species reported in recent molecular-based published phylogenies (Morgan *et al.* 1994; Bayer & Starr 1998; Hardig *et al.* 2000; Goertzen *et al.* 2003; Hilu *et al.* 2003; Urbatsch *et al.* 2003; Soltis & Soltis 2004; Soltis *et al.* 2005). Contrasts were calculated using COMPARE (Martins 2004) and linear regressions were used to examine the relationship between SLA, K_l, K_s, Ψ_{50}, and xylem density contrasts pooled across communities. Correlations among PICs were similarly significant or non-significant to correlations among raw trait values in most cases; therefore, only correlations of raw trait values, which have more easily interpretable values, are discussed in the Results. There was only one case in which the raw trait correlation and PICs correlation did not agree and this is discussed below.

Results

Weather

Spring rains occurred across all sites from February 2006 though May 2006 (Figure 3.1). The summer rainless period began in June 2006 and extended through September 2006 for the Mojave Desert and through October 2006 for the chaparral and coastal sage scrub at which time a few small precipitation events occurred (< 10 mm). Substantial rain did not fall at the sites until the months of December 2006 and January 2007 when several precipitation events (> 20 mm) occurred across the sites. Rainfall during the 2005 to 2006 rainy season was 510 mm for the chaparral site, 460 mm for the coastal sage scrub site, and 230 mm for the Mojave Desert site. Rainfall for the 2006 to 2007 rainy season was considerably lower and was 130, 80, and 100 mm for the chaparral, coastal sage, and Mojave Desert sites, respectively.

Seasonal water potential

All chaparral, coastal sage, and Mojave Desert species experienced significant changes in their water potential over the course of this study (Figure 3.2; $P < 0.05$ for all). Chaparral species reached their maximum Ψ_w (Ψ_{wmax}) during the wet season in February, April, or May 2006. Most chaparral species reached their minimum Ψ_w (Ψ_{wmin}) during the dry season in August, October, or November 2006. *Rhus ovata* differed from these species in reaching its Ψ_{wmin} during the wet winter months (February 2007). All coastal sage scrub species experienced their Ψ_{wmax} during the wet season in April 2006. Most coastal sage scrub species experienced their Ψ_{wmin} at the end of the summer dry season in October and November 2006. Only one species experienced its seasonal low during the winter cool period, *M. laurina* (February 2007). Most Mojave Desert scrub species reached their Ψ_{wmax} during the wet season in April and May 2006 and January and February 2007. *Lepidospartum squamatum* differed from these species in displaying its Ψ_{wmax} during the dry summer period (August 2006). Most species reached their Ψ_{wmin} during the dry summer season in July, August, and September 2006. However, a few species displayed seasonal lows during the wet winter months of January 2007 (*H. salsola*), February 2007 (*L. andersonii*), and March 2007 (*C. ramosissima*).

The average monthly Ψ_{wmax} occurred in the coastal sage scrub and Mojave Desert scrub in April 2006 and in the chaparral in May 2006 (Figure 3.2). The three sites differed in their Ψ_{wmax} (Figure 3.3; $P < 0.001$) with the chaparral and coastal sage scrub species experiencing significantly less negative water potentials than the Mojave Desert species ($P < 0.001$ for both). The chaparral species and coastal sage scrub species did not significantly differ in their Ψ_{wmax} ($P = 0.723$).

The average monthly minimum water potential (Ψ_{wmin}) occurred in the chaparral and coastal sage scrub in November 2006 and in the Mojave Desert in September 2006 (Figure 3.2). There was no difference among sites in Ψ_{wmin} (Figure 3.3; $P = 0.376$).

Seasonal stomatal conductance

All chaparral and coastal sage species experienced significant changes in their stomatal conductance (g_s) over the course of this study (Figure 3.2; $P < 0.05$ for all). All of the chaparral species experienced their maximum g_s (g_{smax}) in the spring in April and May 2006. Several species displayed their minimum g_s (g_{smin}) during the end at the end of the summer dry period in November 2006 (*A. glandulosa*, *C. cuneatus*, *C. megacarpus*, *C.*

oliganthus, and *C. spinosus*). Several other species displayed their g_{smin} in February (*R. ovata*) and April 2007 (*A. fasciculatum*, *M. laurina*, and *Q. berberidifolia*). All of the coastal sage scrub species displayed their g_{smax} during the spring season during April, May, and June 2006. Several coastal sage scrub species experienced their g_{smin} at the end of the summer dry season in November 2006 (*A. californica*, *L. scoparius*, *S. leucophylla*, and *S. mellifera*). However, several other species did not experience their g_{smin} until February (*E. californica* and *M. laurina*) and April 2007 (*E. cinereum*, *H. squarrosa*, and *M. fasciculatus*).

Stomatal conductance for the Mojave Desert species *A. dumosa* and *L. andersonii* did not change significantly over the course of this study (Figure 3.2; $P = 0.449$ and 0.052, respectively) although this includes only two months of data from *L. andersonii* due to it being fully drought deciduous. For the remaining Mojave Desert species, g_s underwent significant seasonal change (Figure 3.2; $P < 0.001$ for all). Most species reached their g_{smax} in the wet spring season in April 2006 (*A. canescens*, *C. ramosissima*, and *L. tridentata*) and May 2006 (*H. salsola* and *G. microcephala*). However, several species reached their g_{smax} later in the dry season including *A. polycarpa* (August 2006), *I. arborea* (November 2006), and *L. squamatum* (November 2006). All species reached their g_{smin} during the cool winter months in December 2006 (*G. microcephala*), January 2007 (*C. ramosissima*, *H. salsola*, *I. arborea*, and *L. tridentata*), and March 2007 (*A. canescens*, *A. polycarpa*, and *L. squamatum*).

The average monthly high stomatal conductance (g_{smax}) occurred in the coastal sage scrub and Mojave Desert scrub in April 2006 but was delayed in the chaparral until May 2006 (Figure 3.2). These months also coincided with the average monthly high water potentials across sites; however Ψ_{wmax} is not correlated with g_{smax} either within or across communities ($P > 0.05$ for all). The three sites differed in their g_{smax} (Figure 3.4; $P = 0.007$) with the coastal sage scrub species experiencing significantly greater g_{smax} than the chaparral or Mojave Desert species ($P = 0.019$ and 0.002 for coastal sage compared to the chaparral and Mojave Desert, respectively). Chaparral and Mojave Desert species did not significantly differ in their g_{smax} ($P = 0.425$).

The average monthly minimum stomatal conductance (g_{smin}) occurred in the chaparral and coastal sage scrub in April 2007 and in the Mojave Desert in January 2007 (Figure 3.2). There was no difference among sites in g_{smin} ($P = 0.267$).

Water potential and stomatal conductance

Among the different sites, the relationship between Ψ_w and g_s varied (Figure 3.5; $P <$ 0.001 for both slopes and intercepts across communities). For the chaparral and coastal sage scrub, Ψ_w was correlated to g_s pooled across species ($P < 0.001$ for both, $r^2 = 0.27$ and 0.22, respectively); however, there was no relationship between Ψ_w and g_s pooled across species from the Mojave Desert ($P = 0.132$). Species from the coastal sage scrub had a significantly greater slope (64.89 ± 13.49) than either the chaparral (27.08 ± 4.60) or the Mojave Desert scrub species (12.07 ± 6.18) (Figure 3.5; $P = 0.007$ and 0.001 for the coastal sage scrub compared to the chaparral and Mojave desert scrub, respectively). Species from the chaparral and Mojave Desert did not differ significantly in their slopes of Ψ_w and g_s pooled across species ($P = 0.264$). Species from the coastal sage scrub also had a significantly greater intercepts (273.77 ± 40.36) than either the chaparral (141.54 ± 13.67) or the Mojave Desert scrub species (106.56 ± 29.91) (Figure 3.5; $P = 0.004$ and

0.001 for the coastal sage scrub compared to the chaparral and Mojave desert scrub, respectively). Species from the chaparral and Mojave Desert did not differ significantly in their intercepts (Figure 3.5; $P = 0.452$).

Specific Leaf Area

Specific leaf area (SLA) was different among sites ($P = 0.014$) with the coastal sage having significantly higher SLA compared to the chaparral ($P = 0.004$) and the Mojave Desert scrub ($P = 0.047$). Specific leaf area was not different between the chaparral and Mojave Desert scrub ($P = 0.321$). Specific leaf area of species was not correlated with Ψ_{wmax}, Ψ_{wmin}, K_s, K_l, or g_{smin} either within or across communities ($P > 0.05$ for all; data not shown). Specific leaf area was also not correlated with g_{smax} across communities ($P > 0.05$ for all; data not shown); however, SLA was correlated with g_{smax} among Mojave Desert species ($P = 0.016$, $r^2 = 0.59$; data not shown) such that species with lower SLA also had lower g_{smax}. Specific leaf area was not correlated with g_{smax} among species in either chaparral or coastal sage scrub communities ($P > 0.05$ for both; data not shown).

Xylem density

Xylem density was significantly different among the sites ($P = 0.007$) with the Mojave Desert scrub having significantly denser xylem than the chaparral ($P = 0.035$) or coastal sage scrub ($P = 0.002$). Xylem density was not different between the coastal sage and chaparral ($P = 0.215$). Xylem density was correlated with Ψ_{wmax} and Ψ_{wmin} when pooled across communities such that species with denser xylem had more negative Ψ_{wmax} and Ψ_{wmin} (Figure 3.6; $P < 0.001$, $r^2 = 0.52$ for Ψ_{wmax} and $P = 0.012$, $r^2 = 0.21$ for Ψ_{wmin}).

Xylem density was not correlated with Ψ_{wmax} and Ψ_{wmin} within individual communities ($P > 0.05$ for all). Xylem density was correlated with g_{smax} across communities such that species with denser xylem had lower maximum stomatal conductance ($P = 0.046$, $r^2 = 0.14$; data not shown). Xylem density was not correlated to g_{smax} within communities ($P > 0.05$ for all; data not shown). Xylem density was not correlated to g_{smin} within or across communities ($P > 0.05$ for all; data not shown).

Hydraulic conductivity

Xylem specific conductivity (K_{s}) did not differ among the three communities ($P = 0.107$; see Jacobsen *et al.* 2007*c*). Xylem specific conductivity was correlated with Ψ_{wmax} ($P < 0.001$, $r^2 = 0.46$; data not shown) across communities and within the coastal sage scrub ($P = 0.036$, $r^2 = 0.55$; data not shown) such that species with greater K_{s} experienced higher Ψ_{wmax}. Xylem specific conductivity was not correlated with Ψ_{wmax} among species within the chaparral or Mojave Desert communities ($P > 0.05$ for both). Xylem specific conductivity was also not correlated with Ψ_{wmin}, g_{smax}, or g_{smin} within or across communities ($P > 0.05$ for all; data not shown); however, K_{s} was correlated with Ψ_{wmin} when data were analyzed using phylogenetic independent contrasts ($P = 0.039$).

Leaf specific conductivity (K_{l}) was correlated with g_{smax} ($P = 0.004$, $r^2 = 0.337$, data not shown) and g_{smin} ($P = 0.030$, $r^2 = 0.206$; not shown) across communities such that species with great K_{l} displayed higher g_{smax} or $\mathrm{g}_{\mathrm{smin}}$. The K_{l} was not significantly different among communities ($P = 0.227$, data not shown).

Xylem vulnerability to cavitation

The xylem and leaf traits included in the present study were not generally good predictors of species vulnerability to xylem cavitation. In contrast, minimum seasonal Ψ_w was predictive of resistance to water stress induced cavitation (Ψ_{50}) across communities (Figure 3.7; $P = 0.013$, $r^2 = 0.22$) and within the chaparral community (Figure 3.7; $P = 0.013$, $r^2 = 0.56$) but not within the coastal sage or Mojave Desert communities ($P = 0.461$ and 0.773, respectively). Other traits included in the present study, including Ψ_{wmax}, g_{smax}, g_{smin}, SLA, xylem density, and K_s, were not predictive of Ψ_{50} either across or within communities ($P > 0.05$ for all; data not shown) with one exception. Minimum g_s was predictive of Ψ_{50} within the coastal sage scrub community with species experiencing lower g_{smin} also having more negative Ψ_{50} ($P = 0.032$; $r^2 = 0.50$; data not shown).

Water use "strategies"

Communities differed in the suites of xylem traits utilized by species ($P = 0.006$). A principle components analysis resulted in two components ($P < 0.001$, Fig. 3.8). Component 1 explained 56.2 % of species variability and was associated mainly with Ψ_{wmin}, K_s, and xylem density. Component 2 explained 22.9% of species variability and was associated mainly with Ψ_{50}. Species from the chaparral, coastal sage, and Mojave Desert communities occupy largely non-overlapping areas when the species values of these components are graphed, consistent with a significant difference between these communities in multivariate strategies utilized by species (Fig. 3.8).

Discussion

Among shrub species occurring in three different winter rainfall plant communities of southern California, plants experienced similar patterns in their seasonal water status and stomatal conductance (g_s) such that across sites species tended to reach their seasonal high water potentials (Ψ_{wmax}) and maximum g_s (g_{smax}) in spring during the wet season. Species tended to reach their seasonal low water potentials (Ψ_{wmin}) at the end of the dry season and seasonal low g_s (g_{smin}) during the cool winter months. This matches the annual precipitation and temperature pattern for these sites and indicates that species are responding similarly to rainfall events and that cooler winter temperatures may limit gas exchange in Californian winter rainfall communities.

Several species from each of the three sites deviated from these average patterns of seasonal water status illustrating phenological diversity among species. These included *R. ovata*, *M. laurina*, *H. salsola*, *L. andersonii*, and *C. ramosissima* which reached their minimum seasonal water potentials (Ψ_{wmin}) during the winter wet season in 2007. This pattern has been described previously for *R. ovata* (Pratt *et al.* 2005) and is likely caused by high winter embolism in the stems of this species which reduces water supply to leaves. However, below ground factors may be involved in other species, including root phenology and soil moisture distribution. *Lepidospartum squamatum* differed from other species in displaying its maximum seasonal water potential (Ψ_{wmax}) during the dry summer period (August). The range of this species extends into summer rainfall regions and this species appears to be tuned to a summer rainfall regime. A similar pattern was also evident in the Mojave Desert shrub *C. ramosissima* which was able to rapidly respond to a small late summer rain pulse in the present study but which slowly responded to a pulse of winter rain. This pattern has been previously described for this species (Lin *et al.* 1996; Gebauer & Ehleringer 2000).

While across sites species tended to respond similarly to seasonal changes in precipitation, plant water status varied significantly among the sites during the wet season. Species in the chaparral and coastal sage scrub experienced similarly high Ψ_{wmax} while species from the Mojave Desert did not reach similarly high water potentials. This may be due to lower wet season soil moisture in the Mojave Desert site compared to the other two sites (Jacobsen, unpublished data). Relatively low soil moisture values of shallow soil layers have been previously reported for the Mojave Desert (Smith *et al.* 1995; Yoder & Nowak 1999) and comparatively higher values have been reported for the chaparral (Johnson-Maynard *et al.* 2004) suggesting this may be a consistent difference among these shrub communities. Additionally, desert shrubs may be accessing deeper soil moisture reserves which are fairly stable and recharge mainly during above average precipitation years as has been described for the Chihuahuan Desert (Snyder *et al.* 2006).

During the dry season, Ψ_{wmin} was not different among the sites suggesting that species at these sites are experiencing a common level of water stress. The across species mean Ψ_{wmin} was -4.6 MPa. This value is similar to the average Ψ_{wmin} reported for 26 chaparral species in years of average precipitation (Jacobsen *et al.* 2007*b*), for 17 species from the winter rainfall region of South Africa (Jacobsen *et al.* 2007*b*), and for 6 species from the Great Basin Desert (Hacke *et al.* 2000). This suggests this Ψ_{wmin} may represent a common physiological limit of arid and semi-arid shrub communities. This is also consistent with a mean cavitation resistance (Ψ_{50}) of approximately -4 to -5 MPa which has been found across Mediterranean-type and Desert ecosystems (Hacke *et al.* 2000; Maherali *et al.* 2004; Jacobsen *et al.* 2007*b*).

Minimum Ψ_w was correlated with Ψ_{50} with a 1:1 relationship between these traits for the chaparral species as has been reported in previous studies (Hacke *et al.* 2000; Jacobsen *et al.* 2007*b*; Pratt *et al.* 2007*a*); however, there was no correlation among these traits within the coastal sage scrub or Mojave Desert scrub. This suggests that these communities may vary considerably in the levels of water stress induced embolism that they experience seasonally. While chaparral shrubs appear to reach approximately 50% native embolism seasonally, it appears that shrubs in other arid systems may have alternative thresholds and may regularly reach significantly higher levels of xylem embolism. Indeed, high seasonal levels of embolism have been reported for species of the Sonoran Desert (Pockman & Sperry 2000) and for the coastal sage (Hargrave *et al.* 1994; Kolb & Davis 1994). Thus, while Ψ_{wmin} and Ψ_{50} may be correlated in some communities it does not appear that these traits are always correlated and Ψ_{wmin} may not be a generally useful predictor of Ψ_{50} in arid and semi-arid communities as has been suggested (Jacobsen *et al.* 2007*b*).

Among these three communities, species displayed differential stomatal sensitivity to water stress with the relationship between Ψ_w and g_s significantly varying among communities. Among coastal sage scrub species, g_s declined rapidly with declining Ψ_w suggesting high stomatal sensitivity to water stress. Consistent with our predictions, the chaparral had a much shallower slope between g_s and Ψ_w when compared to the coastal sage scrub species. This implies that chaparral species, which have stem xylem that is more resistant to cavitation, can tolerant greater declines in Ψ_w before limiting stomatal gas exchange.

In contrast to these first two communities, on average, species from the Mojave Desert scrub showed no change in g_s in response to changes in Ψ_w when data were pooled across species. This suggests that these species do not alter their stomatal conductance in response to water stress and that they may be using alternative strategies to limit water loss. However, it should be noted that while this is the general pattern across species there are some species from the Desert community that displayed rather sensitive stomatal responses. For instance, both *Coleogyne ramosissima* and *Larrea tridentata* displayed relatively steep declines in stomatal conductance with declining water potential (Figure 3.5 C; medium dashed line for *C. ramosissima* and dotted line for *L. tridentata*). Many of the Mojave Desert scrub species may be reducing total leaf area rather than reducing water loss through individual leaves. Indeed, during the course of the present study individuals of many desert species reduced their leaf area, although only one species (*L. andersonii*) is completely deciduous and lost all of its leaves. This pattern of leaf drop as a means of regulating whole plant stomatal conductance appeared most pronounced in *Ambrosia dumosa*. In this species, stomatal conductance increased as water potential declined largely as a result of reduced leaf area which likely increased the water supply to individual leaves even though whole plant water status was reduced (Figure 3.5 C solid line). Significant leaf drop during drought has been described previously in desert shrubs during the dry season (Smith *et al.* 1995).

Consistent with the idea that species in these three communities are employing different suites of traits in response to water stress, species appear to be differentially adjusting leaf area to maintain similar dry season K_l and g_s in response differences in cavitation resistance and therefore seasonal embolism. Stomatal conductivity was correlated with K_l when pooled across sites, with species with greater K_l displaying greater g_{smin} and g_{smax} consistent with previously reported correlations between K_l and g_s (Sperry 2000; Santiago *et al.* 2004). Additionally, dry season K_l and g_{smin} were not different among sites suggesting that perhaps there is a common minimum hydraulic flow which much be sustained in order to maintain evergreen leaves in arid and semi-arid environments. This is consistent with the greater level of leaf drop observed in the coastal sage and Mojave Desert communities concomitant with their greater susceptibility to cavitation and presumably higher levels of dry season embolism. Coastal sage scrub species displayed significantly higher g_{smax} than the other two communities which may partially result from their high stomatal sensitivity to changes in water status. Xylem specific conductivity was not correlated with most water relations and leaf traits, including Ψ_{50}, K_s, K_l, and g_s ; however, across sites Ψ_{wmax} and K_s were correlated.

Thus, species among these three sites are divergent in their functional traits related to available water resources. The pattern of water use displayed by the coastal sage scrub species is consistent with adaptation to utilize shallow temporarily available water (Schwinning & Ehleringer 2001). Coastal sage scrub species tend to have shallow roots (Hellmers *et al.* 1955) and display significantly higher wet seasonal g_s than species in the other two sites, and have high stomatal sensitivity to plant water status. In contrast, shrub species from the desert likely have deeper root systems (Gibbens & Lenz 2001), have low maximum stomatal conductivity, and have low stomatal sensitivity to plant water status consistent with a strategy of tapping mostly deeper soil moisture reserves

(Schwinning & Ehleringer 2001). Chaparral shrub species are a blend of these two extremes with species exhibiting both deep and shallow rooting systems (Cooper 1922; Hellmers *et al.* 1955), intermediate stomatal sensitivity to water stress (Poole & Miller 1975), and low maximum stomatal conductivity not significantly different from that of the Mojave Desert species. Recent work has shown that deep and shallow water use strategies are linked with life history type in the chaparral (Pratt *et al.* 2007*b*). Species that are utilizing suites of traits that occur in overlapping areas between communities (see Fig. 3.8) tend to be species with broad ranges that occur across communities, suggesting there are certain suites of traits that may be successful in many communities.

Specific leaf area (SLA) does not correlate well with plant functional traits among these arid shrub species. SLA was different among the sites, with the coastal sage scrub species having significantly higher SLA compared to the chaparral and Mojave Desert scrub. However, SLA was highly variable among species within a site suggesting lack of convergence to a single SLA within these environments. Additionally, SLA was not correlated with water relations or leaf traits including, Ψ_{50}, Ψ_{wmax}, Ψ_{wmin}, K_s, K_l, and g_s and thus was not useful as a predictor of these physiological traits. SLA is likely correlated to other plant traits not included in the present study such as leaf life span and photosynthetic capacity (Ackerly 2004; Poorter & Bongers 2006; Gulías *et al.* 2003) or may be responding to microsite differences in nutrient availability (Wright *et al.* 2002).

The strong relationship between seasonal water potential and xylem density among data pooled across communities suggests that xylem density may indeed be a useful tool in categorizing plant functional strategies and plant functional variation both across broad regions and within communities (*c.f.* Ackerly *et al.* 2004; Preston *et al.*

2006; Swenson & Enquist 2007). Xylem density was strongly correlated with Ψ_{wmax} and Ψ_{wmin} across communities with species having denser xylem displaying more negative Ψ_{wmax} and Ψ_{wmin}. Xylem density was also correlated with g_{smax} across communities such that species with denser xylem had lower maximum stomatal conductance.

The Ψ_{wmin} that plants experienced was a better predictor of xylem traits than xylem cavitation resistance (Ψ_{50}). Additionally, xylem density was not predictive of xylem cavitation resistance (Ψ_{50}) within any of the three plant communities examined contrary to what has been found in other studies (Hacke *et al.* 2001a; Jacobsen *et al.* 2007*b*). This may be due to the 1:1 relationship between Ψ_{50} and Ψ_{wmin} found in these prior studies. In the present study where Ψ_{50} and Ψ_{wmin} are not correlated or are correlated in a non-1:1 relationship, xylem density appears to be non-predictive of cavitation resistance (Pratt & Black 2006). This suggests that xylem density should not be used to predict cavitation resistance in communities unless the relationship between Ψ_{50} and Ψ_{wmin} has been determined. In contrast, the strong correlation between Ψ_{wmin} and xylem density suggests that these traits may be functionally related and predictive of one another. This is supported by previous studies that have found these traits to be related (Hacke *et al.* 2000; Ackerly 2004; Jacobsen *et al.* 2007*a*, Jacobsen *et al.* 2007*b*). This relationship may be useful in development of global plant functional trait models.

Arid shrub communities appear divergent in their adaptation to water stress even though they experience similar levels of water stress with a community level average Ψ_{wmin} of approximately -4.6 MPa. The relationship between Ψ_{wmin} and Ψ_{50} appears to be community specific and may be related to differential ability of species to tolerate or avoid high seasonal levels of embolism. The relationship between g_s and Ψ_w also appears to be community specific and may reflect differential water use by shrubs in these communities perhaps in response to different rainfall predictability, soil moisture, precipitation, or soil nutrients among sites. Trait relationships that are community specific in the present study may provide the basis for tests of community convergence; it may be that shrub communities in some of the other Mediterranean-type climate regions worldwide display similar relationships as those found in the three communities sampled in the present study.

Table 3.1. Species listed by vegetation type, family, species code, cavitation resistance (Ψ_{50}), xylem specific conductivity (K_s), specific leaf area (SLA), and xylem density for 28 shrub species from three sites in southern California.

Vegetation type and Species	**Family**	**Species Code**
Chaparral		
Adenostoma fasciculatum Hook. & Arn.	Rosaceae	Af
Adenostoma sparsifolium Torrey	Rosaceae	As
Arctostaphylos glandulosa Eastw.	Ericaceae	Ag
Ceanothus cuneatus (Hook.) Nutt.	Rhamnaceae	Cc
Ceanothus megacarpus Nutt.	Rhamnaceae	Cm
Ceanothus oliganthus Nutt.	Rhamnaceae	Co
Ceanothus spinosus Nutt.	Rhamnaceae	Cs
Malosma laurina (Nutt.) Abrams	Anacardiaceae	Ml
Quercus berberidifolia Liebm.	Fagaceae	Qb
Rhus ovata S. Watson	Anacardiaceae	Ro
Coastal Sage Scrub		
Artemisia californica Less.	Asteraceae	Ac
Encelia californica Nutt.	Asteraceae	Eca
Eriogonum cinereum Benth.	Polygonaceae	Eci
Hazardia squarrosa (Hook. & Arn.) E. Greene	Asteraceae	Hs
Lotus scoparius (Nutt.) Ottley	Fabaceae	Ls
Malacothamnus fasciculatus (Torrey & A. Gray) E. Greene	Malvaceae	Mf
Malosma laurina (Nutt.) Abrams	Anacardiaceae	Ml
Salvia leucophylla E. Greene	Lamiaceae	Sl
Salvia mellifera E. Greene	Lamiaceae	Sm
Mojave Desert Scrub		
Ambrosia dumosa (A. Gray) Payne	Asteraceae	Ad
Atriplex canescens (Pursh) Nutt.	Chenopodiaceae	Ac
Atriplex polycarpa (Torrey) S. Watson	Chenopodiaceae	Ap
Coleogyne ramosissima Torrey	Rosaceae	Cr
Gutierrezia microcephala (DC.) A. Gray	Asteraceae	Gm
Hymenoclea salsola A. Gray	Asteraceae	Hs
Isomeris arborea Nutt.	Capparaceae	Ia
Larrea tridentata (DC.) Cov.	Zygophyllaceae	Lt
Lepidospartum squamatum (A. Gray) A. Gray	Asteraceae	Ls
Lycium andersonii A. Gray	Solanaceae	La

Note: Data for Ψ_{50}, K_s, SLA, and xylem density are means ± 1 SE.

[#] from Chapter 2; Jacobsen *et al.* 2007*c*

[§] from Chapter 2; Jacobsen *et al.* 2007*c*

Table 3.1 (cont'd).

Ψ_{50}[#]	K_s[§]	**SLA**	**Density**
-7.33 ± 0.45	0.88 ± 0.13	5.91 ± 0.42	679.3 ± 12.8
-4.65 ± 0.27	1.55 ± 0.19	11.43 ± 1.38	619.8 ± 10.9
-5.09 ± 0.34	1.04 ± 0.18	5.20 ± 0.29	661.5 ± 18.5
-7.19 ± 0.58	0.83 ± 0.19	4.27 ± 0.13	678.1 ± 10.4
-6.44 ± 0.43	1.91 ± 0.27	4.06 ± 0.16	638.9 ± 11.4
-4.05 ± 0.37	1.24 ± 0.11	11.06 ± 1.23	647.1 ± 8.2
-4.14 ± 0.61	1.33 ± 0.21	6.91 ± 0.62	614.9 ± 10.8
-0.52 ± 0.12	5.55 ± 1.05	5.24 ± 0.16	496.6 ± 16.9
-1.51 ± 0.24	1.59 ± 0.51	7.38 ± 0.37	724.2 ± 8.9
-0.56 ± 0.07	1.64 ± 0.36	4.41 ± 0.37	523.1 ± 8.9
-2.15 ± 0.16	1.21 ± 0.20	20.09 ± 1.20	685.7 ± 14.7
-0.82 ± 0.08	1.44 ± 0.29	14.01 ± 0.47	637.8 ± 16.9
-1.97 ± 0.18	0.93 ± 0.13	5.41 ± 0.19	671.2 ± 24.0
-1.42 ± 0.16	0.64 ± 0.08	12.26 ± 0.59	557.9 ± 12.5
-2.34 ± 0.25	1.41 ± 0.25	15.39 ± 0.85	613.7 ± 16.7
-0.94 ± 0.14	0.77 ± 0.13	12.03 ± 0.69	571.8 ± 19.2
-0.68 ± 0.10	4.80 ± 1.67	4.41 ± 0.18	467.2 ± 7.7
-1.70 ± 0.07	2.07 ± 0.28	12.96 ± 1.36	503.1 ± 19.1
-2.39 ± 0.26	1.50 ± 0.21	8.89 ± 0.37	528.9 ± 10.0
-1.05 ± 0.24	0.65 ± 0.12	11.69 ± 1.09	711.2 ± 16.1
-0.97 ± 0.17	0.17 ± 0.03	4.92 ± 0.45	797.8 ± 9.9
-1.98 ± 0.14	0.36 ± 0.05	4.62 ± 0.83	810.7 ± 10.4
-1.50 ± 0.21	0.39 ± 0.05	10.57 ± 1.35	678.3 ± 18.0
no data	*no data*	7.03 ± 0.59	647.2 ± 10.0
-2.09 ± 0.35	1.60 ± 0.40	10.75 ± 0.84	690.2 ± 18.3
-2.51 ± 0.23	0.34 ± 0.04	8.97 ± 0.73	612.6 ± 10.7
-3.31 ± 0.32	0.63 ± 0.10	6.17 ± 0.43	854.1 ± 8.4
-0.79 ± 0.06	1.54 ± 0.39	*no data*	566.1 ± 12.0
-1.02 ± 0.10	0.60 ± 0.16	9.36 ± 0.76	706.9 ± 16.9

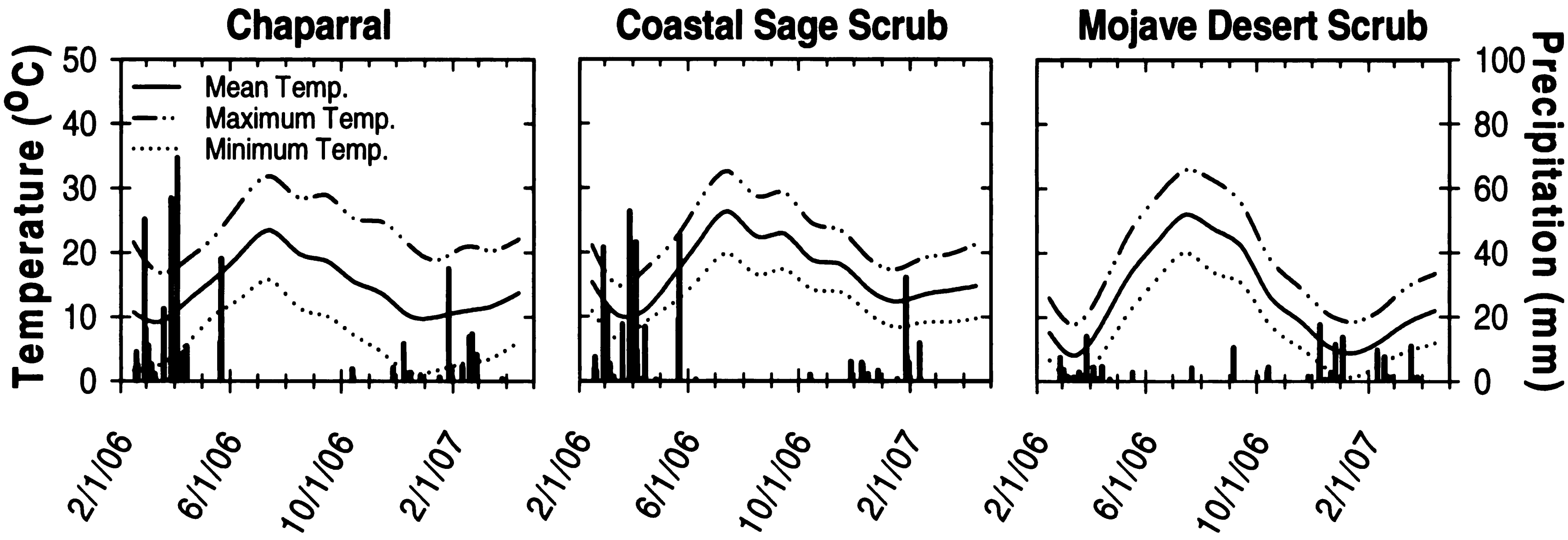

Figure 3.1. Monthly mean, minimum, and maximum temperature and precipitation for three sites located in southern California for February 2006 through April 2007. Each bar represents the date and amount of a precipitation event recorded at a site on that date. Over the period displayed in this figure total precipitation was approximately 360, 330, and 70 mm at the chaparral, coastal sage, and Mojave Desert sites, respectively.

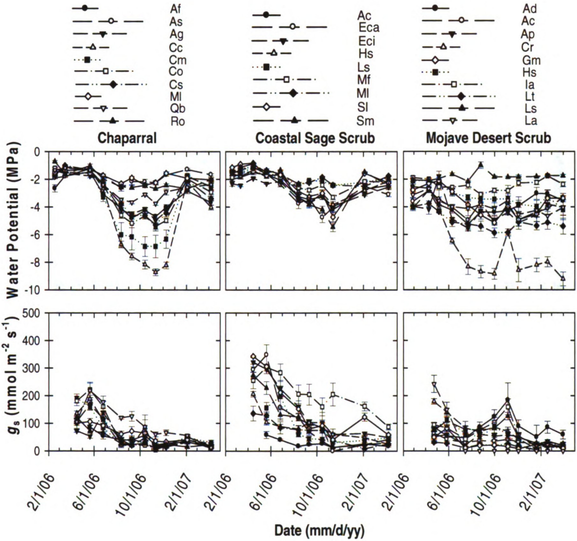

Figure 3.2. Water potential and stomatal conductance (g_s) measured approximately monthly on species from three arid plant communities in southern California. Water potential was measured on 29 species from February 2006 through April 2007 and g_s was measured on 28 species from April 2006 through April 2007. See Table 3.1 for species codes. The same symbols are used to represent the same species for water potential and g_s panels for each community. Symbols are species means ± 1 SE ($n = 6$).

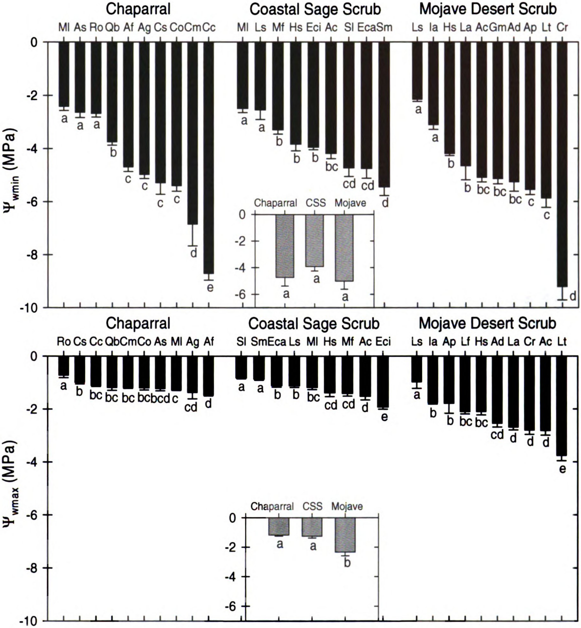

Figure 3.3. Maximum and minimum water potential (Ψ_{wmax} and Ψ_{wmin}, respectively) measured from February 2006 through April 2007 on 28 shrub species from three plant communities in southern California: chaparral, coastal sage scrub (CSS), and Mojave Desert scrub. Each black bar represents the lowest or highest monthly value for a species (mean ± 1 SE; see Table 3.1 for species codes). Different letters below bars indicate significant differences among species within a community. Grey bars in insets represent means ± 1 SE across communities and different letters indicate significant differences.

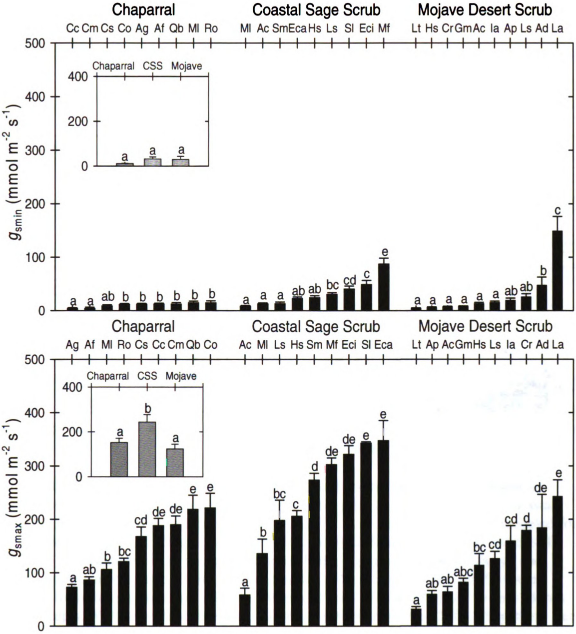

Figure 3.4. Maximum and minimum stomatal conductance (g_{smax} and g_{smin}, respectively) measured from April 2006 through April 2007 on 27 shrub species from three plant communities in southern California: chaparral, coastal sage scrub (CSS), and Mojave Desert scrub. Each black bar represents the highest or lowest monthly value for a species (mean ± 1 SE; see Table 3.1 for species codes). Different letters below bars indicate significant differences among species within a community. Grey bars in insets represent means ± 1 SE across communities and different letters indicate significant differences.

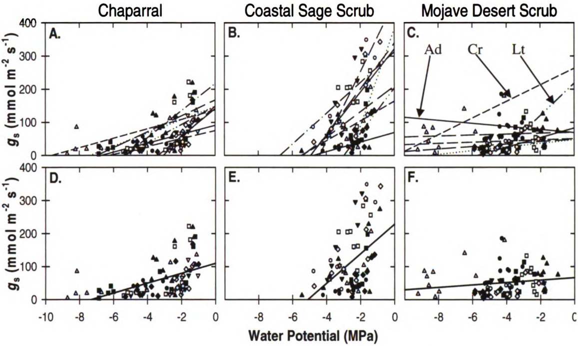

Figure 3.5. Water potential as a predictor of stomatal conductance (g_s) for 28 species from three arid plant communities of southern California: chaparral (A and D), coastal sage (B and E), and Mojave Desert scrub (C and F). Linear regressions were modeled for each species (A, B, and C) and for each community with data pooled across species (D, E, and F). Species from the coastal sage scrub had a significantly greater slopes and intercepts when compared to either the chaparral or the Mojave Desert scrub species. Species from the chaparral and Mojave Desert did not differ significantly in their slopes or intercepts. Within each community, different symbols represent different species. See Table 3.1 for species codes in Panel C and see Figure 3.2 for species legend for A, B, and C.

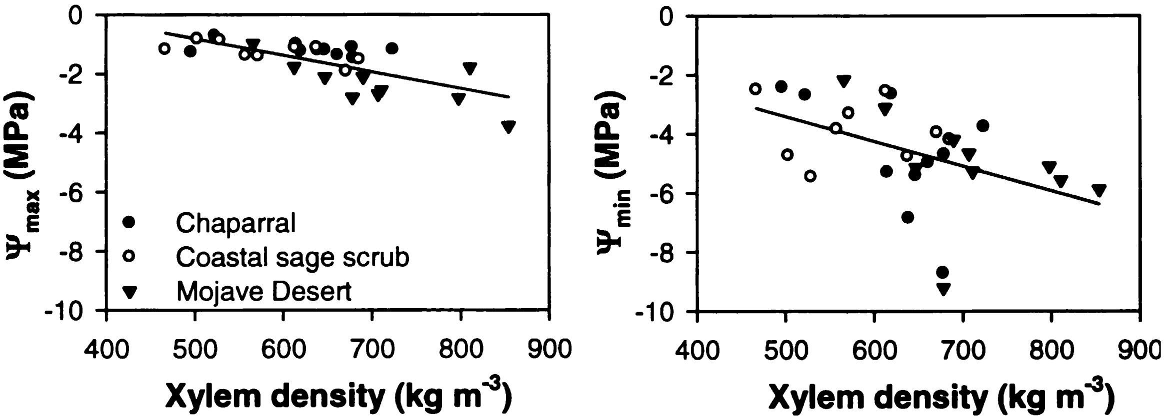

Figure 3.6. Xylem density as a predictor of Ψ_{wmax} and Ψ_{wmin} pooled across communities ($P < 0.001$, $r^2 = 0.52$ for Ψ_{wmax} and $P = 0.012$, $r^2 = 0.21$ for Ψ_{wmin}). Xylem density was not correlated with Ψ_{wmax} and Ψ_{wmin} within individual communities. Different symbols indicate data from the different arid plant communities.

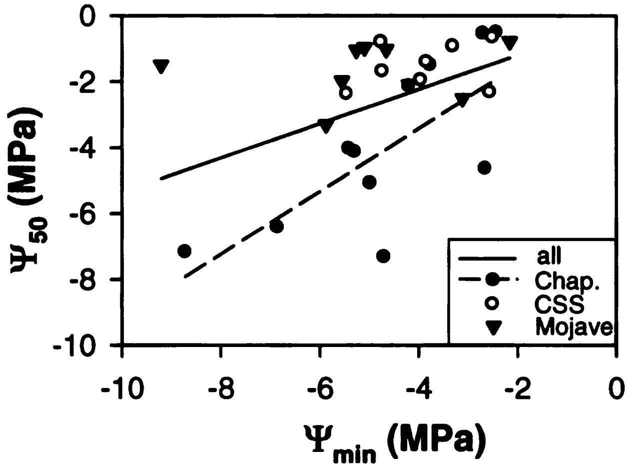

Figure 3.7. Minimum seasonal water potential (Ψ_{wmin}) as a predictor of resistance to water stress induced cavitation (Ψ_{50}) pooled across communities. Different symbols indicate data from a different arid plant community: chaparral (Chap.), coastal sage scrub (CSS), or Mojave Desert scrub (Mojave). Linear regressions were modeled both across all communities and for each community. Only significant regressions are shown ($P < 0.05$).

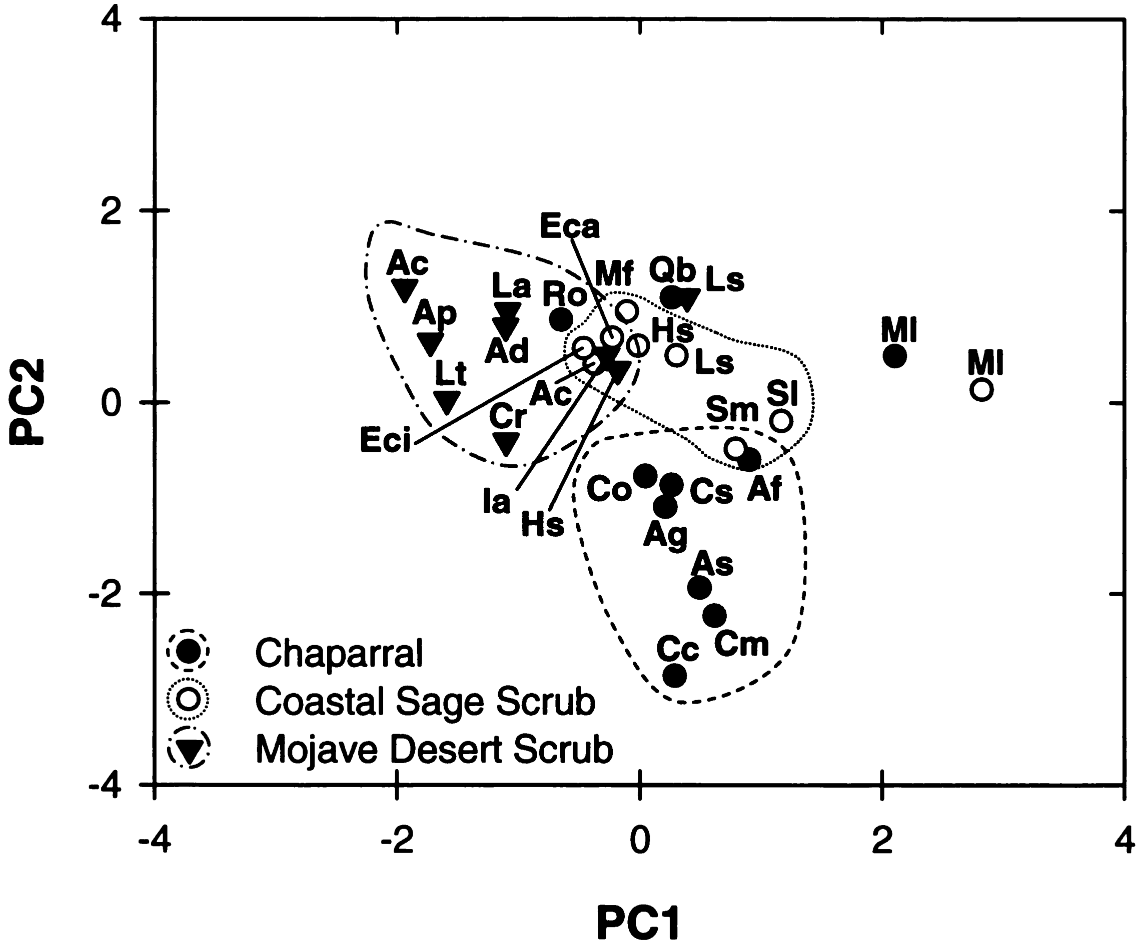

Figure 3.8. Results of a principle component analysis using multiple traits measured on species occurring in three shrub communities of the winter rainfall area of southern California. Component 1 (PC1) is strongly associated with water relations traits including minimum seasonal water potential (Ψ_{wmin}), xylem density, and xylem specific conductivity (K_s) such that species with higher values have greater K_s, lower xylem density, and higher (less negative) Ψ_{wmin}. Component 2 (PC2) is strongly associated with water stress tolerance (Ψ_{50}), such that higher values are less resistant to cavitation (i.e. have higher Ψ_{50}). The suites of traits utilized by each community significantly differ (P = 0.006). Each symbol represents a different community and species codes are shown in Table 3.1.

CHAPTER FOUR

PHYLOGENETIC DISPERSION AND COMMUNITY STRUCTURE IN THREE ARID PLANT COMMUNITIES

Abstract

The phylogenetic relationships among species have the potential to influence community assemblages and community structure. Communities that are the product of environmental filtering will likely contain species that are more closely related than randomly constructed communities, while communities that are biotically shaped through competitive interactions will likely contain species that are more distantly related than randomly constructed communities. These patterns rely on the assumption that more closely related species display more similar functional traits compared to distantly related species. Using woody shrub species from three arid communities of southern California, including the chaparral, coastal sage, and Mojave Desert scrub, I examined whether functional traits related to water stress tolerance and water use were indeed more similar among more closely related species. Additionally, I examined whether phylogenetic relationships among species influence species distributions among and within these communities (at the β and α scales, respectively). At the α level, I examined what functional traits may be associated with species co-occurrence in each community. The three communities examined in this study differed in their species compositions and stand structures, yet they all tended to be composed of species assemblages that were more closely related than expected. This suggests that species were "filtered" into these communities based on preexisting functional traits and that in these stressful arid environments species persistence at the β scale is likely related to traits conserved within certain lineages. Minimum seasonal water potential (Ψ_{wmin}) was significantly correlated with phylogeny and may be related to phylogenetically linked traits important for species persistence in these communities ($P = 0.001$). Within communities, at the α scale, phylogeny was unrelated to species co-occurrence, perhaps because the traits associated with co-occurrence and niche segregation within communities are phylogenetically labile. Traits that varied independent of phylogeny in the present study and which were correlated with species co-occurrence within some communities included water potential at 50% loss in hydraulic conductivity for the coastal sage and stomatal conductance, xylem specific conductivity, and xylem density for the Mojave Desert. Frequent co-occurrence of conspecifics within stands, particularly in the chaparral, and of species displaying similar functional traits, in the Mojave Desert and coastal sage scrub, suggests that habitat heterogeneity or dispersal limitation may influence within community species distributions.

Introduction

In arid communities competition for water is likely one of the key community structuring processes and likely influences within community interactions (Flores-Martínez *et al.* 1998; Vilà & Sardans 1999). Water use differences among species may be involved in community assembly and may facilitate species co-occurrence within communities. Functional traits related to water use and water stress tolerance may also influence species distribution and abundance within a community.

Among 28 shrub species from three arid plant communities in southern California, including the chaparral, coastal sage scrub, and Mojave Desert scrub, species display great variation in water use and response to water stress (Harrison *et al.* 1971; Poole & Miller 1975; Smith *et al.* 1995; Jacobsen *et al.* 2007c; Chapter 3). This divergence in species response includes considerable within and between community variation in species resistance to water stress induced cavitation (Jacobsen *et al.* 2007*c*), water potential (Chapter 3), and stomatal response (Chapter 3). Among these communities, community specific traits and trait relationships may reflect selective filtering of species during community assembly. That is, only species and lineages with certain traits or suites of traits were able to persist within a specific community. Within communities, variation among species in these traits may be related to niche segregation and interspecific partitioning of available resources.

More closely related species likely share more functional traits than distantly related species (Webb *et al.* 2002; Swenson *et al.* 2007) leading to predictions of phylogenetic structuring both between and within communities (at the β and α scales, respectively). At the community (β) level, presence of a functional trait that allows for persistence of a lineage in a given environment may lead to environmental filtering of these species. Thus, communities assembled through filtering would be composed of species that are more closely related than would occur in randomly assembled communities (*i.e.* phylogenetic under-dispersion). Patterns of phylogenetic under-dispersion have been found previously among bacterial communities (Horner-Devine & Bohannan 2006), a neotroprical forest tree community (Kembel & Hubbell 2006), tropical insect herbivores (Weiblen *et al.* 2006), and among diverse Floridian plant communities (Cavender-Bares *et al.* 2006). Alternatively, functionally similar species could competitively exclude one another (Hardin 1960), leading to communities being composed of species that are more distantly related than would occur in randomly assembled communities (*i.e.* phylogenetic over-dispersion). Phylogenetic over-dispersion has been described in Floridian oak communities (Cavender-Bares *et al.* 2004) and among *Adenostoma* species occurring in southern California (Beatty 1987). Both environmental filtering and competitive exclusion could operate to create community level (β scale) species assemblages. Within communities, at the α scale, these same processes can create similar patterns. In an under-dispersed community, more closely related species are more likely to occur near one another whereas in an over-dispersed community, more distantly related species are more likely to occur near one another.

We examined whether, at the community scale, functional traits of species relating to water stress tolerance and water use were correlated with species phylogenetic relationships. Additionally, we examined whether arid communities displayed evidence of the processes of filtering or competitive exclusion in their community composition. We predicted that filtering would be more important than competition in these communities based on the work of Grime (1977) in which it is suggested that competition may be less important in stressful environments (see also Goldberg & Novoplansky 1997). Thus, we would expect phylogenetic under-dispersion among species in these communities if the traits related to persistence within the community are phylogenetically constrained. We also examined whether the phylogenetic relationships among species relates to the distribution of species within these communities. We predicted phylogenetic over-dispersion within communities, consistent with what has been found in prior studies (Beatty 1987; Cavender-Bares *et al.* 2006; Swenson *et al.* 2006) although other phylogenetic patterns have also been found within communities (Horner-Devine & Bohannan 2006; Kembel & Hubbel 2006). We used published values for various physiological and functional traits for the species included in the present study (Jacobsen *et al.* 2007*c*; Chapter 3) to determine which traits may be correlated with species distributions within communities (Redtfeldt & Davis 1996).

We examined the dominant woody shrub species occurring at sites in each of these communities. We focused on woody plant species because these species are of similar form and habit and would likely occupy relatively similar niches and they are the dominant plants in these communities.

Materials and Methods

The dominant woody shrub species in each of three aridland plant communities located in the winter-rainfall region of southern California were sampled. These three sites included a chaparral site where 10 woody plant species were dominant, a coastal sage scrub site where 9 woody plant species were dominant, and a Mojave Desert scrub community where 10 woody plant species were dominant (see Table 4.1 for species names). These species accounted for 89, 99, and 73 % of the woody plants in each community, respectively. The location of these sites is described in Jacobsen *et al.* (2007*c*).

At each site, woody shrub species were sampled using a modified point-quarter sampling method (Cox 1985). Fifty-four to 60 points were established at each site using six randomly chosen individuals of each of the dominant woody species at each site as points. At each point, the distance from the central plant to the nearest plant in each of four quadrants was measured and the species identified. By this analysis a plant had four neighbors but only one nearest neighbor. For each of the five plants at each point, the basal diameter, crown diameter, and crown height were measured. The percentage of co-occurrence of species within each site was calculated as the percentage that species were neighbors in any of the four quadrants measured at each point and also as the percentage that species occurred as nearest neighbors irrespective of quadrant. ANOVAs were used to test for differences in these traits among species and communities. These data were also divided into cases in which the neighbors or nearest neighbors were conspecific or heterospecific. The expected counts of conspecific and heterospecific co-occurrence were predicted based on species abundance within each community and random species distribution within the community. A chi-squared test was then used to determine if the actual co-occurrence of conspecifics and heterospecifics were different than predicted values.

Phylogenetic relationships among species were established using published phylogenetic data (Figure 4.1). The phylogeny was constructed using recent molecular-based phylogenies (Morgan *et al.* 1994; Bayer & Starr 1998; Hardig *et al.* 2000; Goertzen *et al.* 2003; Hilu *et al.* 2003; Urbatsch *et al.* 2003; Soltis & Soltis 2004; Soltis *et al.* 2005). Since we combined several trees to create this phylogeny we lacked branch length information; therefore, all analyses were run assuming equal branch lengths (Ackerly 2000). The phylogenetic distances between species pairs were determined based on the number of steps between each pair. More closely related species pairs have fewer steps between them (small phylogenetic distance), whereas distantly related species have more steps between them (greater phylogenetic distance). The maximum phylogenetic distance in our study was 15. Individuals of the same species would have the minimum phylogenetic distance (zero).

To examine phylogenetic dispersion among communities, phylogenetic distances were calculated between all of the species examined in the present study. From the pool of 28 studied species, species were randomly selected to create hypothetical communities each containing 10 species (n = 1000 randomly assembled communities). Phylogenetic distances among species were calculated for these randomly assembled communities and community average phylogenetic distances determined. Community average phylogenetic distances for the three communities examined in the present study were then compared to the distribution of mean phylogenetic distance values from the randomly assembled communities.

To examine phylogenetic dispersion within communities, phylogenetic distances among species pairs within each community were correlated to the percentage species co-occurrence within each community. We also used published trait values (Chapter 3; Jacobsen *et al.* 2007*c*) to examine if co-occurrence within communities was correlated with species differences in several physiological and functional species traits. The traits included differences among species pairs in minimum and maximum seasonal water potential ($\Delta\ \Psi_{\mathrm{wmin}}$ and $\Delta\ \Psi_{\mathrm{wmax}}$), water potential at 50% loss in hydraulic conductivity ($\Delta\ \Psi_{50}$), minimum and maximum stomatal conductance ($\Delta\ g_{\mathrm{smax}}$ and $\Delta\ g_{\mathrm{smin}}$), xylem density ($\Delta$ density), specific leaf area (Δ SLA), and xylem specific conductivity ($\Delta\ K_s$) among species pairs. All statistical analyses were run using Minitab (Release 14.12.0, Minitab Inc., State College, PA, USA) and the alpha-level was set at 0.05.

Results

Community structure

Within each community the height, crown diameter, and basal diameter of species varied significantly (Table 4.1). Overall, chaparral species were taller and had greater crown diameters relative to the other two communities (Table 4.2). The distance between plants was not different among the chaparral and coastal sage communities although crown overlap was greater in the chaparral due to their significantly wider crown diameters. Individuals occurring at the Mojave Desert site were further apart than in the other two communities and this, combined with their smaller canopy sizes, resulted in an open canopy (Table 4.2).

Community assemblages

Among communities, the chaparral community was phylogenetically under-dispersed compared to the phylogenetic dispersion of randomly generated communities (based on 95% confidence interval; Figure 4.2). The coastal sage and Mojave Desert scrub communities were not different in their phylogenetic dispersion from randomly generated communities (Figure 4.2).

Conspecific occurrence

Within each of the three communities, there were more conspecific neighbors than predicted based on species abundances in the community (Table 4.3). When only nearest neighbors were considered, chaparral species remained more likely to be nearest a conspecific than predicted, while coastal sage and Mojave Desert scrub species were not more likely to be nearest a conspecific than predicted (Table 4.3).

Species distributions within communities

The percent co-occurrence among more closely related individuals increased when conspecifics were included in analyses (Figure 4.3 A, B, C; $P < 0.001$ for the chaparral, $P = 0.023$ for the coastal sage, and $P = 0.014$ for the Mojave Desert). This relationship is also significant among chaparral and Mojave Desert species when only nearest neighbors are included in the analysis (Figure 4.3 A, B, C; $P < 0.001$ for the chaparral, $P > 0.05$ for the coastal sage, and $P = 0.003$ for the Mojave Desert). When conspecifics were excluded from analyses, there was no relationship between either neighbor or nearest neighbor co-occurrence and phylogenetic distance in any of the three communities (Figure 4.3 D, E, F; $P > 0.05$ for all).

Functional traits and phylogeny

Only one trait of those examined, $\Delta \Psi_{\mathrm{wmin}}$ was correlated with phylogenetic distance among species ($P = 0.001$), such that more closely related species had more similar Ψ_{wmin}. All other functional traits included in the present study, including $\Delta \Psi_{50}$, $\Delta \Psi_{\mathrm{wmax}}$, Δg_{smax}, Δg_{smin}, Δ xylem density, Δ SLA, and ΔK_s varied independently of phylogenetic distance ($P > 0.05$ for all).

Functional traits and species co-occurrence

Within the chaparral community, there was no correlation between differences among species pairs in any of the traits included in the present study, including Δ Ψ_{50}, Δ Ψ_{wmin}, Δ Ψ_{wmax}, Δ g_{smax}, Δ g_{smin}, Δ xylem density, Δ SLA, and Δ K_s and the percentage co-occurrence of those species either as neighbors or nearest neighbors ($P > 0.05$ for all).

Within the coastal sage scrub community, Δ Ψ_{wmin} was correlated with co-occurrence as neighbors and nearest neighbors (Figure 4.4 A; $r^2 = 0.146$, $P = 0.022$ for neighbors and $r^2 = 0.166$, $P = 0.014$ for nearest neighbors). Differences between species in Ψ_{50} were also correlated with occurring as neighbors and nearest neighbors (Figure 4.4 B; $r^2 = 0.15$, $P = 0.020$ for neighbors and $r^2 = 0.107$, $P = 0.05$ for nearest neighbors). For both Ψ_{wmin} and Ψ_{50} the species that showed the greatest differences in these traits were less likely to occur near one another. All other traits included in the present study were not correlated with co-occurrence in the coastal sage scrub community ($P > 0.05$ for all).

Within the Mojave Desert scrub community, Δ density was correlated with co-occurrence as neighbors and nearest neighbors (Figure 4.4 C; $r^2 = 0.099$, $P = 0.035$ for neighbors and $r^2 = 0.114$, $P = 0.023$ for nearest neighbors). Differences between species in g_{smax} were also correlated with occurring as neighbors and nearest neighbors (Figure 4.4 D; $r^2 = 0.094$, $P = 0.041$for neighbors and $r^2 = 0.091$, $P = 0.044$ for nearest neighbors). For both g_{smax} and xylem density the species that showed the greatest differences in these traits were less likely to occur near one another. In contrast, Δ K_s was correlated with co-occurrence as neighbors and nearest neighbors (Figure 4.4 E; $r^2 = 0.221$, $P = 0.001$ for neighbors and $r^2 = 0.198$, $P = 0.002$ for nearest neighbors) and species with the greatest differences in K_s were more likely to occur together. All other traits included in the present study were not correlated with co-occurrence in the Mojave Desert scrub community ($P > 0.05$ for all).

Discussion

Community assemblages and structure

Among three arid shrub communities, species utilize community specific suites of traits related to water stress tolerance and water use (Jacobsen *et al.* 2007*c*; Chapter 3). These community specific differences may partially be due to environmental filtering of species with specific traits into these communities. This is evident in the under-dispersion of the chaparral community. Indeed, niche conservatism of many chaparral species has been described previously (Ackerly 2004) suggesting that species may have been "filtered" into the chaparral community based on traits that were already present. The coastal sage and Mojave Desert scrub communities were not significantly more or less phylogenetically dispersed than randomly assembled communities, although they tended to be under-dispersed. This pattern of under-dispersion in arid communities is consistent with the predictions of Grime (1977) that in stressful communities competition may be less important in the determination of community structure and species traits compared to stress tolerance traits. Disturbance in these communities could also mitigate interspecific competition, for instance, the coastal sage scrub and chaparral communities are prone to wildfire. Fire may also lead to under-dispersion due to filtering based on fire-related life history types that are often phylogenetically linked (Verdú & Pausas 2007).

The stand structure of these communities suggests that species occurring in these communities may be more tolerant of stress or disturbance than competition. In the

Mojave Desert scrub, individuals are significantly further apart than in the other two communities and the crowns of individuals do not overlap. This suggests that as adults these individuals are not strongly competing for space and light, although competition for below ground resources may still be great (Casper & Jackson 1997). Above ground, the stands are denser in the coastal sage scrub and chaparral although disturbance by wildfire may mitigate competition in these more productive communities.

Conspecific co-occurrence

Across all three communities, individuals were more likely than predicted to occur near another individual of the same species. This pattern was strongest in the chaparral community where the nearest neighbor of any individual was significantly more likely to be an individual of the same species than would be predicted based on species abundances and random species distribution within the community. This suggests that species distributions and abundances in these communities may be, at least partially, due to α scale environmental heterogeneity within the communities. It also is consistent with evidence of community level environmental filtering.

While conspecific co-occurrence may suggest that certain species possess traits that allow them to best utilize a local abiotic resource, such a pattern could also be explained by dispersal limitation (Levine *et al.* 2003; Levin & Murrell 2003), particularly in the chaparral community. Many of the coastal sage and Mojave Desert species are relatively small seeded (Baker 1972) or have modifications for wind or animal dispersal and thus are able to relatively broadly disperse. Several chaparral species are also broadly dispersed, usually by vertebrate animals, including *Quercus berberidifolia*, *Arctostaphylos glandulosa*, and *Rhus ovata* (Willson 1993); however, many of the species are short to intermediate dispersers including the *Ceanothus* species which utilize ballistic seed dispersal (Evans *et al.* 1987). Localized dispersal may allow species to take advantage of clustered favorable microsites within the chaparral community. Alternatively, the evolution of short range dispersal mechanisms due to other selective pressures may constrain species dispersal across the landscape leading to species clustering in the absence of microsite differences. The high intraspecific competition this pattern creates may also partially explain the high tolerances to water stress found in chaparral shrubs (Jacobsen *et al.* 2007*c*) and selection for increased competitive ability in some of these species (Pratt *et al.* 2007).

Species distributions within communities

By the niche concept, species co-existence is predicted to depend on species specialization resulting in reduced competition within a community (Darlington 1972; Vandermeer 1973; Case 1981; Futuyma & Moreno 1988; Chase & Leibold 2003; Silvertown 2004). This suggests that the distribution of species will be non-random, with functionally dissimilar species more likely to co-occur than species that are functionally more similar. If functional traits are phylogenetically constrained, one would expect a pattern of phylogenetic over-dispersion within communities. This pattern has been found previously among oak species occurring across different habitats (Cavender-Bares *et al.* 2004) and among *Adenostoma* species occurring in southern California (Beatty 1987). Alternatively, heterogeneity across the landscape may combine with niche conservatism (*e.g.* trait conservatism) within lineages resulting in more closely related species co-occurring more often than distantly related species as seen at the across community level (Ackerly 2004). However, in the present study no relationships were found between phylogeny and species co-occurrence within a stand.

The phylogenetic independence of species distributions within these three communities could be due to several factors. It may be that the effects of environmental filtering (which would lead to under-dispersion) and competition (which would lead to over-dispersion) are balancing or weak within these arid communities (Toft & Fraizer 2003; Kembel & Hubbell 2006). Alternatively, it may be that the species specific traits related to resource use segregation are not phylogenetically constrained (Silvertown *et al.* 2006). Indeed, xylem cavitation resistance, a trait that is often used as an estimate of woody plant water stress tolerance, and which is highly variable among species in these communities, has been shown to vary more adaptively rather than be conserved across a broad range of plant taxa (Maherali *et al.* 2004). Xylem specific hydraulic conductivity, a measure of the efficiency that water can be transported through the xylem, also displays more adaptive variability than conservation (Maherali *et al.* 2004). Thus, phylogenetic relationships may not strongly influence plant functional traits and community structure among species within these communities. This is consistent with the findings of Carlquist and Hoekman (1985) that anatomical traits associated with drought tolerance appears to have developed independently in many lineages in these communities. In other communities a poor relationship between phylogeny and co-occurrence at local scales has also been reported (the α scale; Silvertown *et al.* 2006).

Functional traits and community structure

Communities that are not phylogenetically structured may be structured instead by evolutionarily labile traits. We examined the relationship between species co-occurrence and several traits related to water stress and water stress tolerance. In the chaparral community, none of the traits included in the present study were related to species co-occurrence. In contrast, among the coastal sage scrub and Mojave Desert scrub communities, trait relationships among species support habitat heterogeneity as important in driving community structure. Species in these communities are most likely to occur near other species that display similar traits including similar Ψ_{wmin} and Ψ_{50} for the coastal sage and similar xylem density and g_{smin} for the Mojave Desert species. Thus, it is likely that species displaying these traits, regardless of phylogenetic relationships, are able to do well within certain microsites within these communities. One trait, K_s, did appear to display a pattern consistent with niche segregation among co-occurring species with species that were very different in this trait being more likely to co-occur than species with similar K_s.

The three arid communities discussed in this study differ in their species compositions and stand structures, yet they all tend to be composed of species assemblages that are more closely related than expected by chance. This suggests that in these stressful environments species persistence is likely related to traits conserved within certain lineages. These arid systems may offer a unique opportunity to examine the evolution of aridity tolerance of woody species among a diverse range of plant families and to identify which plant functional traits may be important in the filtering of species into arid communities.

Table 4.1. Species and families from three arid plant communities, species codes, and species characteristics including height, crown diameter, and basal diameter. Different letters in each column indicate significant differences among species within each community.

Vegetation type and Species	**Family**	**Species Code**
Chaparral		
Adenostoma fasciculatum Hook. & Arn.	Rosaceae	Af
Adenostoma sparsifolium Torrey	Rosaceae	As
Arctostaphylos glandulosa Eastw.	Ericaceae	Ag
Ceanothus cuneatus (Hook.) Nutt.	Rhamnaceae	Cc
Ceanothus megacarpus Nutt.	Rhamnaceae	Cm
Ceanothus oliganthus Nutt.	Rhamnaceae	Co
Ceanothus spinosus Nutt.	Rhamnaceae	Cs
Malosma laurina (Nutt.) Abrams	Anacardiaceae	Ml
Quercus berberidifolia Liebm.	Fagaceae	Qb
Rhus ovata S. Watson	Anacardiaceae	Ro
Coastal Sage Scrub		
Artemisia californica Less.	Asteraceae	Ac
Encelia californica Nutt.	Asteraceae	Eca
Eriogonum cinereum Benth.	Polygonaceae	Eci
Hazardia squarrosa (Hook. & Arn.) E. Greene	Asteraceae	Hsq
Lotus scoparius (Nutt.) Ottley	Fabaceae	Ls
Malacothamnus fasciculatus (Torrey & A. Gray) E. Greene	Malvaceae	Mf
Malosma laurina (Nutt.) Abrams	Anacardiaceae	Ml
Salvia leucophylla E. Greene	Lamiaceae	Sl
Salvia mellifera E. Greene	Lamiaceae	Sm
Mojave Desert Scrub		
Ambrosia dumosa (A. Gray) Payne	Asteraceae	Ad
Atriplex canescens (Pursh) Nutt.	Chenopodiaceae	Aca
Atriplex polycarpa (Torrey) S. Watson	Chenopodiaceae	Ap
Coleogyne ramosissima Torrey	Rosaceae	Cr
Gutierrezia microcephala (DC.) A. Gray	Asteraceae	Gm
Hymenoclea salsola A. Gray	Asteraceae	Hs
Isomeris arborea Nutt.	Capparaceae	Ia
Larrea tridentata (DC.) Cov.	Zygophyllaceae	Lt
Lepidospartum squamatum (A. Gray) A. Gray	Asteraceae	Lsq
Lycium andersonii A. Gray	Solanaceae	La

Table 4.1 (cont'd)

Height (m)	Crown diameter (m)	Basal diameter (m)
2.51 ± 0.12 b	2.53 ± 0.26 bc	0.31 ± 0.05 bc
5.18 ± 0.23 a	4.37 ± 0.62 a	0.72 ± 0.09 a
1.82 ± 0.14 d	1.62 ± 0.18 cd	0.41 ± 0.07 b
2.96 ± 0.24 bc	1.64 ± 0.20 cd	0.06 ± 0.01 d
2.18 ± 0.16 d	0.80 ± 0.11 d	0.03 ± 0.00 d
2.98 ± 0.19 bc	1.25 ± 0.14 d	0.07 ± 0.02 d
3.16 ± 0.23 b	2.24 ± 0.51 cd	0.14 ± 0.03 cd
3.34 ± 0.31 b	3.37 ± 0.82 ab	0.30 ± 0.09 bc
3.02 ± 0.31 bc	2.39 ± 0.36 bc	0.39 ± 0.12 b
2.41 ± 0.43 cd	2.57 ± 0.39 bc	0.38 ± 0.07 b
1.00 ± 0.10 bc	1.22 ± 0.18 b	0.09 ± 0.01 bc
0.85 ± 0.05 c	1.46 ± 0.21 b	0.13 ± 0.03 bc
0.75 ± 0.08 c	1.04 ± 0.10 b	0.08 ± 0.01 c
0.73 ± 0.08 c	1.32 ± 0.21 b	0.12 ± 0.01 bc
0.89 ± 0.03 c	1.37 ± 0.11 b	0.05 ± 0.01 c
2.07 ± 0.12 a	1.62 ± 0.45 b	0.08 ± 0.02 c
2.22 ± 0.10 a	3.40 ± 0.45 a	0.75 ± 0.14 a
1.03 ± 0.16 ab	1.19 ± 0.24 b	0.16 ± 0.03 bc
0.87 ± 0.07 c	1.66 ± 0.21 b	0.23 ± 0.01 b
0.35 ± 0.03 e	0.62 ± 0.11 f	0.08 ± 0.02 d
0.99 ± 0.06 bc	1.26 ± 0.18 cd	0.25 ± 0.05 bcd
1.07 ± 0.08 bc	1.90 ± 0.12 b	0.35 ± 0.08 b
0.95 ± 0.19 cd	1.42 ± 0.13 bcd	0.20 ± 0.03 bcd
0.61 ± 0.08 de	0.92 ± 0.13 def	0.14 ± 0.03 cd
0.69 ± 0.06 d	1.25 ± 0.09 cde	0.30 ± 0.04 bc
0.90 ± 0.08 cd	1.12 ± 0.20 cdef	0.12 ± 0.03 d
2.11 ± 0.21 a	3.27 ± 0.39 a	0.89 ± 0.14 a
1.26 ± 0.11 b	1.62 ± 0.21 bc	0.23 ± 0.05 bcd
0.48 ± 0.04 e	0.87 ± 0.12 ef	0.13 ± 0.02 cd

Table 4.2. Plant community traits of canopy height, mean species crown diameter, mean basal diameter, and average distance between individual plant bases and crowns in three plant communities, chaparral, coastal sage, and Mojave Desert scrub.

Vegetation type	**Height (m)**	**Crown diameter (m)**	**Basal diameter (m)**	**Distance between bases[1] (m)**	**Distance between crowns[2] (m)**
Chaparral	2.57 ± 0.07 a	1.87 ± 0.08 a	0.17 ± 0.01 a	0.64 ± 0.05 a	-1.15 ± 0.06 a
Coastal Sage Scrub	0.83 ± 0.03 b	0.99 ± 0.05 b	0.10 ± 0.01 b	0.61 ± 0.03 a	-0.47 ± 0.04 b
Mojave Desert Scrub	0.75 ± 0.03 c	1.03 ± 0.04 b	0.18 ± 0.00 a	1.67 ± 0.08 b	0.70 ± 0.08 c

[1] from outside edge of base to outside edge of base

[2] from egde of crown to edge of crown

Table 4.3. Observed and expected numbers of conspecific and heterospecific neighbors and nearest neighbors among the dominant woody plant species from three plant communities, chaparral, coastal sage, and Mojave Desert scrub.

	Neighbors						
Vegetation type	**Conspecific observed (%)**	**Heterospecific observed (%)**	**Conspecific expected (%)**	**Heterospecific expected (%)**	**Significance**	**Neighbors conspecific ± 1 SE (%)**	**Neighbor heterospecific ± 1 SE (%)**
Chaparral	98	142	21.6	218.4	$X^2 = 297.8$ $P < 0.001$	44.8 ± 3.4 a	55.25 ± 3.4 a
Coastal Sage Scrub	51	165	23.7	192.3	$X^2 = 35.0$ $P < 0.001$	23.8 ± 2.9 b	76.2 ± 2.9 b
Mojave Desert Scrub	42	198	17.4	22.6	$X^2 = 37.5$ $P < 0.001$	17.5 ± 2.5 c	82.5 ± 2.5 c

	Nearest Neighbor						
Vegetation type	**Conspecific observed (%)**	**Heterospecific observed (%)**	**Conspecific expected (%)**	**Heterospecific expected (%)**	**Significance**	**Nearest neighbor conspecific ± 1 SE (%)**	**Nearest neighbor heterospecific ± 1 SE (%)**
Chaparral	35	25	21.6	38.4	$X^2 = 13.1$ $P < 0.001$	58.3 ± 6.4 a	41.7 ± 6.4 a
Coastal Sage Scrub	15	39	14.9	39.1	$X^2 = 0.0$ $P > 0.05$	20.4 ± 5.5 b	79.6 ± 5.5 b
Mojave Desert Scrub	16	44	17.4	42.6	$X^2 = 0.2$ $P > 0.05$	26.2 ± 5.6 b	73.8 ± 5.6 b

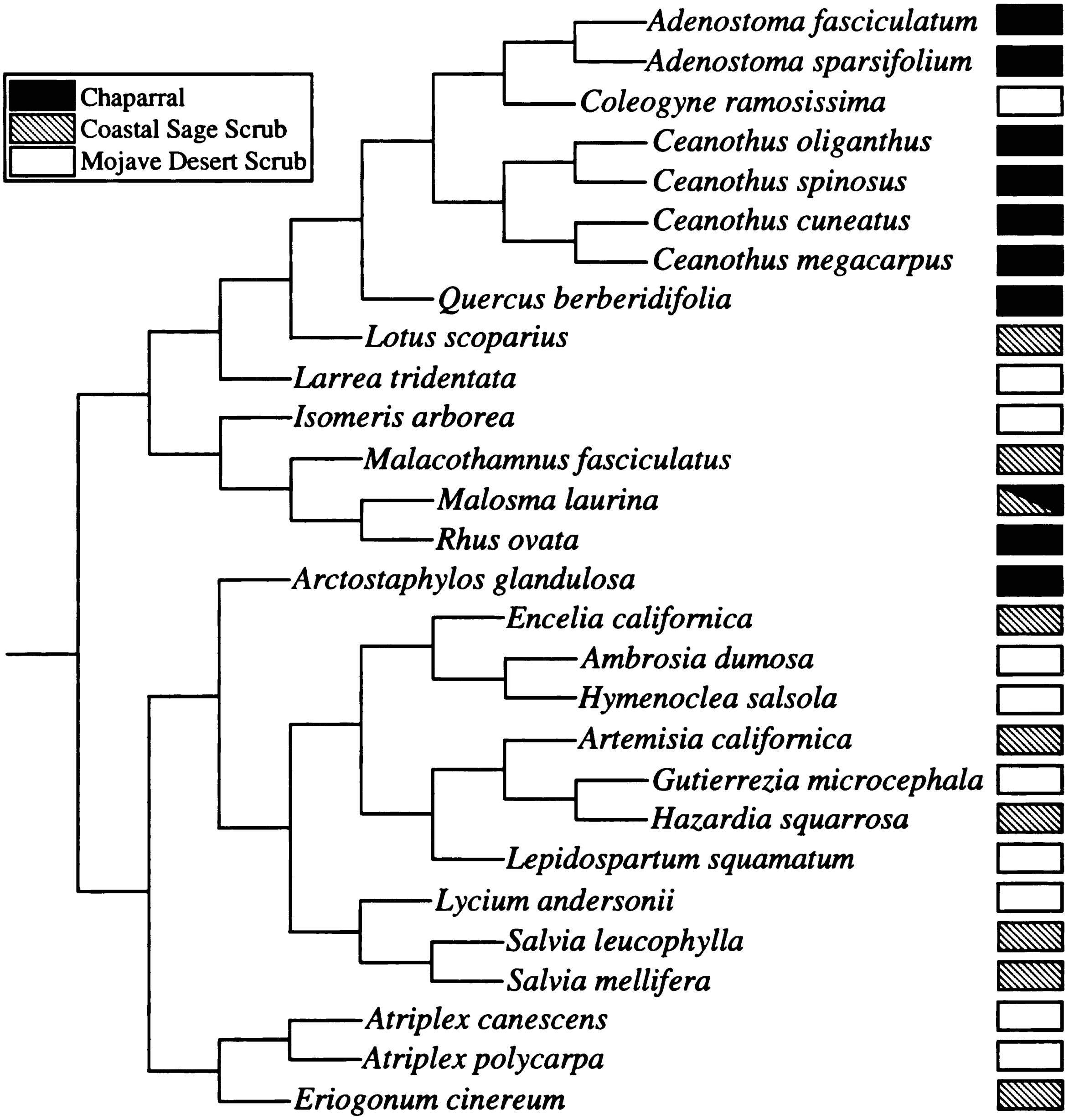

Figure 4.1. Phylogeny of species included in the present study including the plant community in which they occur (chaparral, coastal sage, or Mojave Desert scrub). See Methods for description of how the phylogeny was constructed.

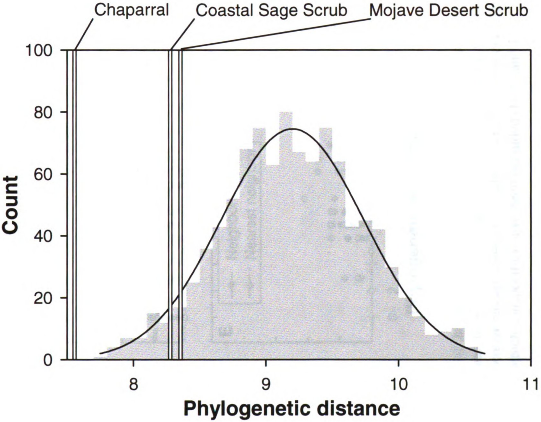

Figure 4.2. Histogram representing the phylogenetic distance of randomly generated communities drawn from a pool of the species analyzed in the present study (grey bars and solid line, $n = 1000$). Mean phylogenetic distances for species occurring within three communities, chaparral, coastal sage scrub, and Mojave Desert scrub, are shown as open bars.

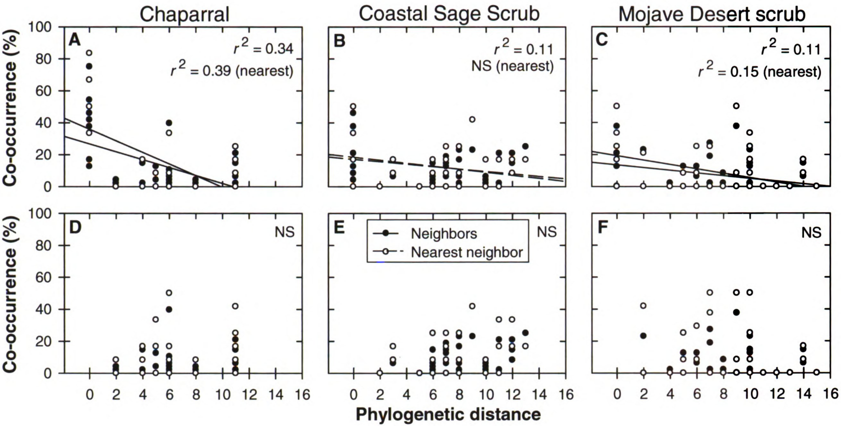

Figure 4.3. Phylogenetic distance as a predictor of the percent co-occurrence among species for neighbors (filled circles and solid line) and nearest neighbors (open circles and dashed line) for three communities, chaparral (A and D), coastal sage (B and E), and Mojave Desert scrub (C and F). Correlations are shown for analyses which include occurrence near conspecifics (A, B, and C) and for analyses in which conspecifics have been excluded (D, E, and F).

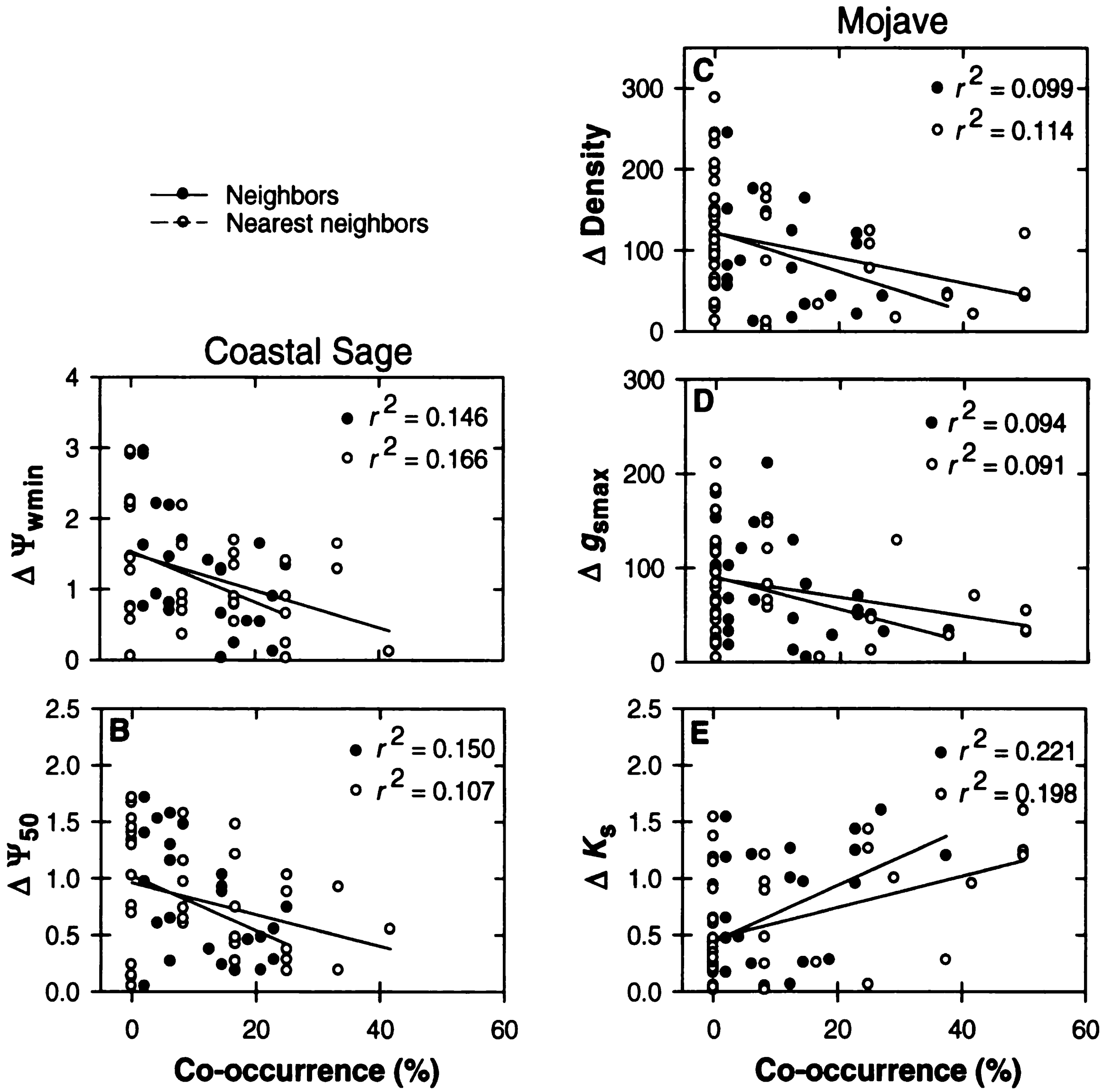

Figure 4.4. The relationship between various functional parameters and species co-occurrence as either neighbors (filled circles) or nearest neighbors (open circles) in the coastal sage (A and B) or Mojave Desert scrub (C, D, and E) communities.

CHAPTER FIVE

DRY SEASON SOIL MOISTURE CONTENT AND INVASIBILITY OF ARID SHRUBLANDS

Abstract

Soil moisture is a limiting resource in arid environments for woody shrub species. We assessed if three arid communities differed in their level of dry season soil moisture content and in their ability to use soil moisture reserves. Water potential of all of the dominant woody plant species occurring in a chaparral, coastal sage, and Mojave Desert community were measured seasonally and dry season soil moisture content was determined. Individuals occurring in the Mojave Desert were able to persist with the lowest water potentials while the coastal sage community displayed the highest minimum water potentials. Volumetric soil moisture content (m^3/m^3) of the Mojave Desert site was lowest during the dry season (7 %), the chaparral site was intermediate (10 %), and the coastal sage scrub site had the moistest dry season soil (20 %). Previous work finds that 18 % soil moisture seems to be an important threshold for woody plant seedling recruitment and the coastal sage community remains above this soil moisture threshold year round. Of the three communities, the coastal sage community may therefore be particularly susceptible to invasion by woody shrub species since its soil moisture content would allow for germination and persistence of a wider range of potential invaders, although all three of these communities are relatively little invader compared to adjacent riparian communities.

Introduction

Competition among species should lead to the exclusion of species that rely on utilization of similar resources (i.e. species that occupy the same niche). This principle was described by Gause and is given the name "competitive exclusion" (Hardin 1960). While a species niche is defined by many variables, it is likely that in any given environment there is one resource that is most limiting to species persistence. Where species are in competition for a single limiting resource, species resource use can be used to predict limiting resource levels and to predict the outcome of competitive interactions (R* rule; Tilman 1982). This pattern of resource competition has also been termed exploitation competition (Levine 1976; Vance 1985).

The R* rule predicts the outcome of competitive interactions based on differential dependence of species on a given resource level for positive population growth (Figure 5.1 A; Tilman 1982). The growth rate of a population will decline as the availability of the limiting resource declines. The level of resource availability at which the population growth rate equals zero is the R*. If the resource is available in levels exceeding the R* the population will grow and if the limiting resource is available in levels less than R* the population will decline. If two species have different R* then the species with the lower R* will be able to competitively exclude the species with the higher R*. This is because the species with lower R* can draw resource availability down to a level that results in a decline in the population of the species with the higher R* (Figure 5.1 A). The same model may also be applied to interactions among plant communities (Figure 5.1 B).

The R* rule and similar models have been used to predict the outcome of competition in controlled experiments (Titman 1976; Ciros-Pérez *et al.* 2001; Fox 2002), patterns of species abundance within communities (Kõiv & Kangro 2005; Harpole & Tilman 2006), and patterns of species succession (Herron *et al.* 2001). Recently, these models have also been applied to species invasions and determination of community invisibility and native versus non-native competitive interactions (Herron *et al.* 2001; Booth *et al.* 2003; Fargoine *et al.* 2003; Krueger-Mangold *et al.* 2006; Funk & Vitousek 2007). While many of these studies have focused on nutrient availability (for plants) or food availability (for animals), moisture availability has also been shown to predict competitive outcomes in some plant communities (Cleverly *et al.* 1997; Booth *et al.* 2003).

We examined differences among three arid plant communities in plant water potential and soil moisture to assess whether these communities differed in their invasibility based on these parameters. Water is likely a limiting resource in these communities, particularly for the evergreen woody shrub species which remain active year-round. Indeed, drought induced mortality of species in these communities at both the seedling and adult stages have been reported (Frazer & Davis 1988; Williams *et al.* 1997; Davis *et al.* 2002; Paddock III 2006). Water potential of woody plant branchlets or leaves estimates the negative pressure in the xylem and is related to the availability of soil moisture. More negative values indicate less available soil moisture reserves. Plants that can tolerate more negative water potentials can continue to extract soil water when plants that are less tolerant no longer are able to obtain enough moisture. Thus, species and communities that tolerate more negative pressure have a lower R* and would be predicted to be invasible by fewer invaders (i.e. only the limited number of potential invaders that are most water stress tolerant) (Pratt & Black 2006). In the present study, I did not attempt to compare across functional groups because predictions of competitive outcomes based on limiting resources appear most useful among similar functional groups (Krueger-Mangold *et al.* 2006). Thus, results are discussed in the context of woody shrub species only.

Materials and Methods

Three diverse arid sites were selected based on their abundance of woody shrub species. These sites represented chaparral, coastal sage, and Mojave Desert plant communities (see Jacobsen *et al.* 2007*c*, Chapter 2 for site descriptions). The average annual precipitation for these sites is approximately 440 mm for the chaparral and coastal sage communities and 180 mm for the Mojave Desert site (average annual precipitation from 1999-2007 based on the July to June rain year).

Volumetric soil moisture content was measured from late August 2006 through April 2007 at each of the three sites. Moisture content of the upper soil layers (upper 30 cm) was measured at the base of the same individuals at each sampling time (n = 14-26) using one TDR probe (CS615, Campbell Scientific, Inc., Logan, Utah, USA) attached to a datalogger (CR23X Micrologger, Campbell Scientific, Inc., Logan, Utah, USA). These sites do not differ in soil texture at this soil depth (Jacobsen *et al.* 2007*c*). Within each site, monthly values were compared using repeated measures ANOVAs. Minimum seasonal soil moisture of each site was determined as a community wide mean from the month with the lowest soil moisture values. An ANOVA was used to compare minimum volumetric soil moisture across communities followed by a Bonferroni/Dunn post-hoc analysis (Statview v. 5.0.1, SAS Institute Inc., Cary, NC, USA).

Water potential was measured at midday on all dominant woody shrub species at each site approximately monthly from February 2006 and continuing through April 2007 (see Chapter 3 for detailed Methods) using a pressure chamber (Model 2000 Pressure Chamber Instrument, PMS Instruments, Corvalis, Oregon, USA). Water potential was measured on leaves or branchlets from six individuals per species at each sampling period. Mean water potentials were calculated for each species at each sampling time. These values were pooled across species and the frequency of water potentials were calculated for each community in order to determine the community wide range in soil moisture resource use.

Results

Dry season volumetric soil moisture of the shallow soil layers significantly differed among the sites and with season (Figure 5.2; Figure 5.3 C). Dry season soil moisture values for fall 2006 followed an average rain year and thus should be representative for soil moisture conditions in normal years during the dry season. The three communities significantly differed in their dry season volumetric soil moisture content (Figure 5.3 C; $P < 0.001$). Minimum volumetric soil moisture content of the Mojave Desert site was the driest (0.071 ± 0.001; $P \leq 0.001$ compared to the chaparral and coastal sage scrub), the chaparral site was intermediate (0.103 ± 0.006; $P < 0.001$ compared to the coastal sage scrub), and the coastal sage scrub site had the moistest dry season soil (0.202 ± 0.010). All of the sites exhibited significantly higher soil moisture values during the wet season from December to March (Figure 5.2); however, rainfall during the winter 2006-2007 wet season was much less than normal (approximately 100-130 mm across all sites), so wet season soil moisture values are likely not as high as would be typical in an average rainfall year.

The three communities examined in the present study differed in the minimum water potentials that could be tolerated by species within each community (Figure 5.3 A and B). The Mojave Desert community experienced the lowest water potentials (-9.2 MPa), the chaparral was intermediate (~-8.7 MPa), and the coastal sage scrub community experienced the least negative water potentials (~-5.4 MPa). All of the communities had some species that maintained relatively high water potentials even in the dry season (Figure 5.3 A and B). These minimum water potential values are consistent with site differences in dry seasonal soil moisture content (Figure 5.3).

Discussion

Arid low productivity sites appear to be less broadly invasible than more moist and productive sites (Stohlgren *et al.* 2002; Otto *et al.* 2006); however, arid plant communities in southern California have still experienced considerable invasion by non-native species. Most of these invasive species are annual herbs and grasses (Knops *et al.* 1995; Sax 2002; Salo 2004; Keeley 2006; Lanbrinos 2006) although there are several woody non-native species naturalized in these communities. In the coastal sage and chaparral communities woody invaders include, *Acacia* spp., *Nicotiana glauca*, *Cytisus* spp. and *Spartium* spp. (Knops *et al.* 1995; Keeley 2006). While annual invasions into arid regions appear to be at least partially dependent on increased nitrogen availability (Brooks 2003), the timing and availability of soil moisture is also important (Salo 2004).

Species of woody shrubs in the chaparral, coastal sage, and Mojave Desert communities are able to tolerate relatively low water potentials (less than -7 MPa in all communities compared to a limit of -2 MPa for most crop plants; Tyree & Zimmerman 2002). Among these three communities, the Mojave Desert contains species that experience the lowest water potentials seasonally and therefore are able to utilize more limited soil moisture reserves than species in the other two communities. This suggests that among these communities the Mojave Desert is the least invasible by woody shrub species if soil moisture is the limiting resource in these communities. In contrast, the coastal sage does not have species that reach as low water potentials as species in the other communities. This suggests that this community may be the most invasible of the three communities examined in the present study.

The dry season soil moisture of these communities is related to the water potentials that the woody shrub species in these communities experience. This is particularly evident when comparing between the chaparral and coastal sage communities. Both of these communities experience similar annual rainfall (approximately 400-500 mm) and have similar soil texture (Jacobsen *et al.* 2007*c*) yet they have very different dry season soil moisture levels. This is presumably because species in the chaparral community are able to attain and survive lower water potentials and are therefore able to draw soil moisture levels down to a lower level.

Differences in dry season soil moisture content may be particularly important in determining the invasibility of these communities. A recent study examined five woody species from the Mediterranean Basin (Spain) and found that among these species at soil moistures over 18% there was 100% survival, while very few seedlings were able to survive dry season soil moistures of less then 12% (Padilla & Pugnaire 2007). If this result is general, this suggests that while the chaparral and Mojave Desert communities would be able to resist seedling establishment by these woody species (10% and 7% dry season soil moisture content, respectively) nearly all seedlings of these Mediterranean species would be able to persist in the coastal sage community through the dry season (20% dry season soil moisture content). Since most of the invasive or naturalized species already present in these communities are native to other regions globally that experience similar environmental conditions including the Mediterranean Basin (Knops 1995; Salo 2004; Keeley 2006) the results of this recent study may be particularly applicable to these southern Californian communities.

Current numbers of non-native woody species in these communities, are consistent with the predicted differences in susceptible to non-native species among these communities. Based on recent species lists and floras of these communities (McAuley 1996 for the chaparral and coastal sage; Faull, unpublished data for Mojave Desert), 15 % of the woody species present in the coastal sage are non-native compared to only 11 % for the chaparral and 8% for the Mojave Desert.

The R* rule and similar models have been useful for prediction of invasibility of some communities. Using this model to predict susceptibility to invasion by woody shrub species in these three woody shrub communities of southern California suggests that the coastal sage community may be most susceptible to woody species invasion if soil moisture is a limiting resource in these communities. This suggests that monitoring and control of woody naturalized shrubs may be most efficient if efforts are focused on this southern Californian plant community, as the chaparral and Mojave Desert communities appear to be more impervious to shrub invasion.

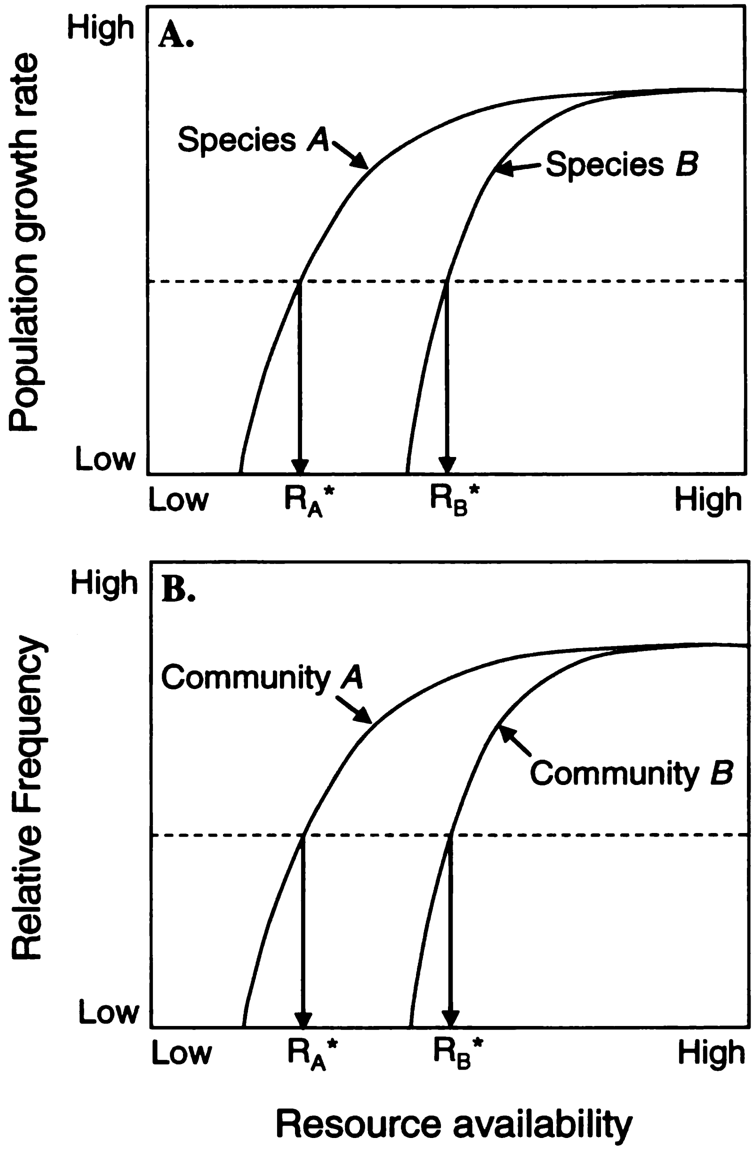

Figure 5.1. Solid curves show the dependence of population growth rate on resource availability (A; based on Tilman 1982) and the dependence of species communities on resource availability (B) if a single resource is limiting. In panel A, the dashed line indicates the point at which the mortality rate in a population equals the replacement rate. The R* indicates the amount of resource needed for the population to increase. Species B requires a higher amount of resource than Species A for the population to increase (i.e. $R_B^* > R_A^*$); therefore, the long term outcome of competition between Species *A* and Species *B* would be the competitive exclusion of Species *B*. This results because Species *A* is able to reduce resources below the level at which Species *B* can maintain positive population growth. In panel B, the dashed line indicates the point at which individuals are so infrequent in the community that the community cannot be sustained. In this example, Community *A* would be able to competitively exclude Community *B*.

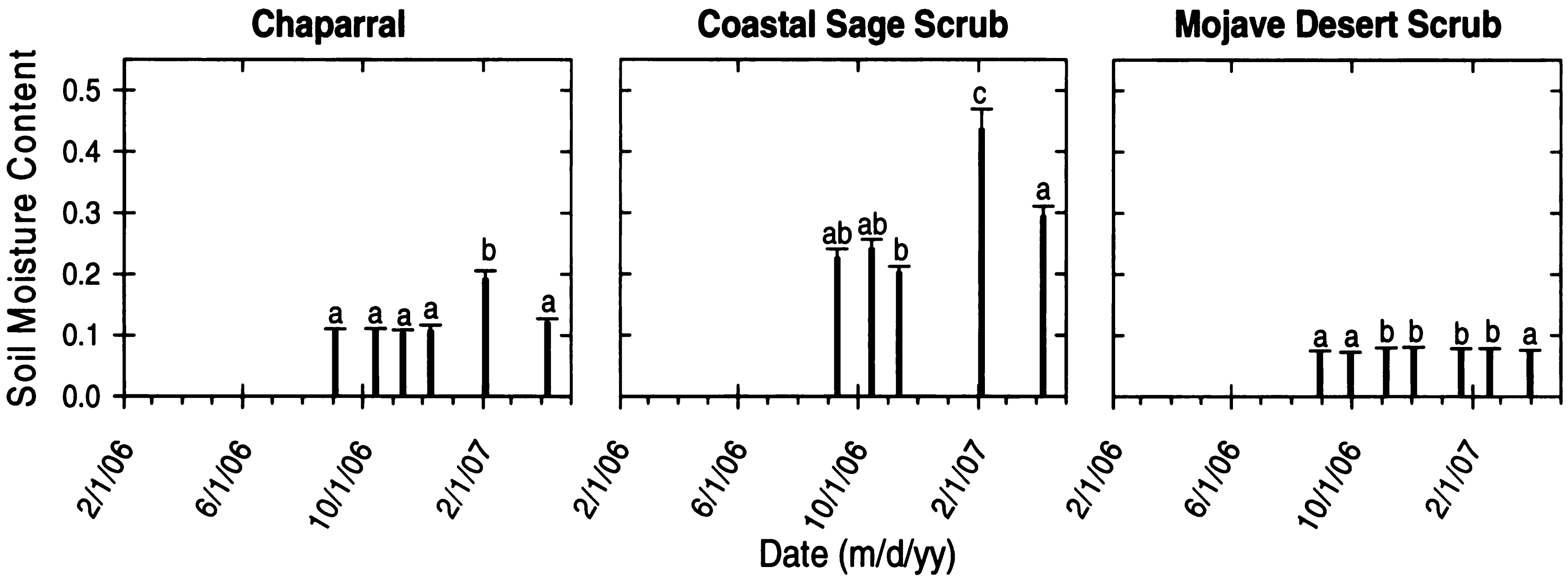

Figure 5.2. Volumetric soil moisture content of the upper soil layers (upper 30 cm) measured from late August 2006 through April 2007 at each of the three arid shrub communities (chaparral, coastal sage, Mojave Desert). Within each panel, different letters indicate soil moisture values that are significantly different.

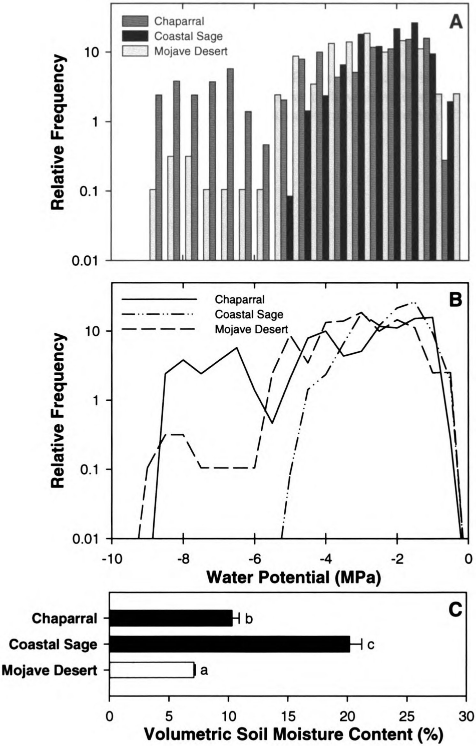

Figure 5.3. The frequency of water potential occurrence in three arid plant communities, the chaparral (medium gray fill, solid line), coastal sage scrub (black fill and dashed line) and Mojave Desert scrub (light grey fill, dashed-dotted line) (A and B) and the minimum seasonal volumetric soil water content for these same three communities (C). Different letters after bars in panel C indicated soil moisture values that are significantly different.

CHAPTER SIX

Jacobsen, A.L., Agenbag, L., Esler, K.J., Pratt, R.B., Ewers, F.W. & Davis, S.D. (2007) Xylem density, biomechanics, and anatomical traits correlate with water stress in seventeen evergreen shrub species of the Mediterranean-type climate region of South Africa. *Journal of Ecology,* **95**, 171-183.

XYLEM DENSITY, BIOMECHANICS, AND ANATOMICAL TRAITS CORRELATE WITH WATER STRESS IN SEVENTEEN EVERGREEN SHRUB SPECIES OF THE MEDITERRANEAN-TYPE CLIMATE REGION OF SOUTH AFRICA

Summary

1 Climate change in South Africa may threaten the sclerophyllous evergreen shrubs of this region. Available data suggest that they are not as tolerant of water stress as chaparral shrubs occurring in climatically similar California, USA.

2 Seventeen species from nine angiosperm families, including both fynbos and succulent karoo species, were studied at a field site in Western Cape Province, South Africa. Minimum seasonal pressure potential ($P_{\min}$), xylem specific conductivity (K_s), stem strength against breakage (modulus of rupture; MOR), xylem density, theoretical vessel implosion resistance ($((t/b)_h^2)$), and several fiber and vessel anatomical traits were measured.

3 Species displayed great variability in $P_{\min}$, similar to the range reported for chaparral and karoo shrub species, but in contrast to previous reports for fynbos shrubs.

4 More negative $P_{\min}$ was associated with having greater xylem density, MOR, and $(t/b)_h^2$. There was no relationship between $P_{\min}$ and traits associated with increased water transport efficiency.

5 Xylem density integrates many xylem traits related to water stress tolerance including $P_{\min}$, MOR, and $(t/b)_h^2$ as well as percent fiber wall, parenchyma, and vessel area, and fiber lumen diameter.

6 Xylem density may be an integral trait for predicting the impact of climate change on evergreen shrubs.

Introduction

The Cape Floristic Region of South Africa is dominated by evergreen shrubs with tough, leathery leaves collectively referred to as fynbos. This region is classified as a Mediterranean-type climate region, referring to its climatic similarity with the Mediterranean Basin Region. This climate type is exemplified by hot dry summers and cool moist winters, with greater than 65% of annual precipitation occurring during the winter months (Aschmann 1973). In the more arid areas adjacent to fynbos, fynbos is replaced by more succulent vegetation called succulent karoo (Cowling & Holmes 1992). These biogeographical regions contain some of the highest levels of regional diversity in the world (Cowling *et al.* 1996) and are particularly threatened by global climate change (McCarthy *et al.* 2001; Lovett, Midgley & Barnard 2005). Predictions for future climate in the region point to significant changes in water availability (Shulze & Perks 2000; McCarthy *et al.* 2001) yet seasonal water relations (Miller, Miller & Miller 1983; Moll & Sommerville 1985; van der Heyden & Lewis 1989; Richardson & Kruger 1990; Smith & Richardson 1990) and associated xylem traits (February & Manders 1999) have only been studied among very few species.

Minimum seasonal pressure potential (P_{min}) is a measure of the maximum water stress that a plant experiences in the field and is often correlated with plant water stress tolerance (Hacke, Sperry & Pittermann 2000; Sperry & Hacke 2002; Jacobsen *et al.* 2005; Jacobsen *et al.* 2006). Differential P_{min} among co-occurring plants may indicate variation in water availability and differential susceptibility to depleted water resources. A less negative P_{min} annually indicates greater access to soil moisture and suggests greater rooting depth (Moll & Sommerville 1985; Smith & Richardson 1990) but may be coupled with greater susceptibility to water stress. Conversely, a lower P_{min} annually and associated greater plant water stress tolerance may be coupled to lower water availability, more extreme drought in dry years (Jacobsen *et al.* 2006), and greater risk of drought induced dieback and mortality (Davis *et al.* 2002; Paddock 2006).

The evergreen shrubs which occur in the Mediterranean-type climate region of South Africa experience a predictable annual summer dry period (Cowling *et al.* 2005). Thus, the evergreen shrubs of this region must be able to both resist the negative pressures that develop within the xylem with water stress as well as maintain water transport to evergreen leaves and actively expanding shoots during the summer dry period (Agenbag 2006). We examined xylem traits associated with water transport efficiency and water stress tolerance as well as P_{min} in 17 evergreen shrub species occurring in the southwestern region of the Western Cape Province, South Africa.

Several xylem anatomical traits were measured to ascertain what xylem structural traits may be associated with decreased P_{min} including increased stem mechanical strength (modulus of rupture), increased vessel and fiber wall thickness, increased percent fiber wall area per transverse area, and decreased fiber lumen diameter. These traits have been shown to be related to P_{min}, perhaps due to the increased structural support needed to buttress xylem against negative pressure (Jacobsen *et al.* 2005). We also estimated the resistance of xylem vessels to implosion under negative pressure as an additional estimate of water stress tolerance, by calculating the square of the ratio of the inter-vessel wall thickness to vessel lumen diameter (*i.e.* $(t/b)_h^2$; Hacke *et al.* 2001). A greater $(t/b)_h^2$ is associated with greater vessel mechanical strength against implosion under negative pressure. Traits associated with greater hydraulic conductivity and capacitance, including increased vessel and fiber lumen diameter and vessel and parenchyma area per transverse xylem area, were also measured.

Sensitivity to water stress varied greatly among fynbos species exposed to an experimental reduction in rainfall (Agenbag 2006). Xylem traits may be useful in explaining this variation. Increased knowledge of xylem traits associated with water stress tolerance in fynbos species may improve our ability to predict how plants will respond to changes in temperature and precipitation in the face of regional climate change.

Materials and Methods

Xylem traits were measured in 17 species of evergreen sclerophyllous shrubs from nine angiosperm families located on Jonaskop in the Riviersonderend Mountain Range in the Western Cape Province, South Africa (Table 6.1; Figure 6.1). This site falls within the Mediterranean-type climate region of South Africa. Individuals were sampled from the north facing slope of the mountain at approximately 33° 56.08' S 19 ° 31.26' E. Eight species were sampled at 920 m elevation (approximately 410 mm annual precipitation), two species were collected from as high as 1160 m elevation, and the remaining 7 species were collected from between 845 and 540 m elevation (Table 6.1). The steep elevational gradient at this site corresponds to considerable variation in precipitation (approximately

315 to 600 mm annual precipitation) and temperature (see Agenbag 2006 for a complete description of the study site).

Species were identified by the Compton Herbarium of the South African National Biodiversity Institute and specimens are housed at the University of Stellenbosch. Identification of one species was not possible, although it was identified as being within the genus *Euclea*.

Twelve individuals of each species were tagged at the site and the same individuals were used for each of the measured parameters. Two of the studied species are dioecious (*Leucadendron laureolum* & *L. salignum* Proteaceae). For these two species, twelve female and twelve male individuals of each were tagged and measured.

Xylem pressure potentials were measured at predawn and midday on 19 February and at midday on 26 February and 24 March 2004 using a pressure chamber (Model 1001, PMS Instrument Company, Corvallis, Oregon, USA). At each sampling time, we measured xylem pressure potentials ($P_{\min}$) on branchlets on a minimum of five plants per species with the pressure chamber technique (Scholander *et al.* 1965). The last rainfall event prior to the onset of the summer dry period occurred on 27 September 2003 (~17 mm). There were several small rain events over the course of the summer (less than 10 mm each) although xylem pressure potentials for most species declined over the measurement period. The summer drought ended on 1 April 2004 with a rainfall event of 21 mm, one week after the 24 March sample date. Thus, the duration of the seasonal drought period was about 6 months.

Xylem density was determined as the dry mass per fresh volume of mature stems from at least six individuals per species or sex (Wagner, Ewers & Davis 1998; Hacke *et* *al.* 2001). The bark and pith were removed from stems prior to determination of xylem density. The stems were saturated in degassed HCl solution adjusted to a pH of 2 until they reached their maximum wet weight. Water-saturated volume was determined by water displacement in a graduated cylinder. The stems were dried at 60°C to a constant weight and dry mass was determined.

Stem xylem modulus of rupture (MOR) was measured using a four-point bending test on an Instron Universal Testing Machine (model 4202, Instron Corporation, Canton, MA, USA) at Michigan State University (East Lansing, MI, USA) following the methods of Jacobsen *et al.* (2005). Twelve stems per species or per sex and approximately 0.3 m in length and 6-8 mm in diameter were collected in the field, wrapped in moist paper towels, and placed in plastic bags. Stems were then express shipped, in a chilled cooler, to Michigan State University for mechanical analysis. All stems were measured within 4 days of field collection. A four-point bending test with a compression load cell of 500 N was conducted following the methods of Jacobsen *et al.* (2005). We were unable to collect stems from four species for mechanical analysis due to difficulty in obtaining straight stems of the required dimensions for measures: *Erica cerinthoides*, *Euclea* sp., *Cliffortia ruscifolia*, and *Metalasia densa*.

Xylem specific hydraulic conductivity (K_s) was calculated following removal of air embolism by high pressure perfusion (Sperry, Donnelly & Tyree 1988). One branch from a minimum of six different individuals per species was collected from the field. The collected branches were approximately 0.3 m in length and 6-8 mm in diameter. They were wrapped in wet paper towels, sealed in plastic bags, and placed in a cooler for transport back to the laboratory. All stems were measured in less than 24 hrs from the time they were collected. Once in the laboratory, branches were submerged in water and trimmed from alternate ends until a final stem segment, 0.1 m in length, was obtained. Stems were connected to a tubing system and flushed with low pH degassed water (pH 2 HCl) that had been passed through a 0.1 μm filter. The stems were flushed at a pressure of 100 kPa for one hour to remove gas emboli from the xylem vessels. The K_h (kg m MPa^{-1} s^{-1}) was then measured gravimetrically on each stem segment (Sperry *et al.* 1988) using an analytical balance (AE260, Delta Range Balance, Mettler Instrument Corp.). The segments were then attached to a tubing system that allowed uptake of a 0.1% (mass/volume) filtered (0.1 μm filter) dye solution of crystal violet under a suction of 4-6 kPa for 20 min (Hargrave *et al.* 1994). The midpoint of each segment was then sectioned to a thickness of 30-40 μm using a sliding microtome. The stained xylem area was then measured using a digital camera (C-730 Ultra Zoom Camedia, Oympus America Inc.) and image analysis software (Image v.1.61, National Institute of Health). The K_s value (kg m^{-1} MPa^{-1} s^{-1}) was calculated as K_h/active xylem area.

The same sections used for determination of active xylem area were also used for xylem anatomical measures. For each stem, images were taken of several wedge-shaped sectors to sample for vessel and fiber features using a digital camera (JVC TK-C1381 Colour Digital Video Camera) attached to a light microscope (DM LB, Leica Microsystems). Vessel lumen diameter (d) and wall thickness, fiber lumen diameter and wall thickness, and percent transverse fiber, vessel, and parenchyma area were measured. All of the vessels and fibers in sectors were measured until 100 vessels and 100 fibers had been measured in a given stem. The hydraulic vessel diameter (d_h) was calculated from the formula $d_h = (\Sigma d^5)/(\Sigma d^4)$, based upon all the sampled vessels in a stem. This parameter weights the vessel diameters by their hydraulic contribution which is a function of the diameter to the fourth power (Tyree & Zimmermann 2002). This was used in our analyses instead of mean *d* because it is more directly related to xylem water transport. The vessel implosion resistance $((t/b)_h^2)$ (Hacke *et al.* 2001) was determined for those vessels, within the sampled 100 vessels per stem, that formed pairs in which one or both vessels fell within ± 5 µm of the calculated d_h, with *t* as the combined wall thickness of adjoining vessels and *b* as the lumen diameter of the selected vessel. Percent transverse area was estimated through measurements of vessel, fiber, and parenchyma area in four randomly chosen cross-sectional sectors per stem.

We calculated phylogenetic independent contrasts (PICs) to test for patterns of correlated evolutionary change between traits (Felsenstein 1985) using COMPARE (v 4.6, EP Martins, Department of Biology, Indiana University). A correlation among PICs for two traits indicates that these traits have undergone changes of similar direction and magnitude across the phylogeny and supports a possible functional link between these traits. The phylogeny used for these analyses was constructed by hand using published phylogenetic data (Fig 1). Relationships among families, genera, and species were based on recent molecular phylogenies (Morton *et al.* 1996; Soltis *et al.* 1997; Bayer & Starr 1998; Soltis *et al.* 2000; Miller, Young & Wen 2001; Hilu *et al.* 2003; Barker *et al.* 2004; Yi, Miller & Wen 2004; McGuire & Fron 2005). All analyses were run assuming equal branch lengths (Ackerly 2000). This is a conservative approach, thus there is less certainty associated with lack of correlation among contrasts but high confidence when there is a significant correlation among contrasts.

Unpaired t-tests were used to compare xylem traits of male and female individuals of dioecious species and linear regression analyses were used to examine interspecific trait correlations (Minitab v.14.12, Minitab, Inc. and Statview v.5.0.1, SAS Institute Inc.). Regressions or differences were considered to be significant if $\alpha \leq 0.05$. Where females were found to differ from males, they are included as separate data points in analyses. A power fit was used to compare d_h and K_s because according to the Hagen-Poiseuille equation K_s is a function of diameter raised to the fourth power (Tyree & Zimmermann 2002). Principle component analysis was used to determine correlation patterns among multiple traits. This analysis is useful in summarizing the relationships between multiple traits into a single two dimensional figure that may be easier to interpret than many separate regressions. Principle component analysis was used to examine both raw data as well as PICs. Data were transformed as necessary to meet the assumptions of statistical models.

Results

Intraspecific comparison of sexes

Two of the studied species, *Leucadendron laureolum* and *L. salignum* are dioecious. Females and males of *L. laureolum* did not significantly differ in any of the xylem traits measured in this study (Figure 6.2; $P > 0.05$ for all comparisons). Data from both sexes of *L. laureolum* were therefore pooled for regression analyses. In contrast, females and males of *L. salignum* were different in their xylem traits related to conductive efficiency, such as xylem specific hydraulic conductivity (K_s), mean hydraulic vessel diameter (d_h), and percent vessel area per transverse xylem area. Males of *L. salignum* had higher K_s

(Figure 6.2a; $P = 0.046$), greater d_h (Figure 6.2b; $P = 0.003$), and greater percent vessel area per transverse xylem area (Figure 6.2c; $P = 0.020$) relative to females. In addition to these hydraulic traits, males also had a lower percent fiber wall area per transverse xylem area compared to females (Figure 6.2d; $P = 0.001$). Female and male *L. salignum* are thus included as separate data points in regressions involving these traits in which they differ significantly. Female and male *L. salignum* did not differ in the other xylem traits measured, including xylem density, modulus of rupture (MOR), $(t/b)_h^2$, minimum seasonal pressure potential (P_{min}), fiber lumen diameter, fiber wall thickness, and percent parenchyma per transverse xylem area ($P > 0.05$ for all).

Minimum seasonal pressure potential

Branchlet pressure potentials (P_x) were measured over three sample dates towards the end of the summer dry period. Over the course of the sample period pressure potentials of most species did not change or declined, indicating steady or declining water status. Pressure potential increased on the sampling date 24 March 2004 for a single species, *Searsia undulata* ($P = 0.016$). Pressure potentials for the two *Pteronia* spp. were approximately -8.5 MPa on 26 February 2004, and declined to below -10 MPa (exceeding the limits of the pressure chamber) on 24 March 2004. Thus, for these two species minimum seasonal pressure potential (P_{min}) is unknown, but is lower than -10 MPa. For the remaining species, the lowest measured pressure potential was used to estimate P_{min}.

Minimum seasonal pressure potentials (P_{min}) varied greatly among the 17 species examined, ranging from a high of -1.89 MPa (*Leucadendron salignum*) to a low of less than -10 MPa (*Pteronia fasciculata* & *P. paniculata*) (Figure 6.3). The mean P_{min} across species, excluding the two *Pteronia* species for which their P_{min} values are lacking was -3.4 ± 0.34 MPa. The mean P_{min} across species, assuming that the two *Pteronia* spp. had P_{min} of -10 MPa, was -4.34 ± 0.61 MPa. Because specific P_{min} values for the *Pteronia* spp. are lacking and they were presumably lower than -10 MPa, the mean P_{min} across all species may actually be more negative.

Correlations of raw trait values

Lower P_{min} was correlated with greater xylem density (Figure 6.4a; Table 6.2), greater stem mechanical strength against breakage (Figure 6.4b; modulus of rupture, MOR; Table 6.2), and increased ratio of vessel wall thickness to lumen diameter (Figure 6.4c; Table 6.2). Minimum seasonal pressure potential was not correlated to other stem xylem properties, including fiber lumen diameter and wall thickness, percent vessel, parenchyma, or fiber wall area per transverse xylem area, K_s, or d_h (Table 6.2).

Xylem density was correlated with stem mechanical and cellular properties. At the stem level, increased xylem density was correlated to increased MOR (Figure 6.5a; Table 6.2), increased percent fiber wall area per transverse xylem area (Figure 6.5b; Table 6.2), and decreased percent parenchyma area and percent vessel area per transverse xylem area (Table 6.2). At the cellular level, greater xylem density was correlated to smaller fiber lumen diameters (Table 6.2) and increased $(t/b)_h^2$ (Figure 6.5c; $r^2 = 0.53$, $P = 0.003$ for 14 species). Three species, *Passerina obtusifolia*, *Pteronia fasciculata*, and *Pteronia paniculata*, had $(t/b)_h^2$ values outside the range previously reported for angiosperms and which significantly impacted the slope of the regression (Figure 6.5c; P

= 0.004). Because of the significant impact of these few points on the regression, values for those species were excluded from the regression analysis, although the regression was still significant when they were included ($r^2 = 0.65$, $P < 0.001$ for all 17 species). Xylem density was not correlated with estimates of hydraulic efficiency, including K_s and d_h, nor to fiber wall thickness (Table 6.2).

Modulus of rupture was negatively correlated with percent vessel area and percent parenchyma area per transverse xylem area, fiber lumen diameter, and d_h (Table 6.2). MOR was positively correlated with percent fiber wall area (Fig 5d) and $(t/b)_h^2$ (Figure 6.5e; Table 6.2). MOR was not correlated with K_s or fiber wall thickness (Table 6.2).

Xylem specific hydraulic conductivity (K_s) was correlated with d_h using a power fit (Figure 6.6; $r^2 = 0.71$, $P < 0.001$) and the exponent was not different from 4 (3.55 ± 0.63). K_s was not correlated to $(t/b)_h^2$, fiber lumen diameter, fiber wall thickness, or percent parenchyma, vessel, or fiber wall area per transverse xylem area (Table 6.2).

Phylogenetic independent contrasts correlations

Phylogenetic independent contrasts (PICs) of P_{min} and xylem density were correlated (Table 6.2), but PICs of P_{min} were not correlated to any other contrasts of measured xylem traits including fiber wall thickness, fiber lumen diameter, K_s, d_h, and percent fiber wall, parenchyma, or vessel area (Table 6.2).

Phylogenetic independent contrasts of xylem density were correlated to contrasts of MOR, fiber lumen diameter, $(t/b)_h^2$, and fiber wall thickness, but not to percent parenchyma, vessel, or fiber wall area per transverse xylem area contrasts (Table 6.2).

Xylem density contrasts were also not correlated to contrasts of hydraulic traits (Table 6.2).

Phylogenetic independent contrasts of MOR were correlated with contrasts of several xylem parameters including fiber lumen diameter, d_h, $(t/b)_h^2$, percent vessel area, percent fiber wall area, and percent parenchyma area per transverse xylem area (Table 6.2). MOR contrasts were not correlated with K_s or fiber wall thickness contrasts (Table 6.2).

Phylogenetic independent contrasts of K_s were correlated with d_h, fiber wall thickness, percent vessel area, and percent fiber wall area per transverse xylem area contrasts, but not to $(t/b)_h^2$, fiber lumen diameter, or percent parenchyma area contrasts (Table 6.2).

Principal components analysis

Principal components analysis of the raw data, not taking phylogenetic relatedness into account, supported a division between pressure potential and structural traits compared to hydraulic traits (Figure 6.7a). The first component described 61.4% of the variation among 11 traits and associated with P_{min}, xylem density, MOR, $(t/b)_h^2$, fiber lumen diameter, and percent fiber wall area and parenchyma area per transverse xylem area. The second component described 17.7% of the variation among 11 traits and was associated with hydraulic efficiency traits including, K_s, d_h, and percent vessel area per transverse xylem area. A third component described 9.4% of the variation and was associated with fiber wall thickness. Xylem density, $(t/b)_h^2$, MOR, and percent fiber wall area are all positively related with one another and are negatively related to P_{min}, fiber lumen diameter, and percent parenchyma. Measures of transport efficiency, K_s, d_h, and percent vessel area, are positively related. This analysis helps elucidate the relationships among many traits simultaneously and summarizes them on a single graph. In this case, this analysis illustrates the relationship among the many xylem structural traits associated with water stress versus the relationships among the vessel traits associated with increased hydraulic efficiency.

Principal component analysis of PICs suggest that the traits that are correlated when raw data are analyzed are also evolutionarily correlated (Figure 6.7b). The first component described 57.8% of the variability among 11 traits and was associated with P_{min}, xylem density, and MOR contrasts as well as the xylem anatomical trait contrasts of percent fiber wall and parenchyma area per transverse xylem area, $(t/b)_h^2$, fiber lumen diameter, and d_h. The second component described 23.2% of trait variability and was associated with trait contrasts of xylem density, K_s, and fiber wall thickness. A third component explained 10% of trait variance and was associated with percent vessel area per transverse xylem area. The positions of traits along the x-axis (component 1) appears to divide traits associated with greater transport efficiency and capacitance (K_s, d_h, percent vessel area, percent parenchyma area, and fiber lumen diameter) from those associated with greater tissue density and increased drought tolerance (P_{min}, xylem density, MOR, percent fiber wall area, and $(t/b)_h^2$).

Discussion

The wide range of P_{min} found among species occurring within close proximity (-1.9 to less than -10 MPa) suggests that these plant species differ in their utilization of water resources. Lower pressure potentials at midday were accompanied by lower predawn pressure potentials. Thus, heterogeneity of $P_{\min}$ indicates variable access to soil moisture and water utilization patterns among species perhaps due to variable rooting depth, root phenology, or soil characteristics among species. This variability may be an important factor in the great species richness of the area (Richardson *et al.* 2001).

All of the species included in this study occur within the Mediterranean-type climate region of South Africa (Aschmann 1973). Because of the similarities in climate among the five Mediterranean-type climate regions of the world, species occurring in these regions have often been compared (e.g. di Castri & Mooney 1973; Cody & Mooney 1978; Cowling & Campbell 1980; Kruger, Mitchell & Jarvis 1983; Cowling *et al.* 1996). Studies that have compared minimum seasonal pressure potentials ($P_{\min}$) of species across regions, specifically to the California chaparral, have found that $P_{\min}$ of South African fynbos are often much less negative (most were higher than -4 MPa; c.f. Miller *et al.* 1983; Moll & Sommerville 1985; Jeffery, Moll & van der Heyden 1987; Davis & Midgley 1990; Smith *et al.* 1992) compared to those found in California (c. f. Miller & Poole 1979; Poole & Miller 1981; Burk 1978; Williams, Davis & Portwood 1997; Davis *et al.* 2002; Jacobsen *et al.* 2005; Jacobsen *et al.* 2006). Other studies have found similarly high pressure potentials among South African fynbos species (van der Heyden & Lewis 1989; von Willert, Herppich & Miller 1989; Richardson & Kruger 1990; Smith & Richardson 1990).

In the present study, we found several species that had $P_{\min}$ lower than -4 MPa and the mean $P_{\min}$ across all species did not differ from the recently published mean $P_{\min}$ of 26 species in California (-4.34 ± 0.61 for South Africa compared to -4.9 ± 0.42 MPa for California, $P = 0.50$; Jacobsen *et al.* 2006). The more negative P_{min} values found in this study than in previous studies are likely due to an inclusion of different and a greater number of species, including some species associated with succulent karoo vegetation. When the two *Pteronia* species (characteristic of the arid, low elevation of the study gradient) are excluded from this analysis the P_{min} for the 15 remaining species do differ from that of the California species ($P = 0.013$). For species that have been included in previous investigations and were part of the current study, our P_{min} are consistent with published values limiting the likelihood that site, sampling technique, or sampling year significantly impacted results (Miller *et al.* 1983; van der Heyden & Lewis 1989; von Willert *et al.* 1989; Richardson & Fruger 1990; Smith & Richardson 1990). For the two *Pteronia* species, whose distributions extend into the more arid succulent karoo shrubland, similarly low values (less than -10 MPa) have been reported for individuals occurring within the succulent karoo (Midgley & van der Heyden 1999).

The greater range of P_{min} found in this study may be due to the steep elevation and precipitation gradient of this site (Agenbag 2006). The five species experiencing the most negative P_{min} occurred at lower elevations (lower than 660 m) at the ecotone between fynbos and succulent karoo and receive less annual precipitation than species occurring at higher elevations. For the remaining 12 species, there does not appear to be any relation between elevation and P_{min}. For instance, the species collected in this study from the highest elevations, *Nebelia laevis* and *Brunia noduliflora* have P_{min} of approximately -4 MPa even though they likely receive more rainfall than many of the other species.

Species that experienced greater seasonal water stress (lower P_{min}) had greater xylem density and greater stem mechanical strength (MOR). The xylem of species experiencing more negative P_{min} also had xylem vessels with greater theoretical implosion resistance (i.e. $(t/b)_h^2$; Hacke *et al.* 2001). These results are consistent with what has been reported in the literature for shrubs of the Mediterranean-type climate region of California (Wagner *et al.* 1998; Jacobsen *et al.* 2005; Jacobsen *et al.* 2006). Interestingly, P_{min} is not correlated with changes in fiber properties, including fiber lumen diameter and wall thickness and transverse fiber wall area. This differs from what has been reported for the chaparral of California (Jacobsen *et al.* 2005; Jacobsen *et al.*2006) and suggests that xylem anatomical traits associated with drought tolerance may differ between these two regions. The relationship between measurements made on bulk xylem tissues and stems, such as density and MOR, and P_{min} appear more consistent between California and South Africa than measurements made on cellular properties (Hacke *et al.* 2001; Ackerly 2004; Jacobsen *et al.* 2005). Thus, increased allocation of carbon resources to support xylem vessels against implosion under increasing negative pressure may be necessary at the bulk tissue level, but this may be accomplished at the cellular level by varied adjustments across cell types (e.g. vessels, tracheids, fibers, or parenchyma). For example, increased cellular support against implosion can result from either increased vessel wall to lumen ratio (South Africa; this study) or increased fiber matrix support (California; Jacobsen *et al.* 2006). Both types of cellular adjustments would result in increased xylem density and MOR. Differences in cellular level adjustments and P_{min} between South Africa and California might be due to phylogenetic differences, differences in the chemical makeup of cell walls or some yet to be determined factor.

While most species had $(t/b)_h^2$ values within the range of previously reported data (approximately 0.01-0.08; Hacke *et al.* 2001; Jacobsen *et al.* 2005; Jacobsen *et al.* 2006), there were three species that had exceptionally high $(t/b)_h^2$ values. Two of these species, *Pteronia fasciculata* and *P. paniculata*, had values that exceed those previously reported for all woody species including conifers ($(t/b)_h^2 = 0.24$ and 0.20, respectively; c. f. Hacke *et al.* 2001). Figure 6.8 shows micrographs of these species in comparison to those of species with $(t/b)_h^2$ values falling within the previously reported range of values. Individuals of the two *Pteronia* species experienced pressure potentials that exceeded the range of our pressure chamber (less than -10 MPa), but their $(t/b)_h^2$ values, as well as their high MOR values, suggest that their xylem could resist implosion at pressures much more negative than -10 MPa (see Figures 6.4b & c). This is consistent with prior reports of great desiccation tolerance in these species (Midgley & van der Heyden 1999). Additionally, it appears that these species have altered patterns of xylem allocation in line with the need to resist greater pressures within the xylem. This is evidenced by the relatively large $(t/b)_h^2$ values of these species for a lesser whole xylem investment compared to other species (see Figures 6.4a & c and Figure 6.5c) which suggests they have preferentially developed vessels that are more implosion resistant without equivalent changes to the whole xylem tissue. This may have been necessary because these species already have the highest percent fiber wall areas (76 and 79%) and the lowest percent parenchyma areas (less than 3%) of all of the species examined. Thus, they may have reached the maximum xylem strength and density possible while still maintaining an adequate conductive area (i.e. vessel area). As a result, *Pteronia fasciculata* and *P. paniculata* differ from other species in the study in the effectiveness of their vessel mechanical resistance for a given xylem density (i.e. these species fall well above the trend line for density versus $(t/b)_h^2$ in Figure 6.5c).

Xylem density and MOR were correlated with xylem tissue and cellular properties, including percent fiber wall, parenchyma, and vessel area, fiber lumen diameter, and $(t/b)_h^2$, consistent with previous studies (Wagner *et al.* 1998; Hacke *et al.* 2001; Woodrum, Ewers & Telewski 2003; Jacobsen *et al.* 2005; Jacobsen *et al.* 2006). However, one species, *Searsia undulata*, appeared to have relatively low MOR, fiber wall area, and $(t/b)_h^2$ for its xylem density. This species also had one of the highest levels of transverse parenchyma area of the species examined. Presumably, the higher percentage of parenchyma and their associated stored starch contributed to xylem density in this species, but did not offer the mechanical strength that displaced fiber area would have, resulting in low MOR. Interesting, while this species has a higher parenchyma and lower fiber area, which may be associated with higher capacitance (Borchert & Pockman 2005), it still experienced a relatively low P_{min} (-6.7 MPa) suggesting high risk of cavitation. It may be that this species differs from other species included in this study in being able to tolerate a greater degree of seasonal embolism such as has been found in the chaparral shrub *Rhus ovata* (Pratt *et al.* 2005).

As predicted, K_s was correlated with increased hydraulic vessel diameter (d_h) and the exponent of the power fit relating these two variables was not different from 4 consistent with the Hagen-Poiseuille equation (Tyree & Zimmermann 2002). Thus, among these species, vessel diameter is a strong predictor of K_s regardless of interspecific differences in vessel area (8.6 – 18.7%) or possible differences in pitted area and vessel sculpturing. Increased K_s, which is associated with greater potential growth rate (Vander Willigen & Pammenter 1998), varied independent of xylem traits associated with strength (fiber and tissue parameters) or drought tolerance (P_{min} and $(t/b)_h^2$) consistent with previous findings (Woodrum *et al.* 2003; Kern *et al.* 2005; Jacobsen *et al.* 2005).

Species of the genus *Leucadendron* are dioecious with some species displaying vegetative dimorphism between sexes, such as differences in leaf morphology and branching, while in other species the sexes display little vegetative divergence (Bond & Midgley 1988; Bond & Maze 1999; Rebelo 2001). The two *Leucadendron* species included in this study, *L. laureolum* and *L. salignum*, are vegetatively dimorphic. Consistent with this, sexes in *L. salignum* also display xylem trait dimorphism. Conversely, sexes in *L. laureolum* do not appear to vary in the xylem traits we examined.

Males of *L. salignum* have greater xylem specific conductivity (K_s), hydraulic vessel diameters (d_h), and percent vessel area, suggesting that for a given xylem area they have more efficient hydraulic conductivity compared to females. Males also have a lower percentage of fiber wall area for a given xylem area due to a combination of fiber displacement in favor of increased vessel area and greater fiber lumen diameter. These changes in cell type abundance apparently do not affect whole stem strength or drastically alter carbon allocation, since males and females do not differ in stem mechanical strength (MOR) or xylem density. Consistent with what has been reported for other *Leucadendron* species (Bond & Midgley 1988; Bond & Maze 1999), males of *L. salignum* at our study site appear to have smaller leaves, thinner branches, and greater branch ramification than females. Selection for greater ramification in males may be due to sexual selection for greater inflorescence number (Bond & Midgley 1988; Geber 1995; Bond & Maze 1999), since inflorescences are terminal in *L. salignum*. It may be that selection for greater inflorescence number has affected xylem hydraulic traits. Males, with more ramified and narrow branches may have increased hydraulic conductivity per xylem area (higher K_s) as a means to maintain flow to leaves through a smaller xylem area. Additionally, although it was not measured in this study, smaller leaves in males may also lead to greater leaf specific hydraulic conductivity (K_l) in males versus females. These results suggest a link between floral and hydraulic traits.

Correlations among phylogenetic independent contrasts (PICs) of xylem characteristics generally agree with correlations obtained from the raw trait values that did not incorporate phylogenetic relatedness. Thus, many of the traits that are correlated using raw data have also experienced correlated evolutionary change across the phylogeny, suggesting that they may be functionally related. Evolutionary changes in xylem density are correlated with evolutionary changes in P_{min}, MOR, fiber lumen diameter, $(t/b)_h^2$, and fiber wall thickness, supporting utility of xylem density in prediction of other xylem traits. Similarly, K_s and d_h contrasts were correlated, supporting a functional relationship between these hydraulic traits.

Unlike the raw trait regression, percent vessel area contrasts were correlated to K_s contrasts. This suggests that these traits may be functionally related as predicted (i.e. greater vessel area could mean either larger vessels or a greater number of vessels, both of which would be predicted to increase flow; Ewers 1985). However, it should be noted that PIC analyses should be viewed with caution, especially where they vary from raw analyses, because of the uncertainty in phylogeny topography and branch length and non-random species sampling (Donoghue & Ackerly 1996; Ackerly 2000).

Principal components analyses of both raw trait values and PICs confirm the above relationships between a suite of mechanical and drought tolerance xylem traits and a suite of hydraulic traits. The mechanical and drought tolerance traits include xylem density, MOR, P_{min}, $(t/b)_{\mathrm{h}}^2$, fiber lumen diameter, percent fiber wall area, and percent parenchyma area. The hydraulic traits include K_{s}, d_{h}, and percent vessel area. We found no evidence of a trade-off between these hydraulic and the mechanical-drought traits.

The great variability in P_{min} found among species suggests that water use and availability may be important factors in the structuring of fynbos and succulent karoo evergreen woody shrub communities of the Mediterranean-type climate region of South Africa. This range of P_{min} among the species included in this study is similar to the evergreen sclerophyllous shrubs of the Mediterranean-type climate region of California and suggests that co-occurring shrubs in these regions may similarly utilize water resources in spite of differences in rainfall reliability among the regions (Cowling *et al.* 2005). The ease of measuring xylem density and its use in predicting xylem characteristics as well as P_{min}, may make it a useful tool in estimating the xylem characteristics and drought tolerance of large numbers of species. This may be particularly useful in South Africa, where the region is vulnerable to changes in climate and where plant distributions and diversity will likely impacted (McCarthy *et al.* 2001; McClean *et al.* 2005; Lovett *et al.* 2005).

Table 6.1. The species of evergreen shrubs located on Jonaskop in the Riviersonderend Mountain Range in the Western Cape Province, South Africa along with the elevation at which the species was sampled (rounded to the nearest 10 m). Nomenclature follows Germishuizen & Meyer (2003) except for *Searsia undulata* which follows Yi *et al.* (2004).

Species	**Elevation (m)**
Brunia noduliflora (E. Mey.) Kuntze	1160
Nebelia laevis O. Kuntze	1020
Aspalathus pachyloba R. Dahlgren	920
Cliffortia ruscifolia L.	920
Erica cerinthoides L.	920
Leucadendron laureolum (Lam.) Fourc.	920
Leucadendron salignum P.J. Bergius	920
Metalasia densa (Lam.) Karis	920
Protea laurifolia Thunb.	920
Protea repens (L.) L.	920
Aspalathus hirta E. Mey.	850
Erica plukenetti L.	850
Passerina obtusifolia Thoday	660
Euclea sp.	540
Pteronia fasciculata L.f.	540
Pteronia paniculata Thunb.	540
Searsia undulata (Jacq.) T.S. Yi, A.J. Miller & J. Wen[1]	540

[1] formerly *Rhus undulata*

Table 6.2. The coefficients of determination (r^2) and probability values (P) for regressions of raw trait values and phylogenetic independent contrast (PIC) values of several xylem structural and functional traits as measured on 17 evergreen shrub species. Bold text indicates a significant correlation. See Methods for abbreviations and trait descriptions.

Traits		**Correlations of Raw Trait Values**		**Phylogenetic Independent Contrast Correlations**	
Independent	**Dependent**	r^2	P	r^2	P
Pmin	Xylem Density	**0.76**	**<0.001**	**0.50**	**0.005**
	MOR	**0.39**	**0.023**	0.07	0.45
	Fiber wall thickness	0.00	0.97	0.22	0.09
	Fiber lumen diameter	0.23	0.06	0.05	0.44
	$(t/b)_h^2$	**0.49**	**0.002**	0.13	0.20
	% Fiber wall area	0.19	0.08	0.00	0.99
	% Parenchyma area	0.03	0.51	0.00	0.88
	% Vessel area	0.15	0.13	0.00	0.86
	dh	0.01	0.73	0.03	0.54
	K_s	0.19	0.08	0.20	0.11
Xylem Density	MOR	**0.72**	**<0.001**	**0.32**	**0.034**
	Fiber wall thickness	0.03	0.46	**0.26**	**0.043**
	Fiber lumen diameter	**0.58**	**<0.001**	**0.31**	**0.026**
	$(t/b)_h^2$	**0.65**	**<0.001**	**0.38**	**0.011**
	% Fiber wall area	**0.52**	**<0.001**	0.14	0.16
	% Parenchyma area	**0.28**	**0.021**	0.13	0.16
	% Vessel area	**0.26**	**0.026**	0.03	0.55
	dh	0.08	0.24	0.00	0.94
	K_s	0.01	0.72	0.06	0.35
MOR	Fiber wall thickness	0.01	0.731	0.02	0.68
	Fiber lumen diameter	**0.67**	**<0.001**	**0.50**	**0.011**
	$(t/b)_h^2$	**0.93**	**<0.001**	**0.86**	**<0.001**
	% Fiber wall area	**0.83**	**<0.001**	**0.78**	**<0.001**
	% Parenchyma area	**0.45**	**0.006**	**0.44**	**0.019**
	% Vessel area	**0.41**	**0.010**	**0.42**	**0.023**
	dh	**0.43**	**0.008**	**0.44**	**0.018**
	K_s	0.11	0.221	0.32	0.06
K_s	Fiber wall thickness	0.10	0.179	**0.35**	**0.015**
	Fiber lumen diameter	0.03	0.500	0.03	0.56
	$(t/b)_h^2$	0.04	0.387	0.08	0.30
	% Fiber wall area	0.16	0.093	**0.38**	**0.011**
	% Parenchyma area	0.04	0.428	0.09	0.25
	% Vessel area	0.17	0.078	**0.33**	**0.019**

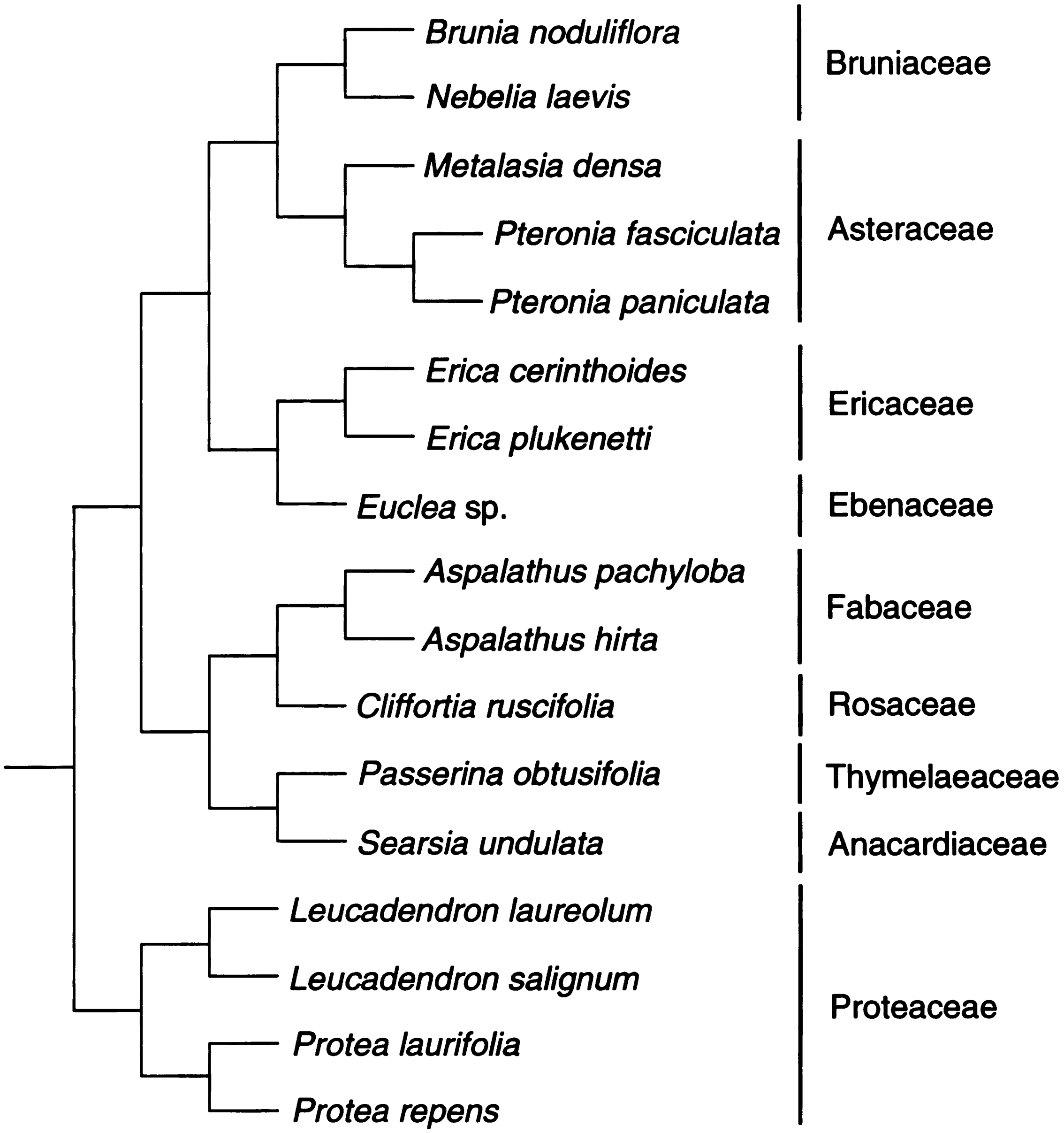

Figure 6.1. Phylogeny of 17 species of evergreen shrubs sampled from Jonaskop, in the southwestern Cape of South Africa. The phylogeny was constructed using published results referenced in the text and all branch lengths are assumed to be equal for determination of phylogenetic independent contrasts. Family divisions are indicated by the broken line on the right side of the figure. We were unable to identify one species (*Euclea* sp.) beyond the level of genus (see Methods for details).

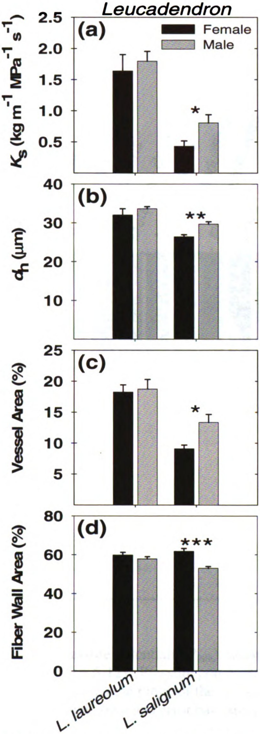

Figure 6.2. Xylem specific hydraulic conductivity (K_s) (a), mean hydraulic vessel diameter (d_h) (b), percent vessel area per transverse xylem area (c), and percent fiber wall area per transverse xylem area (d) of females (black bars ± 1 SE; $n = 6$) and males (grey bars ± 1 SE; $n = 6$) of two dioecious species, *Leucadendron laureolum* and *L. salignum*. Data were analyzed using unpaired t-tests and asterisks indicate significance (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, no asterisk = not significant).

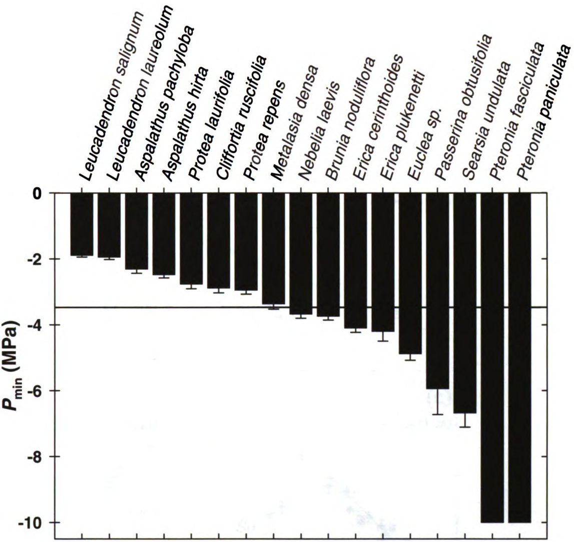

Figure 6.3. Minimum seasonal pressure potentials (P_{min}) measured in 2004 on 17 evergreen shrub species (± 1 SE; $n = 5\text{-}12$). Pressure potentials for two species (*Pteronia fasciculata* and *P. paniculata*) exceeded the range of the pressure chamber (less than -10 MPa) and therefore their P_{min} are unknown and error bars are lacking (see Results for details). The horizontal line indicates the mean P_{min} for 15 species (-3.4 MPa). The two *Pteronia* species have been excluded from this mean because P_{min} for these species exceeded the measurable range (less than -10 MPa) and are therefore lacking.

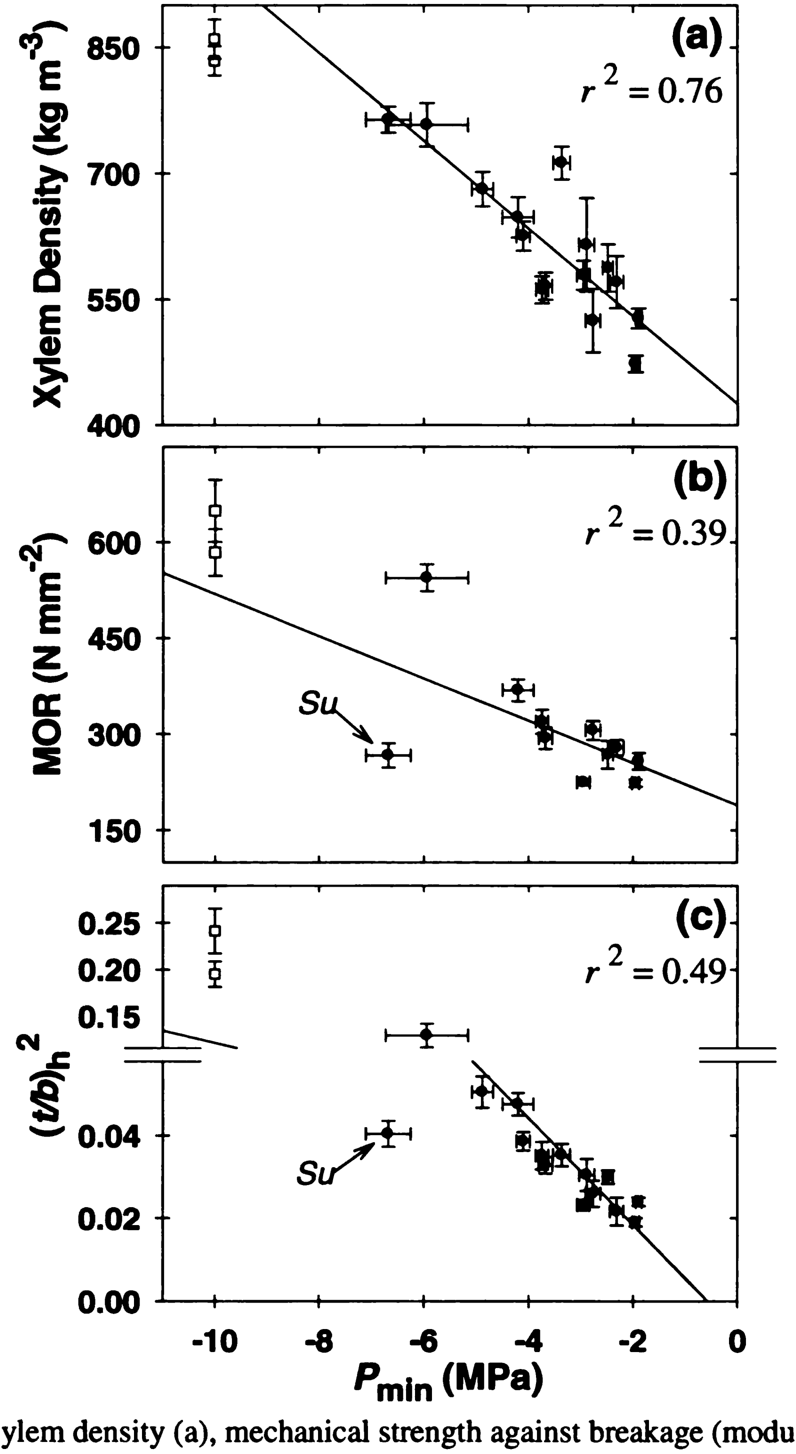

Figure 6.4. Xylem density (a), mechanical strength against breakage (modulus of rupture, MOR) (b) and the ratio of vessel wall thickness to lumen diameter squared, an estimate of vessel implosion resistance ($(t/b)_h^2$) (c), as functions of minimum seasonal pressure potential (P_{min}). Data points are means ± 1 SE. Specific P_{min} values for two species (*Pteronia* spp.) are not known (see Results for details) and thus were not included in regression analyses although they are included in the graphs (open squares). There is a break in the y-axis in panel (c) and a change in scale above the break. In panels (b) and (c) *Su* = *Searsia undulata*. All regressions shown were significant ($P < 0.05$).

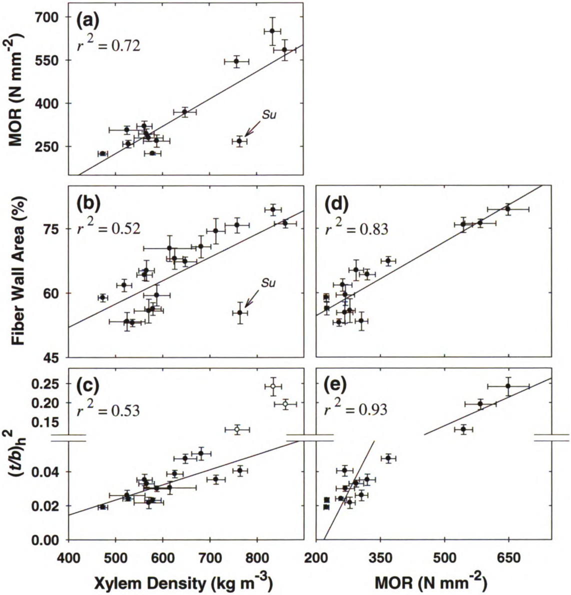

Figure 6.5. Mechanical strength against breakage (modulus of rupture, MOR) (a), percent fiber wall area per transverse xylem area (b & d), and the ratio of vessel wall thickness to lumen diameter squared, an estimate of vessel implosion resistance ($(t/b)_h^2$) (c & e) as functions of xylem density or MOR. Three data points significantly influenced the slope of the regression in panel (c) (open circles) and were excluded from the regression shown (see Results for details). In panels (a) and (b) *Su* = *Searsia undulata*. To accomadate elevated $(t/b)_h^2$ values in three species, there is a break in the y-axis in panels (c) and (e) and a change in scale above the breaks. All regressions were significant ($P < 0.05$).

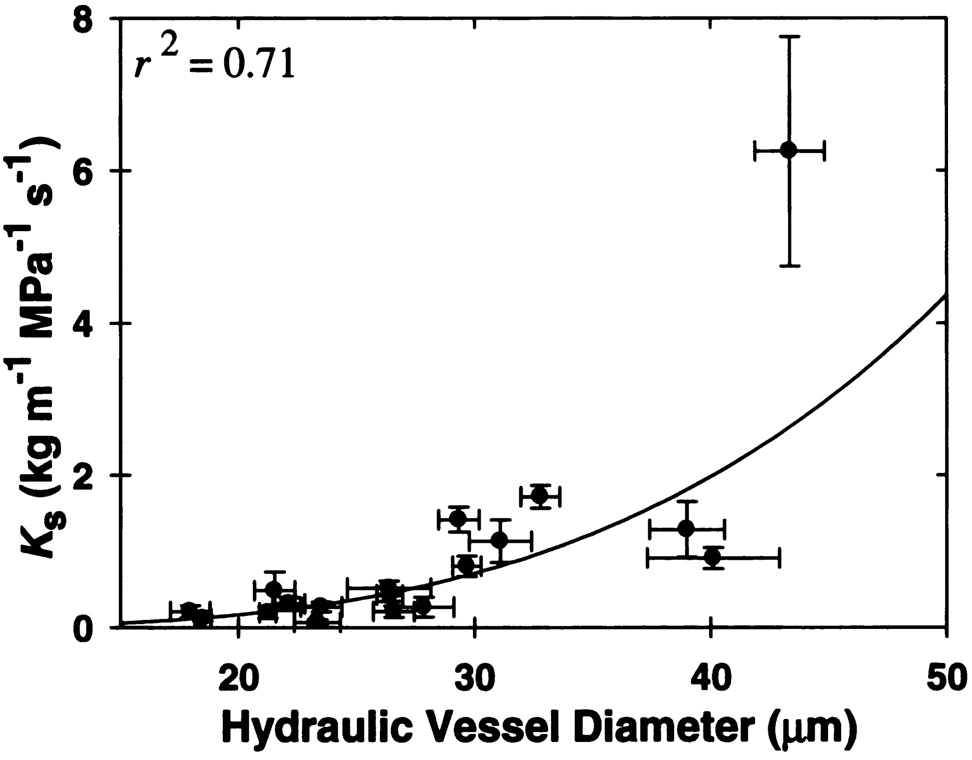

Figure 6.6. Xylem specific conductivity (K_s) as a function of hydraulic vessel diameter (d_h). Data points are shown ± 1 SE. A power model was used to fit the data ($P < 0.001$; $y = 4.115^{-6} x^{3.547}$).

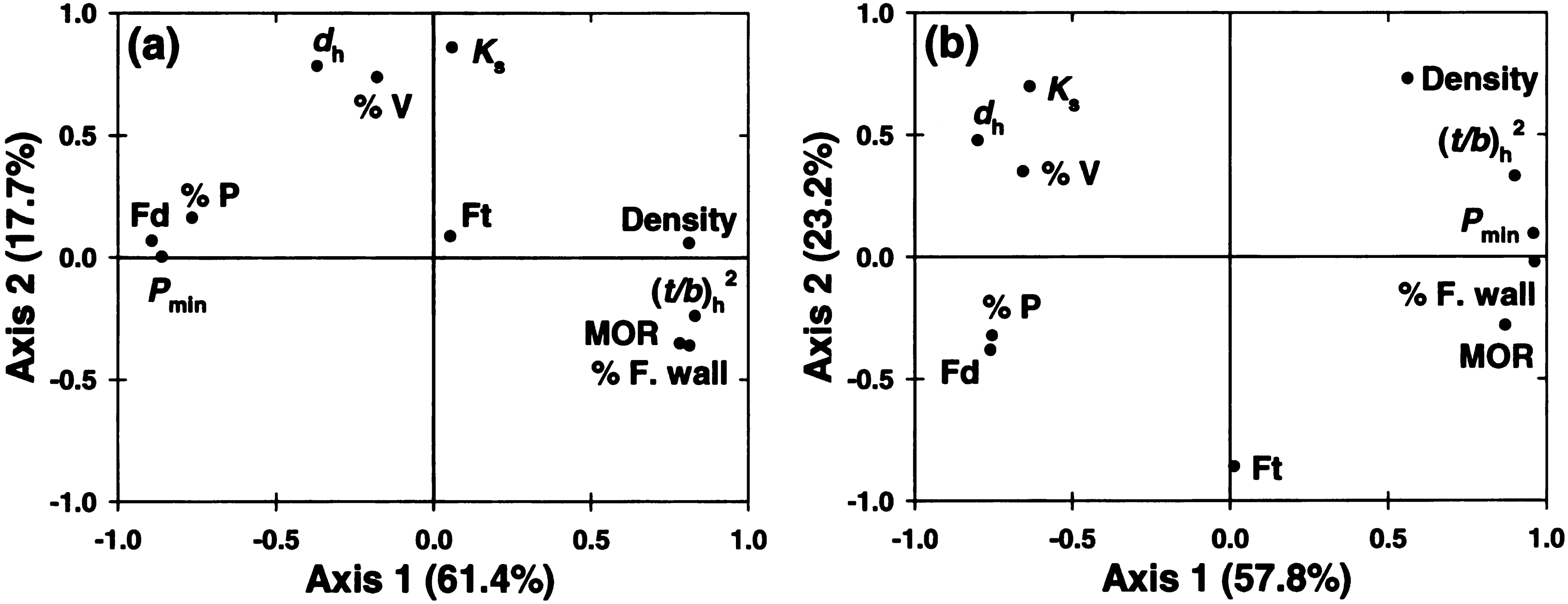

Figure 6.7. Relationship among xylem traits along two axes as determined by principal component analyses on raw data (a) and phylogenetic independent contrasts (b). Xylem traits included in the analyses are as follows: minimum seasonal pressure potential (P_{min}), xylem density, modulus of rupture (MOR), estimated vessel implosion resistance ($(t/b)_h^2$), xylem specific conductivity (K_s), hydraulic vessel diameter (d_h), fiber lumen diameter (Fd), fiber wall thickness (Ft), and percent vessel area (% V), percent parenchyma area (% P), and percent fiber wall area (% F. wall) per xylem transverse area.

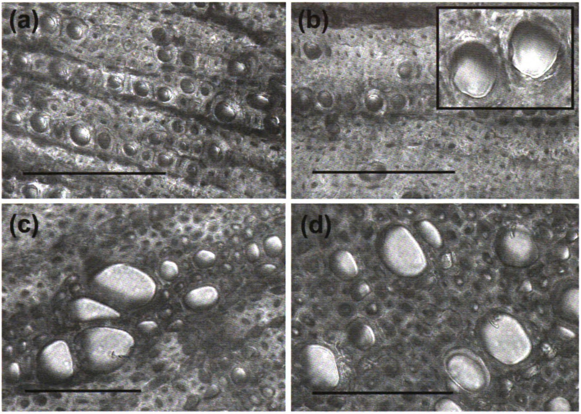

Figure 6.8. Light micrographs of xylem transverse sections illustrating differences in the ratio of the double cell wall between two vessels to the vessel lumen diameter ($(t/b)_h^2$). Two of the species shown have extremely large $(t/b)_h^2$ values, *Pteronia fasciculata* (a) and *Pteronia paniculata* (b), while two other species have $(t/b)_h^2$ values that fall within the range of published values, *Aspalathus pachyloba* (c) and *Protea repens* (d). The inset in panel (b) shows two vessels from a transverse section of *P. paniculata* enlarged to a size comparable to vessels present in panels (c) and (d) to facilitate comparison of vessel wall to lumen ratios. The black bar in each panel is 100 μm in length.

CHAPTER SEVEN

CAVITATION RESISTANCE IN FYNBOS AND SUCCULENT KAROO SHRUB SPECIES AND MEDITERRANEAN-TYPE CLIMATE REGION CONVERGENCE

Summary

1 Arid and semi-arid shrub communities may differ in their water use strategies in response to seasonal water deficit and may be differentially susceptible to extreme weather events.

2 Resistance to water stress induced xylem cavitation (Ψ_{50}) and xylem hydraulic efficiency (K_s) were measured in 15 species from two shrub communities, fynbos and succulent karoo, from the winter rainfall Mediterranean-type climate region of South Africa. These data were correlated with anatomical and physiological data.

3 Species varied in the shape and magnitude of their vulnerability to cavitation curves, with a range in water potentials at 50% loss in hydraulic conductivity (Ψ_{50}) of -1.9 MPa to -10.3 MPa. The mean Ψ_{50} across species was -4.5 ± 1.5 MPa. Fynbos and succulent karoo species were not different in their Ψ_{50}.

4 The males and females of the dioecious species *Leucadendron salignum* were significantly different in their Ψ_{50} suggesting that, even within a species, this trait may be a useful indicator of differential water use strategies.

5 Based on principle components analysis, fynbos species display a similar water use strategy to the vegetative and climatically similar chaparral shrub vegetation community of California as defined by their Ψ_{50}, minimum seasonal water potential (Ψ_{wmin}), xylem

specific hydraulic conductivity (K_s), and xylem density, while succulent karoo species utilize a strategy that overlaps with that of the woody shrubs of the Mojave Desert.
6 The data suggest convergence of community and species specific strategies between these Mediterranean-type climate regions with respect to their xylem traits. The results are discussed in the context of global climate change.

Introduction

The five Mediterranean-type climate regions are among the most biodiverse and threatened in the world. These five regions contain nearly 20% of all vascular plant species but only occupy 5% of the earth's surface (Cowling *et al.* 1996). In a recent study examining global biodiversity conservation priorities, these regions were recognized as highly vulnerable to losses in biodiversity and regions in which recovery of degraded ecosystems was highly unlikely (Brooks *et al.* 2006). Additionally, these regions have been identified as being particullarly threatened by global climate change (IPCC 2007).

The Mediterranean-type climate region of south-western South Africa stands out as being uniquely diverse and particularly threatened. Additionally, predictions for future climate in the region point to significant changes in water availability (Shulze & Perks 2000) and suggest that the ecology of the region may be highly threatened by such changes (Lovett *et al.* 2005; Midgely *et al.* 2006). However, seasonal water relations have only been studied among very few woody plant species of South Africa (Miller *et al.* 1983; Moll & Sommerville 1985; van der Heyden & Lewis 1989; Richardson & Kruger 1990; Smith & Richardson 1990; Jacobsen *et al.* 2007*a*). Additionally, published data on cavitation resistance of woody shrub species from this region is lacking (however see Jacobs *et al.* 2007 for data on four riparian species).

The woody, evergreen sclerophyllous shrubs that typify Mediterranean-type climate regions appear to experience similar levels of water stress during the long summer droughts that characterize the climate of these regions. This is supported by the similar range in and mean minimum seasonal water potential of plants in these regions (Ψ_{wmin}; Martínez-Vilalta *et al.* 2002; Jacobsen *et al.* 2007*a* and 2007*b*). The shrubs of these regions also display similar Ψ_{wmin} to those reported for other arid and semi-arid evergreen shrubland communities including species from the Great Basin Desert (Hacke *et al.* 2000), the Mojave Desert (Chapter 3), the coastal sage scrub (Chapter 3), and the Sonoran Desert (Pockman & Sperry 2000). Although a mean Ψ_{wmin} of approximately -4 to -5 MPa may represent a common physiological limit of these arid and semi-arid shrub communities, it appears that species strategies for coping with this level of water stress vary greatly among these communities (Chapter 3).

The Mediterranean-type climate region of South Africa contains several plant communities which differ in their average precipitation, topography, soil, etc., but which often occur adjacent to one other in a patchwork across the broader landscape. Two such communities are the fynbos and succulent karoo. Fynbos shrub species occur in areas that receive more rainfall than the succulent karoo and the shrub species are usually taller than those which occur in the succulent karoo. Fynbos communities grade into succulent karoo as one travels inland from the coast to areas of less precipitation. It is unknown if shrub species in these communities are utilizing similar xylem strategies in response to protracted summer drought or if they are using different strategies and may be differentially susceptible to climate change.

We examined vulnerability to cavitation of 15 shrub species from South Africa at an ecotone site between fynbos and succulent karoo vegetation communities. These data were analyzed in tandem with previously reported Ψ_{wmin} data on these shrub species in order to examine their water use strategies. Knowledge of the cavitation resistance of these shrubs as it relates to Ψ_{wmin} may be useful in predicting species or communities that are particularly sensitive to extreme water stress events in this region. Additionally, we examined what anatomical traits are associated with increased cavitation resistance among these species. Lastly, data from these South African species were compared to data from three semi-arid plant communities from North America in order to ascertain if communities in these climatically similar regions are utilizing similar strategies in response to water stress, that is, if there is convergence in water stress tolerance among shrub species and plant communities from different Mediterranean-type climate regions.

Materials and Methods

Xylem vulnerability to cavitation was measured in 15 species of evergreen sclerophyllous shrubs located on Jonaskop in the Riviersonderend Mountain Range in the Western Cape Province, South Africa (Table 7.1; see Jacobsen *et al.* 2007*a* for site and species descriptions). Ten species were sampled from between 850 and 1020 m elevation and typically receive around 420 mm of precipitation annually. Five species were sampled from between 540 and 660 ft elevation and typically receive around 315 mm of precipitation annually. This site occurs across an ecotone with the 10 higher elevation species occurring in fynbos vegetation while the 5 lower elevation species are more strongly associated with the succulent karoo vegetation community. These sites are roughly comparable climactically to the chaparral and coastal sage sites (approximately 460 mm of precipitation annual) and a Mojave Desert site (approximately 230 mm of precipitation annually) of the California Mediterranean-type climate region and described in Jacobsen *et al.* (2007*c*). All sites, both in South Africa and California, experience an extended summer dry period and receive primarily winter rainfall as is typical of a Mediterranean-type climate region.

Unbranched, straight stem segments approximately 6-8 mm in diameter and 30 cm long were collected in the field during the early summer from 10 individuals per species. Two species were dioecious. For these species stems were collected from 10 female individuals and 10 male individuals. Stems were trimmed underwater in situ, wrapped in moist paper towels, sealed in plastic bags, and shipped on ice to California State University Bakersfield, USA via international express shipping. Vulnerability to cavitation was determined on stems as soon as they arrived and all stems were measured within a week of being collected in the field.

Stems were trimmed under water from both ends until a segment 4-8 mm in diameter and 14 cm in length was obtained. Stems were connected to a tubing system and flushed for 1 h at 100 kPa and the maximum hydraulic conductivity (K_{hmax}) of stems was measured gravimetrically (Sperry *et al.* 1988) using an analytical balance (CP124S, Sartorius, Goettingen, Germany). Following determination of their K_{hmax}, stems were spun in a centrifuge (Sorvall RC-5C, Thermo Fisher Scientific, Waltham, MA, USA), using a custom made rotor (Alder *et al.* 1997). Vulnerability to cavitation curves were constructed by plotting the water potential (generated using the centrifuge) versus the percent loss in hydraulic conductivity (PLC). For each stem, curves were fit with a second order polynomial model (Jacobsen *et al.* 2007*b*). Cavitation resistance for each species was estimated using the water potential at 50% loss in hydraulic conductivity (Ψ_{50}) calculated from fatigue corrected curves using the conductivity following an initial spin of -0.25 MPa in place of K_{hmax} (Hacke *et al.* 2000; Jacobsen *et al.* 2007*c*). Unpaired t-tests were used to compare xylem traits of males and females of dioecious species (Minitab v.14.12, Minitab, Inc., State College, Pennsylvania, USA).

The xylem specific hydraulic conductivity (K_s) of stems was determined using the methods described in Jacobsen *et al.* (2007*a*). Xylem area (not including the pith) was determined using a digital camera and image analysis software (Olympus SP-500UZ, Olympus Imaging Corp., Center Valley, Pennsylvania, USA and Scion Image v. Beta 4.0.3, Scion Corp., Frederick, Maryland, USA). The K_{hmax} was divided by the xylem area to obtain xylem specific conductivity (K_s).

Several anatomical and mechanical traits have been previously reported for all of the species included in the present study (Jacobsen *et al.* 2007*a*) except for the species *Erica vestita* (see Table 7.2). These include the fiber associated traits of xylem density, modulus of rupture (MOR), fiber lumen diameter, fiber wall thickness, and the percentage fiber wall area per xylem cross sectional area, and the vessel associated traits of vessel lumen diameter, vessel mechanical strength against implosion [$(t/b)_h^2$; Hacke *et al.* 2001], and the percentage vessel area per xylem cross sectional area. We examined which of these xylem traits were associated with Ψ_{50}. We also examined the relationship between Ψ_{50} and Ψ_{wmin} and K_s. Correlations among Ψ_{50} and these traits were examined using both raw trait values and phylogenetic independent contrasts (PICs; Felsenstein 1985). Phylogenetic independent contrasts were run using the phylogeny reported in

Jacobsen *et al.* (2007*a*) and COMPARE (Martins 2004). All interspecific comparisons were analyzed using linear regression analyses (Minitab v.14.12, Minitab, Inc.).

A principle components analysis was used to examine whether South African species were utilizing similar water stress tolerating strategies to those utilized by species from Californian winter rainfall plant communities (Statview v.5.0.1, SAS Institute Inc., Cary, North Carolina, USA). Cavitation resistance (Ψ_{50}), K_s, xylem density, and minimum seasonal water potential data (Ψ_{wmin}) were included in the analyses for 28 species from California from previously published studies (Jacobsen *et al.* 2007*c*, Chapter 3). Data for these same four traits were included for 14 South African species, including Ψ_{50} and K_s from the present study and xylem density and Ψ_{wmin} as reported in Jacobsen *et al.* (2007*a*). A MANOVA was used to examine whether the relationships between these xylem traits differed among these communities and ANOVAs were used to determine if communities' multivariate components differed.

Results

Vulnerability to cavitation

Species varied in the shape and magnitude of their vulnerability to cavitation curves, displaying concave, convex, and linear shapes (Figure 7.1). In general, species displayed very little cavitation fatigue, consistent with our sampling in early summer after the growth of new xylem. Species displayed considerable variation in their resistance to cavitation, with a range in water potentials at 50% loss in hydraulic conductivity (Ψ_{50}) of -1.9 MPa (*Searsia undulata*) to -10.3 MPa (*Passerina obtusifolia*) (Figure 7.2). The mean Ψ_{50} across species was -4.5 ± 1.5 MPa (Figure 7.2). Fynbos and succulent karoo species did not significantly differ in their resistance to cavitation ($P = 0.277$), although the succulent karoo associated species displayed a broader range in cavitation resistances.

The males and females of the dioecious species *Leucadendron salignum* were significantly different in their cavitation resistance ($P = 0.041$), but they displayed similar shapes to their vulnerability curves. The females were more resistant to cavitation than the males, with an average Ψ_{50} of -4.4 ± 0.1 MPa for the females compared to -3.7 ± 0.2 MPa for the males. In contrast, the males and females of the dioecious species *L. laureolum* were not different in their cavitation resistance ($P = 0.699$).

Xylem anatomy and cavitation resistance

Across all species, cavitation resistance was correlated with anatomical traits of xylem fibers (Figure 7.3). Increased resistance to cavitation was associated with increased xylem density (Figure 7.3 A; $P = 0.007$, $r^2 = 0.40$), greater percentage fiber wall area per cross sectional area (Figure 7.3 B; $P = 0.001$, $r^2 = 0.56$), and increased stem mechanical strength estimated by the modulus of rupture (Figure 7.3 C; MOR; $P < 0.001$, $r^2 = 0.79$). Fiber lumen diameter was also correlated with Ψ_{50} ($P = 0.023$, $r^2 = 0.30$; not shown). Fiber wall area and MOR remained correlated with Ψ_{50} when relationships were analyzed using phylogenetic independent contrasts (PICs; $P = 0.024$, $r^2 = 0.33$ and $P = 0.029$, $r^2 = 0.39$, respectively); however, xylem density and fiber lumen diameter were not correlated to Ψ_{50} when PICs were used ($P > 0.05$). Fiber wall thickness was not correlated to Ψ_{50} when either raw traits or PICs were used ($P > 0.05$ for both; not shown). Thus, species with the greatest cavitation resistance have more fiber wall area per cross sectional xylem area and have greater stem mechanical strength against breakage. Species with greater cavitation resistance also tend to have increased xylem density than less resistant species, but these traits are likely not evolutionarily linked and they are therefore likely not functionally linked.

Cavitation resistance was also associated with vessel anatomical and functional traits (Figures 7.3 and 7.4). Vessel lumen diameter (Figure 7.3 D; $P < 0.001$, $r^2 = 0.64$), theoretical vessel implosion resistance (the ratio of the vessel wall thickness to lumen diameter [$(t/b)_h^2$]; Figure 7.3 E; $P = 0.023$, $r^2 = 0.30$), and the percentage cross sectional vessel area (Figure 7.3 F; $P = 0.005$, $r^2 = 0.42$) were correlated with Ψ_{50}. Vessel lumen diameter and percentage vessel area were also correlated with Ψ_{50} when PICs were analyzed ($P = 0.006$, $r^2 = 0.45$ and $P = 0.030$, $r^2 = 0.31$, respectively); however, PICs of $(t/b)_h^2$ and Ψ_{50} were not correlated ($P > 0.05$). Xylem specific conductivity also was correlated with Ψ_{50} when raw traits were analyzed ($P = 0.020$, $r^2 = 0.31$), but not when PICs were analyzed ($P > 0.05$; not shown). Thus, species with the greatest resistance to cavitation have narrower vessel lumen diameters and less vessel area per cross section, these combined traits likely result in more resistant species also displaying decreased xylem efficiency (i.e. lower K_s). The lack of an evolutionary correlation between K_s and Ψ_{50} suggests that there may be changes occurring at the pit-level that uncouple changes in these traits.

Water use "strategies"

Succulent karoo associated shrub species tend to experience lower seasonal water potentials (Ψ_{wmin}) than fynbos shrub species, but are not significantly different in their cavitation resistance (Ψ_{50}). Thus, these vegetation types appear to differ in their water use strategies. Succulent karoo associated shrub species tend to fall above a 1:1 line of Ψ_{50} to Ψ_{wmin}, suggesting that they would typically experience greater than 50% loss in hydraulic conductivity annually (Figure 7.5). In contrast, fynbos shrub species tend to fall below or on this 1:1 line suggesting they would typically experience 50% or less loss in hydraulic conductivity annually (Figure 7.5).

Convergence among Mediterranean-type climate regions

Communities significantly differed in the suites of xylem traits utilized by species ($P < 0.001$). Xylem traits were associated with two axes of variation. The first principle component (PC1) was associated with Ψ_{wmin}, xylem density, and K_s and explained 57% of the variation among species. Positive PC1values are associated with higher Ψ_{wmin}, lower xylem density, and higher K_s. The second principle component (PC2) was associated with Ψ_{50} and explained 20% of the variation among species. Positive PC2 values are associated with lower cavitation resistance.

Fynbos and chaparral shrub species utilize similar suites of xylem traits and did not differ in calculated values of either the first or second components of a principle components analysis (Figure 7.6; $P > 0.05$). Fynbos and chaparral species both tend to fall in the lower right corner of this figure (Figure 7.6), consistent with a relatively safe strategy of high cavitation resistance and comparatively low stress. The fynbos "strategy" significantly differed from the Mojave Desert species in both PC1 and PC2 ($P > 0.05$ for both). Fynbos species did not differ from coastal sage in PC1 ($P > 0.05$) but did differ in values of PC2 ($P = 0.006$).

The succulent Karoo shrub species display widely variable strategies, but are most closely associated with Mojave Desert species in PC1 (Figure 7.7; $P > 0.05$ between the Mojave Desert and succulent karoo) and with the chaparral and fynbos in PC2 ($P > 0.05$ for PC2 between the both the chaparral and fynbos compared to succulent karoo). These species experience high water stress levels annually but are not very resistant to cavitation relative compared to these water stress levels. Succulent karoo species differed from coastal sage species in values of both PC1 and PC2 ($P > 0.05$ for both).

Discussion

Resistance to cavitation

Cavitation resistance varied broadly among 15 species from the Mediterranean-type climate region of South Africa. Species displayed cavitation resistances (estimated as the water potential at 50% loss in hydraulic conductivity; Ψ_{50}) ranging from -1.9 to -10.3 MPa and displayed as much variation within microsites as across the sampled ecotone. Species displayed a mean cavitation resistance of -4.5 MPa which is not different from that reported for angiosperms from other Mediterranean-type climate regions (Martínez-Vilalta *et al.* 2002; Maherali *et al.* 2004; Jacobsen *et al.* 2007*b*).

Cavitation resistance was correlated with various xylem fiber and vessel anatomical and functional traits. Xylem density, percentage fiber wall area, and modulus of rupture (MOR) were all correlated with cavitation resistance. Fiber wall area and MOR were also correlated with Ψ_{50} when data were analyzed taking into account the evolutionary relationship among species (i.e. phylogenetic independent contrasts), supporting a functional relationship among these traits. This is consistent with previous studies that have found strong and phylogenetically independent correlations between fiber traits and cavitation resistance (Jacobsen *et al.* 2005; Jacobsen *et al.* 2007*b*) and between stem mechanical properties and cavitation resistance (Jacobsen *et al.* 2005; Jacobsen *et al.* 2007*b*) in semi-arid shrubs. These correlations suggest that xylem fibers, which largely determine xylem density and mechanical strength in angiosperms, may play a role in cavitation resistance, perhaps through reinforcement of xylem vessel walls against implosion (Jacobsen *et al.* 2005). This points to a mechanical cost to increased xylem cavitation resistance.

Vessel lumen diameter, vessel cross sectional area, and the theoretical implosion threshold of vessels ($[t/b]_h^2$; Hacke *et al.* 2001*a*), were also correlated with cavitation resistance. Decreased vessel lumen diameter was associated with increased cavitation resistance and it may also be the driver for the relationship between $(t/b)_h^2$ and cavitation resistance since this variable is partially dependent on lumen diameter (b). These vessel traits increase the mechanical strength of vessels and, as with the fiber traits above, are likely important in the resistance of vessel implosion in response to the extreme negative pressures that develop in the xylem of arid and semi-arid shrubs. Smaller vessel lumen diameters and reduced cross sectional vessel area both contributed to declined xylem specific conductivity (K_s) in species with greater resistance to cavitation, leading to a weak tradeoff between conductive efficiency and cavitation resistance. However, there is no tradeoff between these traits when data are analyzed using phylogenetic independent contrasts suggesting that this relationship may be due to community filtering or selection for some alternative trait, such as pit membrane area or structure, rather than a direct functional relationship between cavitation resistance and conductivity. However, there may also be a conductivity efficiency cost to increased cavitation resistance.

Vessel and fiber anatomical traits may be useful in predictions of inter- or intra-specific differences in cavitation resistance. For instance, *Leucadendron salignum* females and males were found to differ in their cavitation resistance in the present study. The sexes of this species co-occur in the same microsites and experience similar water potentials suggesting that they may share similar resistances to cavitation; however, they have previously been reported to vary in several xylem traits including K_s, vessel diameter, vessel area, and fiber wall area (Jacobsen *et al.* 2005). In contrast, the K_s, vessel diameter, vessel area, and fiber wall area of females and males of *Leucadendron laureolum* were not reported to differ and so it is not surprising that the sexes of this species are also not different in their resistance to cavitation.

Previous studies have examined the differences in water use between males and females of dioecious species. These studies have found that females often occur in more mesic areas compared to males of the same species (Dawson & Bliss 1989; Dawson & Ehleringer 1993) although females have also been reported to have a higher water use efficiency than males in some species (Correia & Diaz Barradas 2000). In the present study, we found that females of the species *Leucadendron salignum* were more resistant to cavitation than males of the same species. To our knowledge, this is the first time that a difference in cavitation resistance has been reported between the sexes of a dioecious species. Additionally, the greater cavitation resistance of the females in this species suggests that females may incur additional costs as carbon to xylem tissue to increase cavitation resistance in order to maintain a steady water supply to developing fruit. This is inconsistent with maternal investment hypotheses and may alter our understanding of drivers and limits of sexual dimorphism in this genus (Bond & Midgley 1988; Bond & Maze 1999).

Water use "strategies" of fynbos and succulent karoo shrubs

The fynbos and succulent karoo species included in the present study appear to be utilizing differential strategies for coping with summer water stress. The fynbos species all experienced minimum seasonal water potentials that were the same or higher (less negative) than their Ψ_{50}. This suggests that in a year of normal precipitation these species will have dry season losses in hydraulic conductivity that are near or less than 50%. In contrast, the succulent karoo species are not more resistant to cavitation than the fynbos species, yet these species experience much more negative dry season water potentials. In a typical year, these species are likely to reach higher percent losses in hydraulic conductivity during the dry season, especially *Searsia undulata* and the *Euclea* species.

The fynbos "strategy" appears similar to that of other Mediterranean-type climate region sclerophyllous shrublands including the California chaparral and mixed oak forests of the Mediterranean Basin. Shrubs in these regions also typically reach water potentials equivalent to 50% loss in hydraulic conductivity or less during the dry season (Martínez-Vilalta *et al.* 2002; Jacobsen *et al.* 2007*b* and 2007*c*). Thus, in typical years these species employ a relatively safe strategy consistent with their maintenance of full canopies of leaves year round; however, this may be a risky strategy during extremely dry years.

Evergreen shrubs in California and the Mediterranean Basin appear susceptible to drought induced mortality during dry years (Lloret *et al.* 2004; Paddock 2006). Additionally, individuals occurring at ecotones with drier vegetation communities may be particularly susceptible as suggested by the high mortality observed among chaparral species at a chaparral-desert ecotone during a drought year in California (Paddock 2006). Shrubs in these communities may rely on avoidance of large declines in hydraulic conductivity and most may not be able to adjust other traits, such as canopy area, in response to declining water availability. Indeed, species that retain a nearly full canopy often exhibit dieback, while co-occurring species that are able to facultatively thin their canopy appear to survive in greater numbers (Pratt, *personal communication*).

The succulent karoo "strategy" appears most similar to that of desert shrubs which also undergo high levels of embolism seasonally (Pockman & Sperry 2000; Chapter 3). In drier areas, woody plants may be unable to avoid high levels of water stress and concomitant losses in hydraulic conductivity and must therefore rely on traits other than increased cavitation resistance, such as tight stomatal control or adjustment of leaf area seasonally (Chapter 3). Succulent karoo shrub species may therefore be more able to deal with annual rainfall variability than fynbos species.

The coastal sage community of California is typified by facultatively deciduous species which are sometimes referred to as "soft chaparral" because they are similar in form but lack the highly sclerophyllous leaves of the chaparral. We did not examine any soft leaved species from South Africa and there is little overlap between the coastal sage and any of the studied sclerophyllous South African species examined in the present study. This may be a separate water use "strategy" that is not utilized by species of the fynbos or succulent karoo communities. However, there are some morphologically similar plant communities in South Africa which may utilize a similar water use strategy such as the coast Renosterveld.

Convergence among South African and Californian plant communities

Morphologically and climactically similar plant communities from the Mediterranean-type climate regions of South Africa and California appear to be convergent in the suites of xylem traits utilized by species in relation to water stress. The plant communities of the Mediterranean-type climate regions of South Africa and California have been compared previously, resulting in mixed conclusions regarding plant convergence. Most of these studies have focused on the dominant vegetation communities of these regions, the fynbos and chaparral communities, and on plant structure and diversity rather than physiological strategies of plants (*e.g.* Cody & Mooney 1978; Barbour & Minnich 1990; Keeley & Bond 1997). Limited comparisons have also been made between plants of the succulent karoo and Mojave Desert (Esler & Rundel 1999).

Chaparral and fynbos communities, which are morphologically similar (*i.e.* they are woody, evergreen, sclerophyllous shrubs) appear to converge in their water use "strategy" as defined by their cavitation resistance, minimum seasonal water potential, xylem density, and hydraulic efficiency. The five shrub species from the succulent karoo included in the present study appear to be utilizing relatively divergent strategies in regards to their xylem water use, although they share some overlap with sclerophyllous shrubs from the Mojave Desert consistent with these being drier and more variable regions.

The apparent convergence in woody shrub physiological strategies between these climactically and morphologically similar plant communities suggests that these communities may be similarly susceptible to climate change. The ability to compare community and even species specific strategies between these regions could provide useful predictions for species and communities most likely to be affected by global climate change. In addition, the ability to apply data measured in more thoroughly studied Mediterranean-type climate regions and vegetation communities to those that have been less studied could greatly enhance our ability to minimize the impact of climate change on biodiversity in these regions.

Table 7.1. Species of evergreen shrubs located on Jonaskop in the Riviersonderend Mountain Range in the Western Cape Province, South Africa including species families and the code used for each species in Figures 7.1, 7.2, 7.5, and 7.6.

Species	Family	Code
Fynbos		
Aspalathus hirta E. Mey.	Fabaceae	Ah
Aspalathus pachyloba R. Dahlgren	Fabaceae	Ap
Cliffortia ruscifolia L.	Rosaceae	Cr
Erica plukenetti L.	Ericaceae	Ep
Erica vestita Thunb.	Ericaceae	Ev
Leucadendron laureolum (Lam.) Fourc. Females	Proteaceae	LlF
Leucadendron laureolum (Lam.) Fourc. Males	Proteaceae	LlM
Leucadendron salignum P.J. Bergius Females	Proteaceae	LsF
Leucadendron salignum P.J. Bergius Males	Proteaceae	LsM
Metalasia densa (Lam.) Karis	Asteraceae	Md
Nebelia laevis O. Kuntze	Bruniaceae	Nl
Protea repens (L.) L.	Proteaceae	Pr
Succulent karoo		
Euclea sp.	Ebenaceae	E
Passerina obtusifolia Thoday	Thymelaeaceae	Po
Pteronia fasciculata L.f.	Asteraceae	Pf
Pteronia paniculata Thunb.	Asteraceae	Pp
Searsia undulata (Jacq.) T.S. Yi, A.J. Miller & J. Wen[1]	Anacardiaceae	Su

[1] formerly *Rhus undulata*

Table 7.2. Xylem functional and anatomical traits for several xylem functional and anatomical traits previously published in Jacobsen *et al.* (2007*a*) and included in analyses in the present study. See Chapter 6 for detailed methods and descriptions of data.

Species	**Xylem Density**	**MOR**	Ψ_{min}	d_h
Fynbos				
Aspalathus pachyloba	570.5 ± 31.0	279.2 ± 12.2	-2.31 ± 0.13	40.1 ± 2.8
Aspalathus hirta	587.6 ± 28.1	268.4 ± 21.6	-2.48 ± 0.10	26.4 ± 1.8
Cliffortia ruscifolia	615.0 ± 55.5	*no data*	-2.89 ± 0.15	31.1 ± 1.3
Erica plukenetti	647.9 ± 24.1	368.8 ± 17.0	-4.20 ± 0.30	26.6 ± 0.9
Leucadendron laureolum (Females)	487.3 ± 14.8	223.8 ± 8.6	-2.06 ± 0.11	32.0 ± 1.6
Leucadendron laureolum (Males)	459.2 ± 11.9	223.9 ± 8.4	-1.84 ± 0.04	33.5 ± 0.5
Leucadendron salignum (Females)	518.6 ± 15.9	262.1 ± 21.8	-2.13 ± 0.15	26.4 ± 0.5
*Leucadendrom salignum (*Males)	536.1 ± 18.1	253.7 ± 14.1	-1.87 ± 0.07	29.7 ± 0.6
Metalasia densa	712.7 ± 19.5	*no data*	-3.37 ± 0.16	23.5 ± 0.9
Nebelia laevis	565.8 ± 16.5	293.7 ± 17.0	-3.68 ± 0.13	21.2 ± 0.3
Protea repens	579.2 ± 17.4	225.1 ± 4.7	-2.95 ± 0.12	29.3 ± 0.9
Succulent karoo				
Euclea sp.	681.5 ± 20.6	*no data*	-4.88 ± 0.20	39.0 ± 1.6
Passerina obtusifolia	757.8 ± 25.7	544.4 ± 20.9	-5.94 ± 0.79	21.5 ± 0.9
Pteronia fasciculata	834.1 ± 17.4	649.1 ± 48.5	-10	18.0 ± 0.8
Pteronia paniculata	860.0 ± 23.3	583.9 ± 36.4	-10	18.5 ± 0.3
Searsia undulata	764.0 ± 15.5	267.1 ± 19.1	-6.68 ± 0.43	43.3 ± 1.5

Table 7.2 (cont'd)

$(t/b)_h^2$	Fiber Lumen Diameter	Fiber Wall Thickness	Parenchyma (%)	Vessel (%)	Fiber Wall (%)
0.021 ± 0.003	6.19 ± 0.61	3.57 ± 0.15	13.6 ± 0.7	15.5 ± 1.5	55.8 ± 2.8
0.030 ± 0.002	5.13 ± 0.38	3.65 ± 0.19	13.4 ± 0.8	15.0 ± 2.2	59.5 ± 2.4
0.031 ± 0.004	5.69 ± 0.37	4.34 ± 0.38	4.5 ± 1.1	11.6 ± 0.9	70.4 ± 3.0
0.048 ± 0.003	5.18 ± 0.14	3.66 ± 0.27	4.7 ± 0.2	13.8 ± 1.0	67.3 ± 1.1
0.019 ± 0.002	5.95 ± 0.41	3.76 ± 0.13	7.4 ± 2.1	18.2 ± 1.2	59.9 ± 1.3
0.019 ± 0.002	6.28 ± 0.28	3.42 ± 0.07	6.3 ± 0.9	18.7 ± 1.6	57.8 ± 1.2
0.026 ± 0.001	6.51 ± 0.33	3.23 ± 0.04	8.3 ± 1.6	9.1 ± 0.6	61.8 ± 1.4
0.021 ± 0.002	8.40 ± 0.75	3.10 ± 0.09	7.6 ± 1.0	13.3 ± 1.3	53.0 ± 0.9
0.035 ± 0.003	4.91 ± 0.21	4.35 ± 0.32	4.9 ± 1.2	8.9 ± 1.7	74.4 ± 3.0
0.033 ± 0.002	6.89 ± 0.24	5.03 ± 0.53	10.7 ± 1.1	10.6 ± 0.8	65.2 ± 2.4
0.023 ± 0.001	6.63 ± 0.25	3.54 ± 0.21	9.7 ± 1.7	16.9 ± 1.1	56.3 ± 1.5
0.051 ± 0.004	3.45 ± 0.26	3.50 ± 0.14	9.3 ± 0.8	11.4 ± 1.0	70.8 ± 2.6
0.129 ± 0.013	4.34 ± 0.32	3.75 ± 0.19	3.9 ± 0.7	8.6 ± 1.5	75.8 ± 1.8
0.241 ± 0.024	3.02 ± 0.04	3.40 ± 0.10	2.4 ± 0.3	9.9 ± 1.4	79.3 ± 1.3
0.195 ± 0.014	3.13 ± 0.10	3.16 ± 0.14	3.0 ± 0.4	11.4 ± 0.9	76.1 ± 1.0
0.040 ± 0.003	6.31 ± 0.27	3.05 ± 0.20	10.6 ± 1.5	14.6 ± 1.5	55.3 ± 2.5

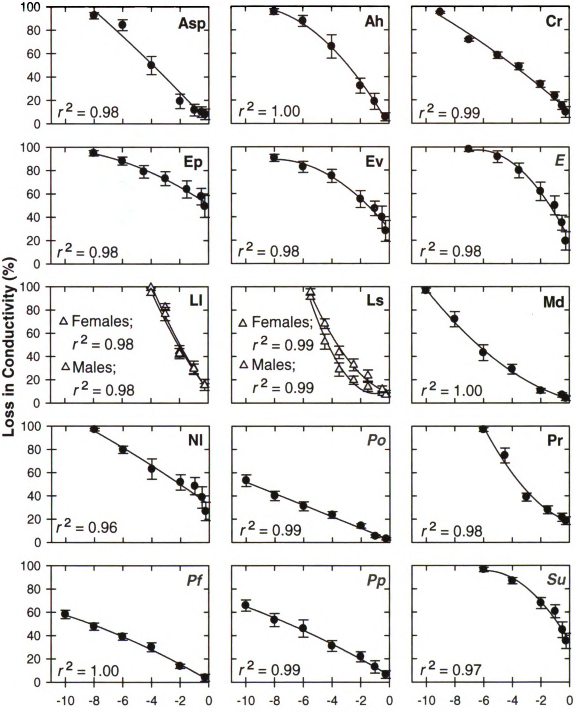

Figure 7.1. Vulnerability to cavitation curves for 15 evergreen shrub species from South Africa depicting the percentage loss in hydraulic conductivity for a given decrease in water potential generated using a customized rotor and centrifuge (see Methods for details). Curves for monoecious species are shown with black filled circles, and for dioecious species, curves from females are shown with grey triangles and for males with open triangles. Species abbreviations are in black text for fynbos species and in grey italics text for succulent karoo species. For key to species abbreviations, see Table 7.1.

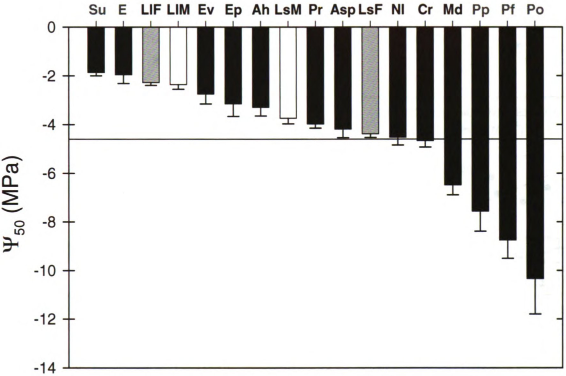

Figure 7.2. Cavitation resistance (Ψ_{50}) of 15 evergreen shrub species from South Africa, estimated from fatigue-corrected vulnerability to cavitation curves (see Methods for details). Bars represent species means ± 1 SE (monoecious species values are represent as black bars, females from dioecious species as grey bars, and males from dioecious species as open bars). Horizontal line indicates the across species mean cavitation resistance of -4.5 MPa. Species abbreviations are in black text for fynbos species and in grey text for succulent karoo species. For key to species abbreviations, see Table 7.1.

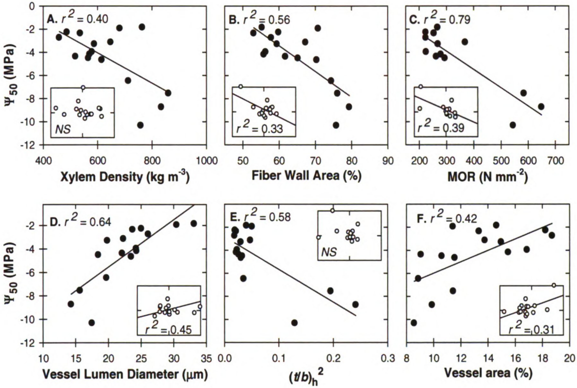

Figure 7.3. Xylem fiber and vessel anatomical traits as predictors of cavitation resistance (Ψ_{50}) among 15 evergreen shrub species from South Africa. Xylem fiber associated traits include xylem density (A), percentage fiber wall area per xylem cross sectional area (B), and stem mechanical strength against breakage estimated by the modulus of rupture (MOR) (C). Xylem vessel associated traits include vessel lumen diameter (D), theoretical vessel implosion resistance $[(t/b)_h^2]$ (E), and percentage vessel area per xylem cross sectional area (F). Regressions of raw trait values are shown as filled circles and of phylogenetic independent contrasts in insets as open circles (see Methods). Only significant regressions are shown.

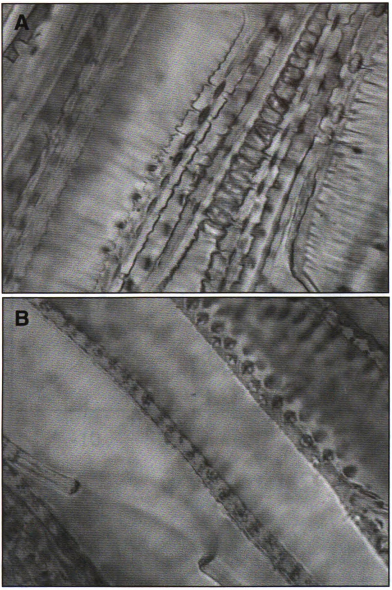

Figure 7.4. Light micrographs of xylem longitudinal sections demonstrating some of the xylem vessel anatomical differences among species. *Passerina obtusifolia* xylem (A) displays the relatively narrow xylem vessels and thick vessel walls typical of very resistant species. *Leucadendron salignum* male xylem (B) displays intermediate cavitation resistance and has xylem vessel diameters and wall thickness typical of the "average" species. Both micrographs were taken at the same magnification (1000x).

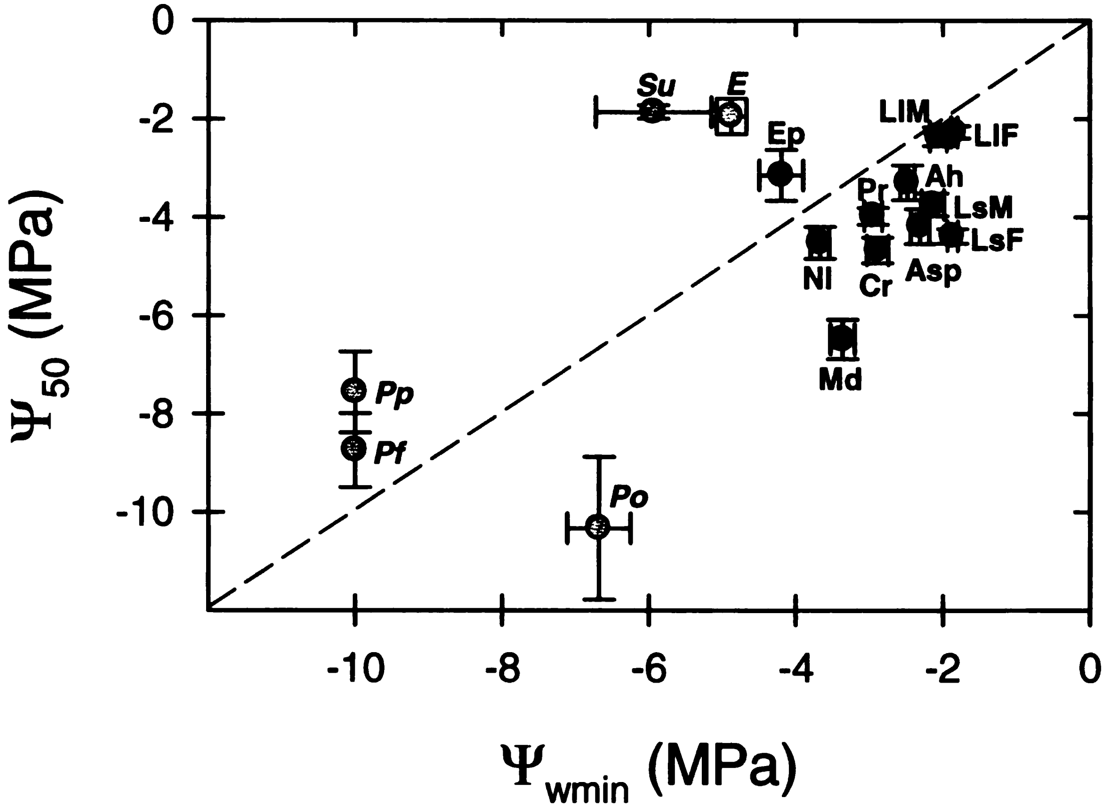

Figure 7.5. Cavitation resistance (Ψ_{50}) plotted against minimum seasonal water potential (Ψ_{wmin}) for 15 species of evergreen shrubs from South Africa. Succulent karoo species are represented by grey filled circles and italics text and fynbos species are represented by black filled circles. Note, the Ψ_{wmin} values for the two *Pteronia* species (Pf and Pp) shown are only approximate because they exceeded the limits of the pressure chamber ($\Psi_{wmin} < -10$ MPa). The dashed line represents a 1:1 line. For key to species abbreviations, see Table 7.1.

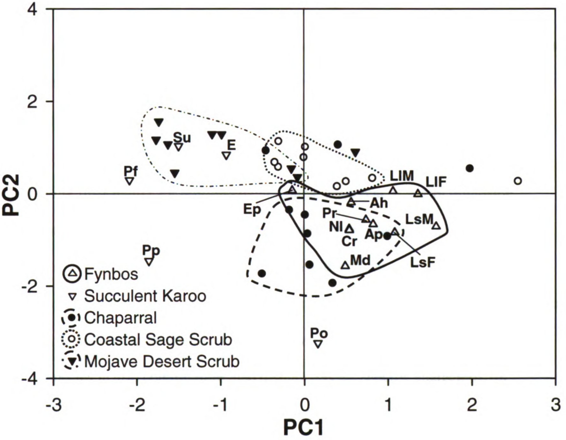

Figure 7.6. Relationships among species from South Africa to those from climatically similar plant communities in California, USA as determined by principle components analysis. The first principle component (PC1) was associated with minimum seasonal water potential (Ψ_{wmin}), xylem density, and xylem specific conductivity (K_s) and the second principle component (PC2) was associated with cavitation resistance (Ψ_{50}). Positive PC1 values are associated with high seasonal Ψ_{wmin}, relatively high K_s, and low xylem density. Positive values of PC2 are associated with low cavitation resistance (high Ψ_{50}). Species from South Africa are shown as open triangles, with fynbos species as upward pointing triangles and succulent karoo species as downward pointing triangles. For key to South African species abbreviations, see Table 7.1. Communities included from California include the chaparral (filled circles), the coastal sage scrub (open circles), and the Mojave Desert scrub (filled triangles). See Chapter 3 for identification of species within the California plant communities.

CHAPTER EIGHT

CONCLUSION

Across arid communities, woody shrub species experience similar levels of water stress and share several xylem traits associated with increased water stress tolerance. Species that experience very low xylem water potentials seasonally have dense xylem and are likely relying on mechanical reinforcement of both xylem vessel walls and the surrounding fiber matrix for reinforcement against the threat of vessel implosion. Additionally, there is a trend for species experiencing the lowest water potentials and having the greatest resistance to cavitation to have decreased hydraulic efficiency compared to less resistant species, suggesting that loss of conductivity efficiency may be an additional cost of increased drought tolerance.

Species in arid communities also display species specific characteristics, including differences in cavitation resistance and stomatal sensitivity to water stress among three California arid communities. These community specific differences are likely important in species response to biotic and abiotic differences among communities. These community specific water use traits may also play a role in community assembly. The trend for California aridland shrub communities (at the β level) to contain species that are relatively closely related suggests that there are lineage specific functional traits important for persistence in each community. Additionally, within communities (at the α level) species tend to co-occur with other species utilizing similar xylem traits suggesting that there may be microsite specific traits as well.

At the ecosystem level, comparable plant communities from the Mediterranean-type climate regions of California and South Africa appear to be share many of the same xylem structural and functional traits and to be utilizing similar water use strategies. This suggests that these strategies may be common to winter rainfall aridland shrub communities, even among species that are geographically and phylogenetically distant.

The apparent presence of community divergence and ecosystem convergence in woody shrub physiological strategies between these aridlands shrub communities suggests that these ecosystems and communities may be physiologically structured. Future study of additional plant communities and ecosystems may enhance our understanding of community assembly in arid shrublands and aridland ecosystem functioning. Additionally, tests of physiological convergence among plant communities may provide a use framework for understanding the relative roles of biotic and abiotic as well as biogeographical influences on plant functional strategies.

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