ABSTRACT

ABIT, PAMELA P. Ecological and Physiological Basis for the Distribution of Woody Plants along Water Availability Gradients in the Southeastern United States Mixed Forest. (Under the direction of Dr. William A. Hoffmann.)

Higher temperatures under climate change are likely to result in greater evaporation and increased soil moisture deficits. Increased drought will likely cause a shift in the vegetation distribution. This study focused on woody plants of the Southeastern United States mixed forest. Although water is not a primary limiting factor in this forest, species distribution suggests that water availability exerts a strong control on the success of woody plants. I used a comparative approach to understand environmental factors and corresponding species traits that determine species composition across a gradient of water availability. I compared hydraulic architecture, vulnerability to cavitation, and the ability of xeric and mesic species to germinate, grow and survive under varying levels of water stress. Congeneric pairs composed of one xeric and one mesic species were used. Seeds were subjected to polyethylene glycol solutions of different water potentials to compare the effects of water availability on germination of xeric and mesic species. I used understory saplings to compare the difference in the xylem hydraulic properties between xeric and mesic species and the air-injection method was used to determine differences in their xylem vulnerability to cavitation. I performed a dry-down experiment to compare the ability of the xeric and mesic species to survive extreme drought by evaluating survival after re-watering subsequent to drought exposure. Growth performance was evaluated by measuring electron transport rate (ETR), stomatal conductance (g_s), shoot and root biomass of seedlings that were exposed to different levels of water (well-watered, dry, and flooded) and nutrients (high and low). Results

indicate that the ability to germinate under drought did not differ consistently between xeric and mesic species, but, germination ability under drought was associated with the ability to maintain turgor. Stems of xeric species were less vulnerable to wilting than mesic species under drought stress. I found greater resistance to xylem cavitation in xeric species than mesic species. It appears that cavitation resistance was independent of specific conductivity and wood density since I did not find any trade-off between specific conductivity and vulnerability to cavitation among the woody plants examined. In the growth performance study, xeric species tend to have lower whole plant biomass, higher Root:Shoot (R/S) ratio, higher coarse root mass ratio (CRMR) and less reduction in g_s under drought stress than mesic species. The higher stomatal conductance and lower photorespiration rates among the mesic species may have influenced their increased photosynthetic rates, thereby producing a greater total plant biomass than xeric species. Species distribution along water availability gradients appears to be better explained by the ability of seedlings to resist cavitation, to tolerate and survive water stress, and by their biomass allocation patterns, rather than by their ability to germinate under drought.

Ecological and Physiological Basis for the Distribution of Woody Plants along Water
Availability Gradients in the Southeastern United States Mixed Forest

by
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DEDICATION

For Serge and Andre

When I felt like giving up,
you helped me hold on for so long…
to keep our dreams alive.

BIOGRAPHY

On December 9, 1975, the author was born to Wilson Paterno Po Jr. and the late Mercedes Milan Po of Baybay City, Philippines. She is married to Sergio M. Abit Jr., whom she has a son, Serge Andre. She completed her Bachelor of Science degree in Agriculture major in Plant Protection and specializing in weed science and graduated *Magna cum laude* from the Visayas State College of Agriculture (ViSCA) now Visayas State University (VSU), Baybay, Leyte, Philippines. She was a consistent University scholar and was awarded a two-year scholarship of the Weed Science Society of the Philippines (WSSP) during her college days. After graduation, the author joined the Department of Plant Protection at ViSCA as a graduate teaching assistant. In 1997, she was awarded a scholarship by the flanking program of the ViSCA-gtz Applied Tropical Ecology Program to pursue Masters of Science in Plant Taxonomy (minor in Environmental Science) at the University of the Philippines at Los Baños (UPLB), Laguna, Philippines. In 2000, she joined ViSCA as a project development officer and later on joined the Department of Biological Sciences in ViSCA as a junior faculty member. She was awarded a full scholarship in 2003 by the Fulbright-Philippine Agriculture Scholarship Program (FPASP) to pursue her PhD. in Botany. In the fall of 2003, she started her PhD. program in the Department of Botany (now Plant Biology) at North Carolina State University, Raleigh, North Carolina, where she specialized in plant physiological ecology under the guidance of Dr. William A. Hoffmann. She is a member of the Phi Kappa Phi Honor Society (UPLB and NCSU chapters), Gamma Sigma Delta (UPLB chapter), Ecological Society of America, Association of Southeastern Biologists, Pest Management Council of the Philippines, and Weed Science Society of the Philippines.

culturally and to deal other's viewpoints as I work with them, live with them, learn with them and learn from them.

To the Department of Biological Sciences, Visayas State University, Philippines, for granting me a study leave to pursue my PhD. degree.

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To the Almighty Father who gave me strength, sound mind, and good health.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER VI. Summary and Conclusion

LIST OF FIGURES

CHAPTER II. Drought Tolerance of Xeric and Mesic Southeastern US Mixed Forest
Species

CHAPTER I

Ecological and Physiological Basis for the Distribution of Woody Plants Along Water Availability Gradients in the Eastern United States Mixed Forest

INTRODUCTION

Rationale of the Study

Plant communities are formed by successful germination, survival, growth, and establishment of plant species (Donovan et al., 1993), which eventually affects floristic composition, species ranges, structure of the landscape, and overall species distribution. Occurrence of plant species can be largely controlled by environmental factors, such as water availability, that may constrain seed germination, seedling survival, growth performance, and establishment (Schupp, 1995).

Water availability is one of the most important factors influencing plant species distribution at a global scale (Bahari et al., 1985; Woodward, 1987). Other factors that may co-vary with water availability and shape vegetation distributions include nutrient availability, light, temperature, competition, and pathogen or herbivore pressure (Schupp, 1995; Engelbrecht, 2007). Among these factors, nutrient availability is closely associated with water availability, since this can also be influenced by soil characteristics, variation in temperatures, and rainfall patterns in the same way as water availability. Water and nutrient availability are also known to affect biomass allocations and tolerance to resource limitation. Tolerances of species to certain environmental or edaphic conditions are reflected in the variation in species composition along a water availability gradient. For instance, under low soil resources, species composition may indicate tolerance to poor soil conditions (Goldberg, 1985), while rapid growth in response to high soil resource availability could enable

particular species to out compete less responsive but physiologically tolerant species (Chapin et al., 1993; Schreeg et al., 2005).

Variation in the distribution of vegetation on earth corresponded to past climate change (DeHayes et al., 2000). It is expected that future climate change will have a major impact on the forest composition (Condit, 1998) and cause shifts in vegetation distribution (Hanson and Weltzin, 2000). Climate change is expected to cause an increase in global temperature, resulting in greater evaporation and increased soil moisture deficits. An increase in global temperatures is therefore expected to increase the severity or duration of drought conditions, which will likely shift vegetation patterns (Graham and Grim, 1990; Hanson and Weltzin, 2000).

It has been projected that a mean temperature increase of about 3-5 ^{o}C is likely to occur in the US within the next 100 years and a 2-3 ^{o}C increase in the eastern US by 2100 (IPCC, 2001 and NAST, 2001). NAST (2001) also found that precipitation is very likely to increase in some areas of the US. However, this is likely to be offset by greater evaporation rates brought about by higher air temperatures (NAST, 2001). Drought is a common occurrence in the longer historical record (Cook et al., 1988, Leuscher et al., 2001). For example, southeastern mixed forests experienced prolonged dry spells and above normal temperatures in 1995 (Baldocchi, 1997) and 2007.

Drought is a major abiotic stress that plants encounter and can be responsible for the inhibition or delayed seed germination, poor seedling growth, and establishment. Drought has been associated with decreased tree growth, increased tree die-back, and mortality in temperate forests (Allen and Breshears, 1998; Villalba and Veblen, 1998) and in tropical

forests (Condit, et al., 1995; Fensham and Holman, 1999; Williamson, 2000; Gilbert et al., 2001; Engelbrecht et al., 2005). Drought-induced mortality can cause shifts in vegetation distribution and such shifts have been documented in the pinyon-juniper/ponderosa ecotone (Allen and Breshears, 1998) and pinyon-juniper woodland (Mueller et al., 2005).

Tree die-back and tree mortality can be caused by drought-induced cavitation (Rice et al., 2004 and Mueller et al., 2005), which leads to tissue desiccation and death. Cavitation is the abrupt transition of xylem water from metastable liquid state to vapor, resulting from the large negative pressures induced by water stress (Tyree and Dixon, 1986; Tyree et al., 1994). One mechanism by which cavitation occurs during drought (Tyree and Dixon, 1986) is by air-seeding (Zimmerman, 1983). Because of the high tension in water-filled vessels, air is pulled from neighboring vessels through the pores located in the pit membrane of the primary cell walls. Once air enters the vessel, it disrupts the cohesion of water molecules and the water column breaks and retracts, filling the vessel first with water vapor. Ultimately, as air comes out of solution from the surrounding water, the vessel completely fills with air (Kozlowski and Pallardy, 1997). Xylem cavitation is a major potential problem in plants (Dickison, 2000), causing xylem dysfunction (Tyree and Sperry, 1989) and disrupting the hydraulic pathway from the soil to the leaf (Zimmerman, 1983). The resulting increased resistance to water flow in the sapwood can eventually limit plant growth and lead to death of the plant (Tyree and Sperry, 1988), since cavitation reduces the ability of plants to transport water to the leaves and induces greater water stress, and reduces transpiration and photosynthesis (Pockman et al., 1995 and Sperry, 1995).

Future change in landscape hydrology due to climate change is likely to have an impact on species ranges, species composition, and ecosystem function (Engelbrecht et al., 2007). Future shifts in species composition of a plant community in response to climate change may depend on their physiological traits that allow them to germinate, grow, establish, and survive under water stress.

This study focused on woody plant species found in the SE US mixed forest dominated by broad-leaved deciduous trees and is classified by Delcourt and Delcourt (1981) into 3 vegetation types: oak-hickory-southern pine (*Quercus-Carya-Pinus*), southern pine (*Pinus*), and cypress-gum (*Taxodium-Liquidambar*) forests. Deciduous forest in the SE US is composed of tree genera that are diverse and widespread, both geographically and ecologically (Barnes, 1991). For example, the oaks (*Quercus*), many of which are xerophytic (*Q. incana, Q. prinus, Q. marilandica*), are also adapted to mesic conditions (*Q. nigra, Q. palustris,* and *Q. phellos*) whereas *Q. alba, Q. rubra,* and *Q. velutina* are species that are widely distributed (Barnes, 1991) along gradients of water availability. On the other hand, the hickories (*Carya*), which are typically associated with oaks, are also abundant and widespread on xeric and mesic sites (Barnes, 1991). *Carya glabra* dominates the xeric sites, while *C. aquatica* and *C. cordiformis* dominate the hydric and mesic sites, respectively. Other genera that dominate the mesic sites are *Acer, Fagus,* and *Tilia* (Barnes, 1991). In the SE US, xeric forest is typically dominated by drought-tolerant species. Conversely, mesic forest is dominated by drought-sensitive hardwood forest species (Braun, 1950; Schafale and Weakley, 1990). Although the SE US mixed forest is not considered to be a water-limited ecosystem, such distribution of species suggests that water availability does exert a strong

control on the success of the woody plants in their current distribution.

Woody plants form a conspicuous cover and important component in an ecosystem. The environmental tolerances of woody species are important in vegetation models to predict climate-vegetation interaction at local, regional and global scales (Williams and Snyder, 2003). It is important to study distribution of plants in relation to water availability, since water availability is one of the factors that restrict terrestrial production at the global scale, though it is the most abundant resource on earth (Lambers, et al., 1998). Understanding the factors, which may restrict the species from its distribution, its consequences on plant species survival, and the physiological adjustments that the plant make could be a way of explaining natural patterns in productivity of a natural ecosystem.

Attempts to understand the distribution of plant species in different habitats have focused mainly on relating the distribution of plant species to edaphic, hydrological or climatic factors. Little is known on the appropriate physiological traits that enable the plant species to survive in its physical environment. Broadening our knowledge and understanding the ecophysiological factors that determine the current distribution of plant species or vegetation along a water availability gradient is an essential step in predicting how the global distribution of vegetation is likely to respond to changes in climate.

The objective of this study was to determine and understand the ecological and physiological basis underlying the distribution of woody plants along water availability gradients of eastern US mixed forest. This was done by testing hypotheses on the hydraulic properties and water relations of woody plants, the germination, survival and growth of established individuals as well as their differences in response to water and nutrient

availability. I hypothesized the following: a) xeric species have greater ability to germinate under low water potential, b) xeric species exhibit greater survival under extreme drought stress, c) seedling performance such as growth, stomatal conductance, and electron transport rate of xeric species is less sensitive to chronic drought stress and is more sensitive to flooding, and d) xylem of xeric species is less vulnerable to cavitation relative to mesic species.

Thesis Summary

Chapter two describes experiments testing the effect of substrate water potential on the germination and osmotic adjustment of four congeneric species pairs, each composed of one xeric and one mesic forest species. I used different concentrations of polyethylene glycol 8000 (PEG) to simulate various substrate water potentials. I found that the ability to germinate under drought did not differ consistently between xeric and mesic species, nor did xeric species consistently exhibit greater capacity for osmotic adjustment.

I suspected that drought may continue to act as a filter to plant survival after germination and initial seedling establishment. In chapter three, I performed a dry-down experiment to evaluate the drought tolerance of seedlings of three congeneric pairs of xeric and mesic species. Complete and partial stem survival rates were determined after the plants were re-watered subsequent to drought exposure. I observed that different wilt stages closely corresponded to the different leaf water potentials of the species and could be used as a good visual tool in assessing the stress level of the plants (Engelbrecht et al., 2007). Overall, xeric species had higher stem survival under drought than mesic species.

Chapter four describes the relative growth performance responses to water and nutrient availability between xeric and mesic seedlings. A factorial experiment (2 species types x 3 water levels x 2 nutrient levels) was performed on each of the four congeners used. Stomatal conductance (g_s), electron transport rate (ETR), and root and shoot biomass were measured. I observed that growth performance of xeric and mesic species was not only controlled by drought, but, by flooding as well. Xeric and mesic species had contrasting biomass allocation patterns and tolerance to resource limitation (i.e water and nutrients). Species adapted to low-resource environments commonly have low growth rates. Xeric species had lower whole-plant biomass than mesic species at the end of the experiment. The low growth of xeric species is a consequence of a drought-tolerant strategy. Xeric species had a higher root:shoot ratio, higher coarse root mass ratio and less reduction in stomatal conductance under drought than mesic species. Such traits may allow the xeric species to tolerate low water and low nutrient availability. Mesic species, on the other hand, showed flood tolerance, as demonstrated by higher stomatal conductance and higher whole-plant biomass under flooding, relative to xeric species. It can be deduced from this study that traits for drought tolerance, which are more strongly manifested by xeric species than mesic species, seem to influence the exclusion of mesic species from xeric sites. The traits for flood tolerance were more apparent in mesic species relative to xeric species. Such traits may allow mesic species to successfully occur in mesic sites than xeric species.

Chapter five compares hydraulic traits and cavitation resistance in xeric and mesic species. I suspected that drought still continues to act as a filter in the sapling stage in the life history of a plant. Hence, I used saplings of seven congeneric species pairs to test whether

xeric species are more able to withstand drought than mesic species. I measured xylem specific conductivity (K_s), leaf specific conductivity (K_l), Huber value (HV= sapwood area/leaf area), wood density, and specific leaf area (SLA). Vulnerability curves were also determined using the air-injection method (Cochard et al., 1992). I found a greater resistance to xylem cavitation among xeric species than mesic species. I did not find any evidence of trade-off between specific conductivity (K_s) and cavitation resistance. There was only a significant negative correlation between K_s and wood density across all species; neither of these traits differed significantly between xeric and mesic species. There were no significant correlations between wood density and K_l, HV, and SLA across all species.

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CHAPTER II

Substrate water potential constraints on the germination ability and osmotic adjustment of xeric and mesic Southeastern US mixed forest species

INTRODUCTION

Low soil water potential is a major abiotic stress that plants encounter. Low water availability (more negative water potential) leads to drought-stressed conditions, which can be responsible for inhibition or delayed seed germination and seedling establishment. Successful germination and seedling establishment are primary determinants of species distribution.

This study focused on woody plant species found in the SE US mixed forest dominated by broad-leaved deciduous trees and is classified by Delcourt and Delcourt (1981) into 3 vegetation types: oak-hickory-southern pine (*Quercus-Carya-Pinus*), southern pine (*Pinus*), and cypress-gum (*Taxodium-Liquidambar*) forests. Deciduous forest in the SE US is composed of tree genera that are diverse and widespread, both geographically and ecologically (Barnes, 1991). For example, the oaks (*Quercus*), many of which are xerophytic (*Q. incana, Q. prinus, Q. marilandica*), are also adapted to mesic conditions (*Q. nigra, Q. palustris,* and *Q. phellos*), whereas *Q. alba, Q. rubra,* and *Q. velutina* are species that are widely distributed (Barnes, 1991) along gradients of water availability. On the other hand, the hickories (*Carya*), which are typically associated with oaks, are also abundant and widespread on xeric and mesic sites (Barnes, 1991). *Carya glabra* dominates the xeric sites, while *C. aquatica* and *C. cordiformis* dominate the hydric and mesic sites, respectively. Other genera that dominate the mesic sites are *Acer, Fagus,* and *Tilia* (Barnes, 1991). Although the SE US mixed forest is not considered to be a water-limited ecosystem, such

distribution of species suggests that water availability does exert a strong control on the success of the woody plants in their current distribution. The confinement of the xeric species and mesic species to their respective edaphic conditions could be determined by a physiological filter such as seed germination.

Seed germination and early seedling growth are two very important stages in the establishment of any plant species that are significantly influenced by water availability (Kozlowski and Pallardy, 1997; Turk et al., 2004). Seed germination is controlled by both internal and external factors and water is one of the most important external factors controlling it. The availability of adequate water is necessary during the first stage of germination (imbibition), in breaking dormancy and for the cell division and cell elongation to proceed. Germination and early seedling growth are among the most drought-sensitive of the life history stages because they are dependent upon substrate water potential in the surface soil, which is the first to dry during drought. Differences in the ability of seeds to germinate under water stress between xeric and mesic species may contribute to the separation of species along water availability gradients (Oberbauer and Miller, 1982). In addition, the different degree of soil moisture tolerances may inhibit or delay germination (Bewley and Black, 1994; Eneas-Filho et al., 1995; Swagel et al., 1997; Uniyal and Nautiyal, 1998; Murillo-Amador et al., 2002).

Plants that are adapted to drought conditions would germinate, grow, and survive by maintaining cell turgor through osmotic adjustment (OA). Osmotic adjustment (OA) is a mechanism that enables the plants to acclimate to drought conditions by regulating their osmotic potential with a net increase in cell solute concentration and turgor pressure

14

(Morgan, 1984, Kramer and Boyer, 1995, Babu et al., 1999). This mechanism allows plants to increase turgor pressure that allows continued cell elongation (Nguyen and Lamant, 1989; de Lacerda et al., 2003; Kusaka et al., 2005) even under drought stress, and therefore germination (Morgan, 1984 and Kusaka et al., 2005) and growth. The greater the capacity for solute accumulation in roots, the greater is the capacity for plants to take up water from drying soil and the longer these plants are able to delay stomatal closure and continue photosynthesizing (Hsiao, 1973; Nguyen and Lamant, 1989).

Drought tolerance of poplar (Gebre et al., 1994), oak (Epron and Dreyer, 1996), and pines (Nguyen and Lamant, 1989 and Meier et al., 1992) has been attributed to osmotic adjustment (OA). However, most of these studies were focused on leaf OA (Gebre et al., 1994; Epron and Dreyer, 1996; Meier et al., 1992) except for the root OA in pines (Nguyen and Lamant, 1989). Abrams (1988) observed that trees growing in xeric habitats have leaf osmotic potentials at full turgor ranging from -1.51 to -3.25 MPa, lower than those from mesic habitats which ranged from -0.72 to 2.0 MPa. In addition, drought-tolerant white oak and chestnut oak had lower osmotic potentials than the drought-sensitive dogwood and beech (Abrams, 1988). Among pine provenances, OA was higher in drought-adapted than a drought-sensitive provenance (Nguyen and Lamant, 1989). Greater OA was also found in agronomic crops grown under drought conditions and upland sites than in irrigated conditions or wetland sites (Babu et al., 1999; Bajji et al., 2001; Kusaka et al., 2005). In contrast, Turner et al. (1986) found a greater OA in wetland cultivars than in upland cultivars due to greater diurnal change in water potential observed in wetland cultivars compared to upland cultivars. Studies on leaf OA may provide little insight for root OA, which should be

15

of greater importance for understanding the effects of drought on seed germination and early seedling growth. Expanding plant tissues, such as a growing root tip, rely on turgor pressure for continued growth, and therefore growth is very sensitive to drought. Capacity for OA should be critical for permitting rapid germination and early seedling growth, which are extremely critical for successful establishment in habitats where water availability is unreliable. Producing deep roots quickly under moderate drought will be important for survival if drought conditions intensify.

This study focused on the effect of water stress on seed germination of four congeneric species pairs, each composed of one xeric and one mesic forest species. I determined differences in seed germination ability, osmotic adjustment, and radicle elongation rates under drought stress to provide a basis for predicting future seedling establishment, survival and distribution of xeric and mesic species in SE US mixed forest. I hypothesized that: a) drought would reduce the germination and seedling growth of mesic species more than the xeric species, and b) xeric species have greater ability for osmotic adjustment under drought than do mesic species.

MATERIALS AND METHODS

Study species

Congeneric species pairs of *Fraxinus, Quercus, Vaccinium,* and *Nyssa* were selected for study (Tables 1 and 2) to ensure phylogenetic independence, which is an important condition for making inferences in comparative studies and improving the statistical power of comparison between two groups (Ackerly, 1999). In each pair, one species is adapted to xeric conditions, while another is adapted to mesic conditions (Radford et al., 1964 and Schafale and Weakley, 1990). I used xeric and mesic in relative terms, wherein xeric species are those common in well-drained sites while mesic species are those which are more frequent in moist sites. I used Radford et al. (1964) and Schafale and Weakley (1990) to choose study species that are typical of xeric or mesic forests. The *Fraxinus, Quercus,* and *Nyssa* seeds were obtained from Sheffield's seed company, who collected seeds from the Southeastern US. Vaccinium seeds were obtained from the Department of Horticultural Science, NCSU.

Table 1 shows the wetland indicator status of the study species. *Fraxinus americana* is a facultative upland species and is typical of rich upland soil (Lance, 2004), which is dry and well-drained (Radford et al., 1964; Burns et al., 1990; Schafale and Weakley, 1990). Natural stands of *Fraxinus pennsylvanica*, a facultative wetland species, are almost completely confined to moist bottomlands (Burns et al., 1990), swamps, and along streams (Lance, 2004). Another facultative upland species is *Quercus alba*, which is found on either sandy plains, gravelly ridges, rich uplands and well-drained soils, but is common in driest shallow soil (Burns et al., 1990; Schafale and Weakley, 1990). Moreover, *Q. alba* grows in association with other tree species such as *Liriodendron tulipifera, F. americana,* and

17

Table 1. Wetland indicator status of study species based on USDA NRCS Plants
Database, USFWS wetland indicator status (http://plants.usda.gov/wetland.html,
accessed May 20, 2008).

Species	Wetland indicator status (Region 2-SEUS)*
Fraxinus americana	FACU
Fraxinus pennsylvanica	FACW
Quercus alba	FACU
Quercus nigra	FAC
Vaccinium stamineum	FACU
Vaccinium corymbosum	FACW
Nyssa sylvatica	FAC
Nyssa aquatica	OBL

* **OBL** (obligate wetland)- Occurs almost always (estimated probability 99%) under natural conditions in wetlands; **FACW** (facultative wetland)- Usually occurs in wetlands (estimated probability 67%-99%), but occasionally found in non-wetlands; **FAC** (facultative)- Equally likely to occur in wetlands or non-wetlands (estimated probability 34%-66%); **FACU** (facultative upland)- Usually occurs in non-wetlands (estimated probability 67%-99%), but occasionally found on wetlands (estimated probability 1%-33%); **NI** (no indicator)- Insufficient information was available to determine an indicator status; +- the positive sign indicates a frequency toward the higher end of the category (more frequently found in wetlands)

N. sylvatica, which also occurs in well-drained sites. Although *Quercus nigra* appears on a wide variety of sites, which ranges from wet bottomlands to well-drained uplands, this species is commonly found and grows very well in moist soils such as those along streams (Radford et al., 1964 and Burns et al., 1990). *Vaccinium stamineum* is typical in dry upland sites, while *V. corymbosum* occurs commonly in moist to boggy soils (Schafale and Weakley, 1990; Lance, 2004). *Nyssa sylvatica*, which is considered to be facultative, grows very well on well-drained light textured soils and on drier upper slopes and ridges (Burns et al., 1990; Schafale and Weakley, 1990). Conversely, *N. aquatica*, which is an obligate wetland species, can also grow in moist sites, which remain near saturation level (Burns et al., 1990). There may be an overlap in the distribution of each pair, but relatively, one species is more adapted to well-drained site and the other to moist site.

Germination experiment

This experiment was performed to compare the ability of xeric and mesic species to germinate under drought stress. Prior to germination, the seeds of *Fraxinus americana, F. pennsylvanica, Vaccinium corymbosum, Nyssa sylvatica,* and *N. aquatica* were stratified as whereas no treatment was required by *Quercus alba*, *Q. nigra,* and *V. stamineum,* as recommended by USFS (1974). Seeds were stratified by placing in moist sand in black plastic trays or on pre-soaked blotter in Petri dishes for the *Vaccinium corymbosum*. The stratification requirements are as follows:

Table 2. Stratification requirements for the species used in the germination experiment.

Species and Species type	Warm Period Temperature (^{o}C)	Warm Period (Days)	Cold Period Temperature (^{o}C)	Cold Period (Days)
F. americana L. (xeric)	30/20	30	4	60
F. pennsylvanica Marsh. (mesic)	25	60	4	60-90
Q. alba L. (xeric)	none		none	
Q. nigra L. (mesic)	none		none	
V. stamineum L. (xeric)	none		none	
V. corymbosum L. (mesic)	none		4	60
N. sylvatica Marsh. (xeric)	none		5	30-90
N. aquatica L. (mesic)	none		5	30-90

Prior to setting up the germination experiment, the seeds were surface sterilized with 0.6 % sodium hypochlorite solution and rinsed thoroughly with sterile deionized water. Control seeds were germinated on blotter paper in Petri dishes which were kept moistened with sterilized deionized water. Treated seeds were also placed on blotter paper in Petri dishes and moistened with sterilized polyethylene glycol 8000 (PEG) solutions of different concentrations to simulate various soil water potentials of -0.2 MPa, -0.4 MPa, -0.6 MPa, and -0.8 MPa.

19

Ten replicates of ten seeds each were used for each treatment for each species. The Petri dishes containing the seeds were placed in a growth chamber and maintained at 30 $^\circ$C (day) and 20 $^\circ$C (night) with 8 hours daylight and 16 hours of dark period. The number of germinated seeds was recorded daily until no further germination occurred for at least 7 days. To account for differences in viability across species, relative final germination percentage was calculated as follows:

Relative final germination (%) = $\dfrac{\text{final germination (\%) in each species per water potential}}{\text{mean final germination (\%) of the control}}$

Ten representative seedlings were randomly chosen and marked from each treatment to measure root elongation. Root length was measured daily for 6 days using digital calipers (Fisher scientific 14-648-17).

Osmotic adjustment

Measurements of osmotic potential were taken on each of ten primary root tips of seedlings that germinated at each water potential (0 MPa, -0.2 MPa, -0.4 MPa, -0.6 MPa, -0.8 MPa). The apical 10 mm of the primary root was removed and re-hydrated for 10 minutes in deionized water and was frozen at -20 $^\circ$C. After 2 days, the frozen root was thawed at room temperature and centrifuged at 10,000 rpm for 20 minutes. Ten µl of cell sap from the root tip was collected and placed in a filter paper disc. Osmotic potential was measured with a vapor pressure osmometer (VAPRO 5520, Wescor, Inc., Logan UT, USA). Osmotic adjustment (OA) is calculated as the difference in osmotic potential of roots at full turgor between the control and stressed plants.

OA = ψ_π (100) control - ψ_π (100) stressed seedling

20

where: ψ_π (100) = osmotic potential at full turgor

Osmotic adjustment measurement was not possible for *Vaccinium* spp. because it was not possible to extract sufficient sap from their very small primary root.

Turgor potential for each root grown in particular substrate water potential was calculated based from the osmotic potential measurement using the following equation:

$\psi_p = \psi_{total} - \psi_\pi$

where: ψ_p – root turgor potential in a specific substrate water potential

ψ_{total} – substrate water potential

ψ_π – root osmotic potential

Statistical analysis

Statistical analyses were performed using JMP version 6.0 (SAS institute, Cary, NC, USA). Relationships between germination, osmotic adjustment, and substrate water potential, as well as the correlation between the root elongation and turgor potential and root elongation versus substrate water potential were evaluated with analysis of covariance (ANCOVA) using species type (xeric vs. mesic) as a categorical variable and substrate water potential as covariate.

RESULTS

Relative final germination rates declined with substrate water potential for each congeneric species pair of *Fraxinus, Nyssa, Vaccinium,* and *Quercus* examined (Fig. 1, $p<0.0001$). The low germination was more apparent when the levels in water potential were below -0.4 MPa though the reduction in germination percentage was also evident even at -0.2 MPa.

There was a significant interactive effect between species type and substrate water potential in all species (Fig. 1A, C-D, $p<0.0001$ for all congeners), except *Nyssa* (Fig. 1B, $p=0.452$) although the trends are contrasting. The interaction effect reflects the relative germination rate response to drought between the xeric and mesic species. There was no consistent tendency for mesic species to be more sensitive to water stress. While the mesic species of *Fraxinus* showed a significantly higher relative germination percentage than their xeric species pair (Fig. 1A, $p<0.0001$), *Vaccinium* exhibited the opposite trend (Fig. 1C, $p<0.0001$). Although the xeric and mesic *Quercus* spp. responded differently to water potential (Fig. 1D, $p<0.0001$), the direction of the difference was not consistent. However, we found a sharp decline in the germination of mesic *Q. nigra* at -0.6 MPa relative to xeric *Q. alba,* but at -0.8 MPa, *Q. alba* had the lower germination. The relative germination rates of xeric and mesic *Nyssa* spp. did not vary significantly (Fig. 1 B, $p=0.252$) in response to decreasing water potential.

It was also evident that germination was delayed by drought stress (Fig. 2A-C, $p<0.0001$), except for *Quercus* (Fig. 2D, $p=0.204$). There were significant differences in germination time between the xeric and mesic species within genera (Fig. 2A-D, $p<0.018$),

but the direction of this difference was not consistent. The only significant interactions between species type and different levels of water stress were in *Fraxinus* spp. and *Nyssa* spp. (Fig. 2A, p=0.027 and B, p<0.001, respectively).
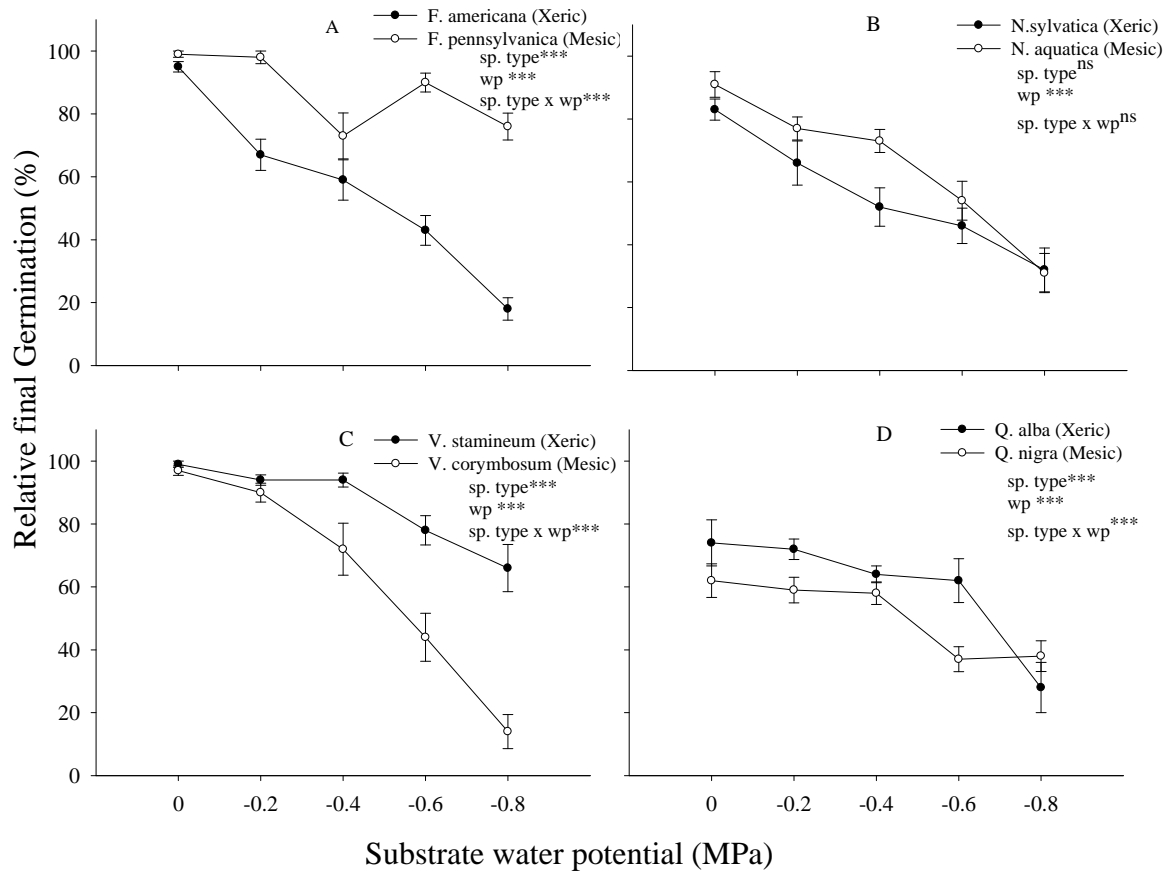
Figure 1. Relative final germination percentage (mean + SE) of congeneric species of A) *Fraxinus,* B) *Nyssa,* C) *Vaccinium* and D) *Quercus* grown in various water potentials. Relative final germination percentage is the mean final germination (%) observed in each species grown in various substrate water potential relative to mean the final germination (%) of the control. Symbols for comparison: sp. type-species types (xeric and mesic spp.), wp-water potential, ns- not significant, * p<0.05, ** p<0.01, *** p<0.005
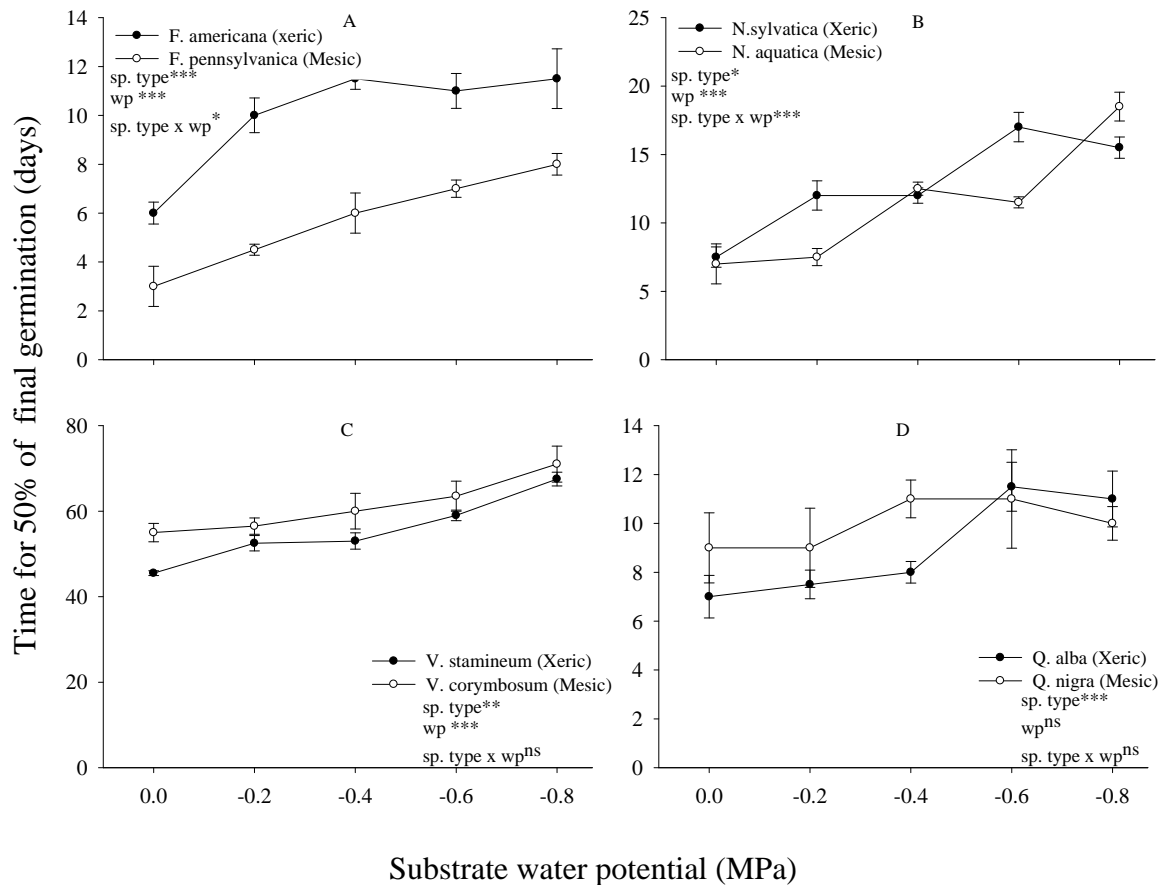
Figure 2. Mean number of days it takes to reach fifty percent of final germination in each congeneric species pair of A) *Fraxinus,* B) *Nyssa,* C) *Vaccinium* and D) *Quercus* grown in various water potentials. The number of days recorded was taken when 50% of final germination was observed in each treatment in all species examined. Symbols for comparison: sp. type-species types (xeric and mesic spp.), wp-water potential, ns- not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.005$

The roots of xeric and mesic species did not significantly differ in their OA except for *Quercus* (Fig. 3A-B, $p>0.175$; 3C, $p<0.0001$). OA significantly increased with an increase in levels of water stress across species (Fig. 3 A-C, $p<0.0001$). We only found a significant interaction between species and substrate water potential in *Quercus* (Fig. 3C, $p=0.036$).
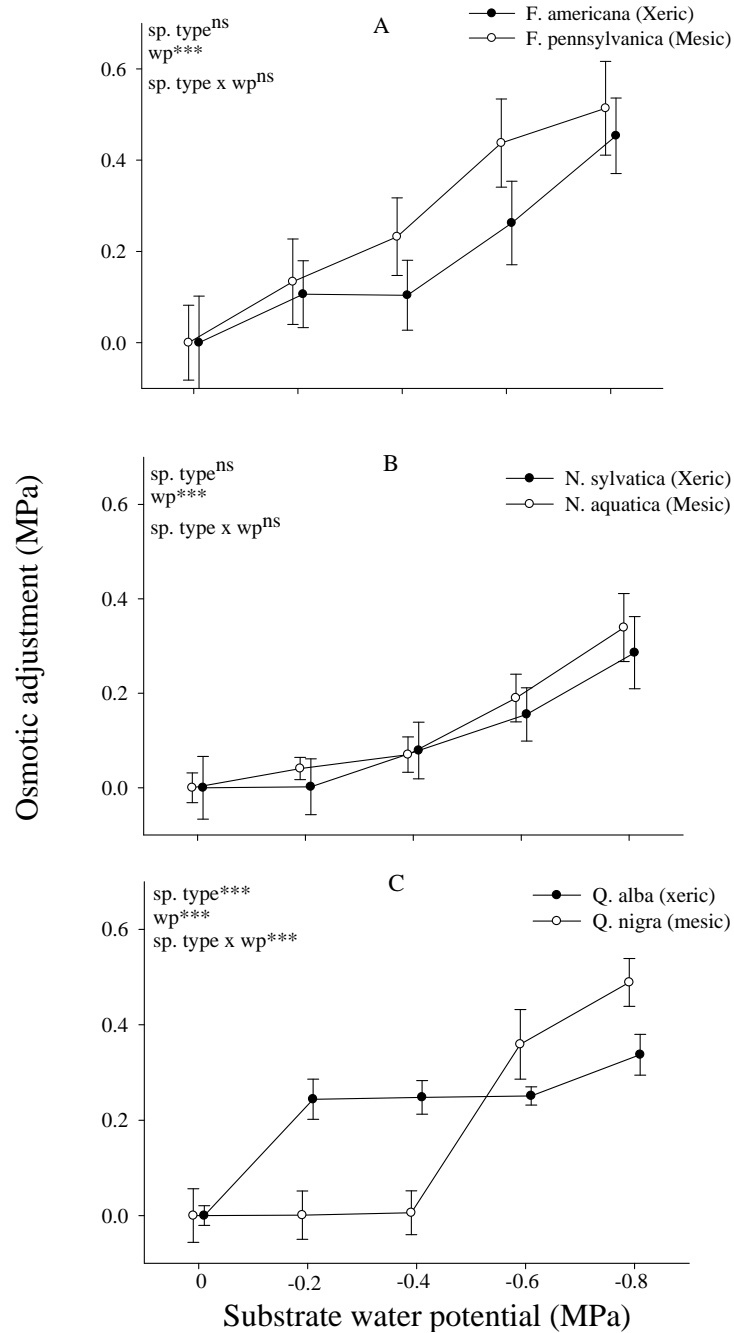
Figure 3. Osmotic adjustment (OA) in A) *Fraxinus* spp., B) *Nyssa* spp. and C) *Quercus* spp. under different levels of substrate water potential (MPa). Standard error for each point in was calculated using the following equation: $SE(X-Y_i) = \sqrt{(SE(X))^2 + (SE(Y_i))^2}$; where: $SE(X)$ is the standard error for osmotic potential at 0 MPa (control) and $SE(Y_i)$ is the standard error of osmotic potential at -0.2 to -0.8 MPa, respectively. Symbols for comparison: sp. type-species types (xeric and mesic spp.), wp-water potential, ns- not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.005$

There was a significant decline in the relative root elongation rate with decreasing substrate water potential across species (Fig. 4 A-D, p<0.0001 and Fig. 5 D, p=0.018). In each congeneric pair, xeric and mesic species significantly differed in their relative root elongation rates (Fig. 4 A-B & D, p<0.038), except *Vaccinium* (Fig. 4C, p=0.874). On the other hand, a positive correlation between the root length and relative rate of root elongation versus root cell turgor across all species was observed (Fig. 5A, p=0.042 and B, p<0.001). There were no significant differences between xeric and mesic species when I tested for correlation between root length and relative root elongation rate with turgor potential.
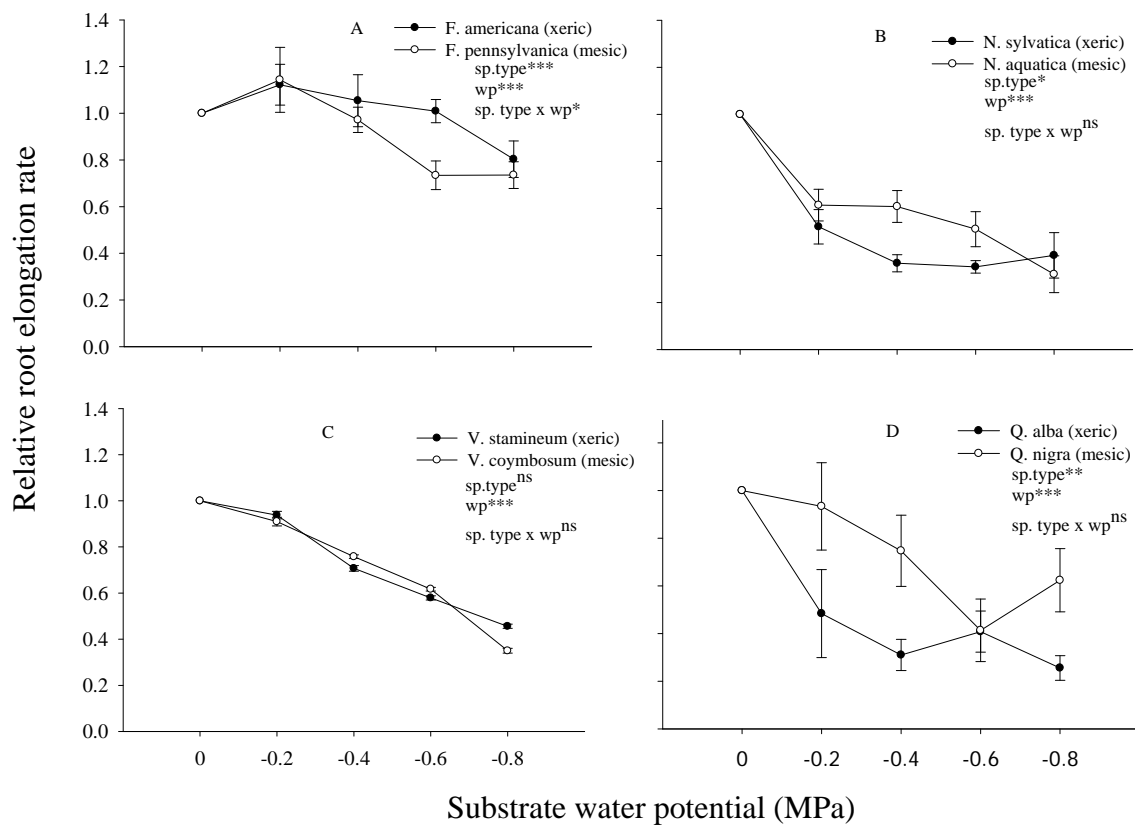
Figure 4. Relative root elongation rate 6 days after germination (mean +SE) of congeneric species of A) *Fraxinus*, B) *Nyssa*, C) *Vaccinium,* and D) *Quercus* grown in various water potentials.
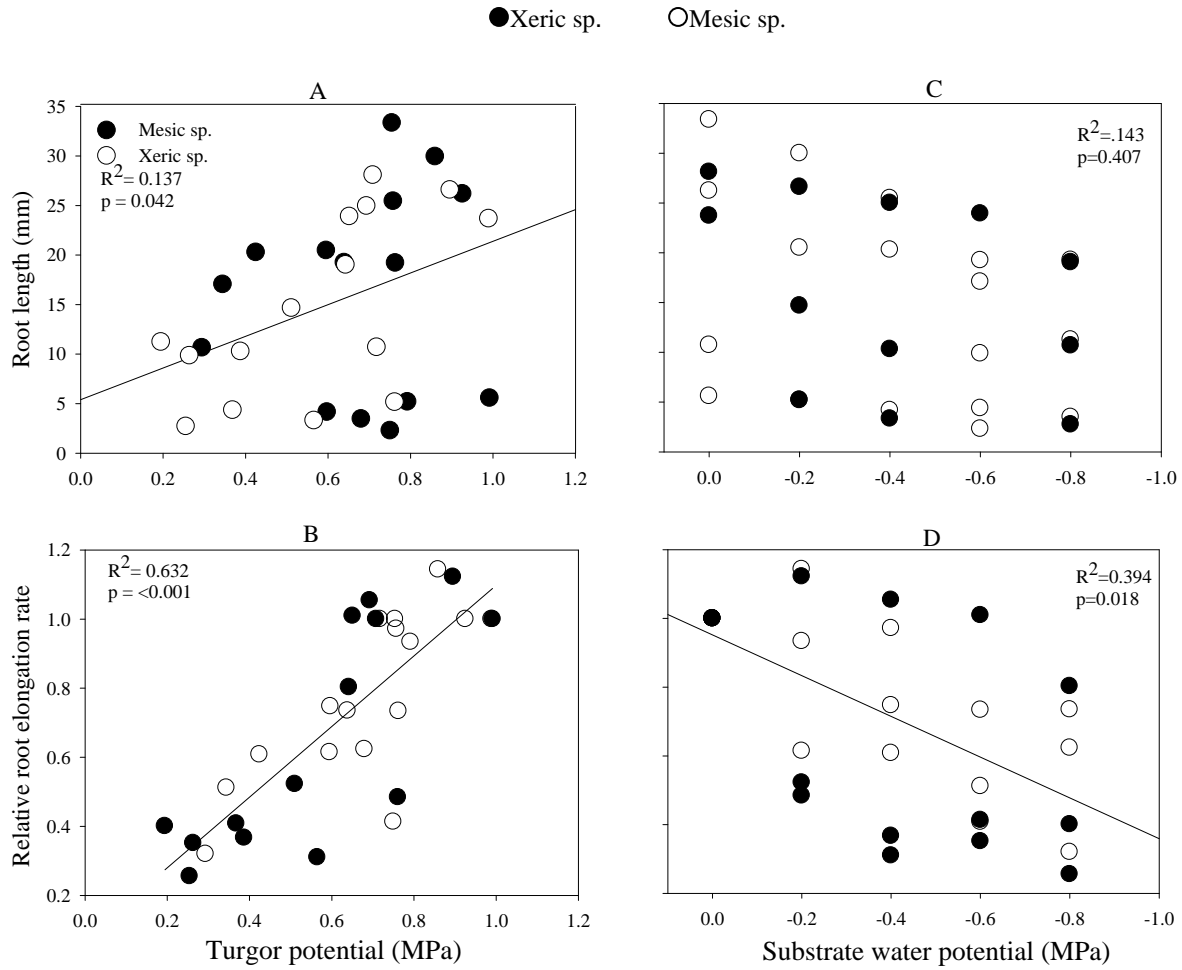
Figure 5. Correlation between (A & C) absolute root length (mm) and (B & D) relative root elongation rate taken 6 days after initial germination and root turgor potential (MPa) across all species examined. Rate of root elongation = (final root length (mm)-initial root length)/maximum root length of the control. Turgor potential = substrate osmotic potential (MPa) minus the root osmotic potential (MPa). There was no significant difference between xeric and mesic species in any cases presented. Each point represents the mean for one species in one treatment.

DISCUSSION

Seed germination and early seedling growth may only occur when a specific level of substrate moisture is available. Water availability may limit the emergence, growth and survival of seedlings, which may reduce the establishment of some species in an area (Villalobos and Pelaez, 2001). The results indicate that water availability is influential in the germination across all species pairs. There was a reduction in the final germination percentages in all species especially at a very water-stressed condition and these findings are similar to the findings of Uniyal and Nautiyal (1998), de Souza et al. (1999), Duan et al. (2004), Socolowski and Takaki (2004), and Turk et al. (2004). There was evidence for differences across species in the ability to germinate under drought, but contrary to predictions, this ability did not differ consistently between xeric and mesic species (Fig. 1). It seems that high final germination percentage is not a common occurrence for all the xeric species tested especially under drought stress. The inconsistent trends in the ability of xeric and mesic species to germinate under osmotic stress may suggest that there may be other factors related to water availability that could indirectly cause the species segregation at the germination stage (Oberbauer and Miller, 1982) such as duration of favorable moisture period, oxygen levels, and soil pH. The lack of tendency for xeric species to always germinate better under drought stress may reflect the fact that drought avoidance is another drought resistance strategy other than drought tolerance as manifested by delaying germination. Seeds may be ready for the first phase of germination (imbibition) when conditions will be favorable (i.e. water is available) for germination, however, these conditions may rapidly become unfavorable for the subsequent germination stages.

28

Thus, some seeds may undergo dormancy until the next prolonged period of favorable conditions (Fitter and Hay, 2002). For some seeds to have high germination success under low water potentials or drier habitats, drought stress adaptation either consists of avoiding drought or tolerating it.

More negative substrate water potential indicates low water availability. These increased levels of water stress imposed on seeds reduced germination success across species. Changes in the levels of water stress reduce water uptake as well as the enzymatic activity needed to break dormancy (Delachiave and Pinho, 2003; Socolowski and Takaki, 2004). The elevated drought may limit the imbibition of water (first phase in germination) in seeds of *F. americana* (xeric sp.) and *V. corymbosum* (mesic sp.) more than the *F. pennsylvanica* (mesic sp.), *V. stamineum* (xeric sp.), *Q. nigra* (mesic sp.) (Fig. 1 A,C-D), respectively. Inhibition of the imibibition of water may deactivate the synthesis of enzymes that function in the breakdown of storage materials in seeds, which are utilized by the embryo to initiate germination (Kozlowski and Pallardy, 1997). Thus, increased levels of drought may reduce germination success due to the deactivation of the initiation phase of germination. These findings support the initial hypothesis that germination rate is significantly reduced by drought stress. The high germination of xeric species at various levels of water-stressed conditions was only observed in *Vaccinium* (Fig. 1 C). The trend of high germination of xeric species versus the mesic species under water stress was observed by Oberbauer and Miller (1982) and Claus and Venable (2000). On the other hand, mesic *Fraxinus* germinated more than the xeric *Fraxinus* (Fig. 1 A) across all drought conditions. The mesic *Fraxinus* may need to germinate more seeds than xeric *Fraxinus* in order to

establish a pool of seedlings because the later stages of their life cycle may be more vulnerable to drought stress. There was a trend showing an abrupt decline in the germination success of *Q. nigra* (mesic sp.) at a lower level of drought stress compared to *Q. alba* (xeric sp.) (Fig. 1 D).

Remaining dormant or rapid germination could be adaptive mechanisms under water-stressed conditions and are efficient ways to successfully get established in areas with harsh environments (Naido and Naicker, 1992; Clauss and Venable, 2000). The low germination percentage and delayed germination in xeric *Fraxinus* and *Nyssa* (Fig. 1A & B, Fig. 2 A & B, respectively) could indicate signs of dormancy as a mechanism to avoid drought or spread out the risk. On the other hand, the high germination of xeric *Vaccinium* and *Quercus* (Fig. 1 C & D) may allow seedlings to establish under modest drought. The rapidity of germination may be an important survival strategy of xeric *Vaccinium* and *Quercus* under drought stress to enable them to produce extensive roots before drying of the substrate occurs. Dormancy as an avoidance strategy to drought stress may be used by xeric *Fraxinus* as well as for some xeric species in their success in habitats exposed to drought conditions. In field conditions, the seeds of xeric species may be retained in the soil and remain dormant until adequate moisture is already available. Seeds of some xeric species are innately dormant initially when exposed to drier habitats due to the uncertainty of favorable conditions for germination (Fitter and Hay, 2002). The favorable conditions that the seeds are exposed to may rapidly become unfavorable for the subsequent growth of seedlings (Fitter and Hay, 2002). So, delaying germination may be advantageous for the survival and establishment of xeric species exposed to periodic drought conditions. The greater germination percentage and

faster germination time of some mesic species is an advantageous strategy that enables them to establish a seedling bank especially if their seedling stage would be more vulnerable to water stress in the later stages of seedling establishment. Hence, the ability to germinate in xeric habitats may be dependent on the drought tolerance strategy that the species adapt, so species distribution is not just influenced by the species selection for habitats suitable for their germination.

Osmotic adjustment is known to be a means of drought tolerance (Kusaka et al., 2005). The ability to adjust osmotically under water stress, is a trend observed among all the species tested. All species varied considerably in their OA under increasing osmotic stress (Fig. 3C). However, OA between species types only differs significantly between the xeric and mesic *Quercus* sp. The greater OA of *Q. alba* (xeric sp.) than *Q. nigra* (mesic sp.) corresponds to a higher germination rate up to -0.6 MPa (Fig. 3C and Fig. 1D, respectively). Placing a fully hydrated seed in the media with osmotic potential of -0.8 MPa should result in a less than -0.8 MPa decline in pressure potential, if no osmotic adjustments were to occur. Overall, the average osmotic adjustment was approximately 0.4 MPa, resulting in an equivalent increase in turgor pressure. The net result is that the net decline in turgor pressure is less than half of that which would have occurred if osmotic adjustment were not possible.

Water deficit reduces growth in plants due to a decline in cellular expansion (Kramer, 1983) as a consequence of loss in cell turgor (Hsiao, 1973; Kowlowski and Pallardy, 1997, Neto et al., 2004). In addition, the inhibition of cell expansion due to loss of turgor under extreme water stress will eventually affect the species ability to germinate and get established as a consequence of limited access to available moisture due to reduced root growth (Swagel

et al., 1997; Delachiave and Pinho, 2003; Socolowski and Takaki, 2004; Turk et al., 2004).

Although there was no significant difference in OA between species types except for

Quercus spp., our results indicate that across species (Fig. 4), the capacity for OA to maintain

cell turgor (Kusaka et al, 2005) allows root growth to continue even under drought conditions

(Delachiave and Pinho, 2003). By allowing root cell elongation, OA increases the ability of a

plant to exploit limited water during drought, which in turn enables the roots to sustain cell

turgor and maintain water uptake necessary for survival.

Therefore, we conclude that the ability to germinate under drought conditions cannot

alone account for the observed distribution of the species along a water availability gradient.

Higher osmotic adjustment under water stress is not a trend observed among all xeric

species tested. There could be other strategies for drought tolerance and OA is just one

mechanism, which is not expressed in all xeric species. However, osmotic adjustment

contributed to maintaining the root cell turgor, which enables the plant species to continue

root growth even under water stress.

The relative germination rate results between xeric and mesic species suggest that the

occurrence or survival of a species in its current habitat relies on the species adaptation for

drought resistance. Seeds of adapted species may be ready to germinate at a time when

conditions may still be advantageous for germination, but these conditions may rapidly

become very unfavorable for the subsequent growth of seedlings. Hence, it is not unexpected

to find seeds of some successful species from xeric sites to be innately dormant for some

time, and not germinating until the start of the next extended period of favorable conditions,

as a means of drought avoidance. Maintaining high germination and high root elongation

rates can allow seedlings to establish despite modest drought and may be associated with a strategy of drought tolerance. Either dormancy or quick germination could be a key to high survival rates of xeric species (see Chapter 3) in dry environments. In the context of global warming due to climate change, the germination ability is only one factor determining future species distribution. Other morphological, ecological, and physiological factors such as regulation of stomatal conductance, development of deep root systems, seed dispersal, development of wood with high density, and resistance to xylem cavitation, play an equally important role in the seedling establishment and survival of the species. These factors are also crucial in determining the potential shift of species distribution.

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CHAPTER III

Drought Tolerance of Xeric and Mesic Southeastern US Mixed Forest Species

INTRODUCTION

Increase in drought frequency and intensity due to global climate change may lead to changes in floristic composition (Condit, 1998) and cause a shift in vegetation distribution (Graham and Grim, 1990; Hanson and Weltzin, 2000; Tyree et al., 2003). A temperature increase of about 3 to 5 ^{o}C by 2100 due to global warming has been predicted (IPCC, 2001 and NAST, 2001), which would result in greater evaporation and increased soil moisture deficits. For example, southeastern US mixed forests experienced prolonged dry spells and above normal temperatures in 1995 (Baldocchi, 1997) and 2007. Drought is a common occurrence in the longer historical record (Cook et al., 1988 and Leuscher et al., 2001).

Drought, as a form of water stress, is one of the environmental factors that is commonly associated with increased tree die-back (Condit et al., 1995), and decreased tree growth rates. Increased tree mortality and decreased tree growth rates influence shifts in vegetation distribution. Extreme drought may affect the seedling bank of a species, the number of individuals in an area and seedling survivorship, thus, altering landscape vegetation structure and species composition (Edwards and Krockenberger, 2006). Drought stress as indicated by low water potentials and wilting is associated with mortality of seedlings (Condit et al., 1995; Veenendaal et al., 1995). Wilting is the most commonly used indicator of plant stress under drought (Engelbrecht et al., 2007). The seedling stage is the most critical stage commonly impacted by resource shortages, such as low water availability (Prider and Facelli, 2004), because of their less developed and shallow root system that may have limited access to soil water (Poorter and Hayashida-Oliver, 2000; Tyree et al., 2003).

So, the differential ability of seedlings to withstand water stress may influence plant survival, and ultimately species distribution in the landscape. Plants surviving in habitats with low water availability have different strategies to cope with water stress that involves either drought avoidance or drought tolerance strategy. Drought avoidance includes traits, which increase access to water and limit water loss, such as deep roots, early stomatal closure, low cuticular conductance, water storage in plant organs, osmoregulation, and drought deciduousness (Tyree et al., 2003). On the other hand, drought tolerance involves traits that permit continued water uptake, gas exchange, and survival at low water availability. Moreover, increased resistance of xylem to cavitation and the ability of cells and meristematic tissues to survive low water potentials are also traits that correspond to drought tolerance (Tyree et al., 2003). Drought tolerance is a strategy that plants adapt to allow them to maintain survival (Slot and Poorter, 2007) or productivity even during drought (Jones, 1992).

This study focused on woody plant species found in the SE US mixed forest dominated by broad-leaved deciduous trees and is classified by Delcourt and Delcourt (1981) into 3 vegetation types: oak-hickory-southern pine (*Quercus-Carya-Pinus*), southern pine (*Pinus*), and cypress-gum (*Taxodium-Liquidambar*) forests. Deciduous forest in the SE US is composed of tree genera that are diverse and widespread, both geographically and ecologically (Barnes, 1991). For example, the oaks (*Quercus*), many of which are xerophytic (*Q. incana, Q. prinus, Q. marilandica*), are also adapted to mesic conditions (*Q. nigra, Q. palustris,* and *Q. phellos*), whereas *Q. alba, Q. rubra,* and *Q. velutina* are species that are widely distributed (Barnes, 1991) along gradients of water availability. On the other hand,

the hickories (*Carya*), which are typically associated with oaks, are also abundant and widespread on xeric and mesic sites (Barnes, 1991). *Carya glabra* dominates the xeric sites, while *C. aquatica* and *C. cordiformis* dominate the hydric and mesic sites, respectively. Other genera that dominate the mesic sites are *Acer, Fagus,* and *Tilia* (Barnes, 1991). Although the SE US mixed forest is not considered to be a water-limited ecosystem, such distribution of species suggests that water availability does exert a strong control on the success of the woody plants in their current distribution.

I aimed to compare the ability of xeric and mesic species to survive extreme water stress. I hypothesized that xeric species are more likely to withstand drought conditions than mesic species. This study could be used to inform modeling of the future landscape of the SE US mixed forest and in predicting how the global distribution of vegetation is likely to respond to climate change. Ability of a species to tolerate drought has a direct role in determining its distribution (Engelbrecht and Kursar, 2003), especially for the plants growing along a gradient of water availability. Tolerance to drought is likely to be advantageous in surviving future climatic shift in the SE US mixed forest ecosystem.

MATERIALS AND METHODS

Study species

Congeneric species pairs of *Fraxinus* (*F. americana*; *F. pennsylvanica*), *Quercus* (*Q. alba; Q. nigra*), and *Nyssa* (*N. sylvatica; N. aquatica*) were used. In each pair, the first species listed is adapted to xeric conditions, while the second is adapted to mesic conditions. The *Fraxinus, Quercus* and *Nyssa* seeds were obtained from Sheffield's seed company, who collected seeds from the Southeastern US. In this study, the categorization into xeric and mesic species is used on a relative basis, wherein xeric species are more common in well-drained sites while mesic species are more frequent in the moist sites. I used Radford (1964) and Schafale and Weakley (1990) to choose species typical of xeric and mesic forests. Congeneric species were used to ensure phylogenetic independence, which is an important condition for making inferences in comparative studies and improving the statistical power of comparison between two groups (Ackerly, 1999).

Table 1 shows the wetland indicator status of the study species. *Fraxinus americana* is a facultative upland species and is typical of rich upland soil (Lance, 2004), which is dry and well-drained (Radford et al., 1964; Burns et al., 1990; Schafale and Weakley, 1990). Natural stands of *Fraxinus pennsylvanica*, a facultative wetland species, are almost completely confined to moist bottomlands (Burns et al., 1990), swamps, and along streams (Lance, 2004). Another facultative upland species is *Quercus alba*, which is found on either sandy plains, gravelly ridges, rich uplands and well-drained soils, but is common in driest shallow soil (Burns et al., 1990; Schafale and Weakley, 1990). Moreover, *Q. alba* grows in association with other tree species such as *Liriodendron tulipifera, F. americana,* and

Table 1. Wetland indicator status of study species based on USDA NRCS Plants
Database, USFWS wetland indicator status (http://plants.usda.gov/wetland.html,
accessed May 20, 2008).

Species	Wetland indicator status (Region 2-SEUS)*
Fraxinus americana	FACU
Fraxinus pennsylvanica	FACW
Quercus alba	FACU
Quercus nigra	FAC
Nyssa sylvatica	FAC
Nyssa aquatica	OBL

* **OBL** (obligate wetland)- Occurs almost always (estimated probability 99%) under natural conditions in
wetlands; **FACW** (facultative wetland)- Usually occurs in wetlands (estimated probability 67%-99%), but
occasionally found in non-wetlands; **FAC** (facultative)- Equally likely to occur in wetlands or non-wetlands
(estimated probability 34%-66%); **FACU** (facultative upland)- Usually occurs in non-wetlands (estimated
probability 67%-99%), but occasionally found on wetlands (estimated probability 1%-33%); **NI** (no indicator)-
Insufficient information was available to determine an indicator status; +- the positive sign indicates a
frequency toward the higher end of the category (more frequently found in wetlands)

N. sylvatica, which also occurs in well-drained sites. Although *Quercus nigra* appears on a

wide variety of sites, which ranges from wet bottomlands to well-drained uplands, this

species is commonly found and grows very well in moist soils such as those along streams

(Radford et al., 1964 and Burns et al., 1990). *Nyssa sylvatica*, which is considered to be

facultative, grows very well on well-drained light textured soils and on drier upper slopes and

ridges (Burns et al., 1990; Schafale and Weakley, 1990). Conversely, *N. aquatica*, which is

an obligate wetland species**,** can also grow in moist sites, which remain near saturation level

(Burns et al., 1990). There may be an overlap in the distribution of each pair, but relatively,

one species is more adapted to well-drained site and the other to moist site.

Dry-down experiment

　　　　This experiment compared the ability of xeric and mesic species to survive extreme

water stress by evaluating survival after re-watering subsequent to drought exposure. I used

the seedlings grown in Petri plates from the germination study described in Chapter II. The

seedlings were transplanted to a 6.35cm x 6.35cm x 8.9 cm pots with a substrate composed

of 50% gravel and 50% peat-lite and were grown in the growth chamber maintained at 30 $^{\circ}$C

(day) and 20 $^{\circ}$C (night) with 8 hours daylight and 16 hours of dark period. When the

seedlings had four to six leaves, the pots were soaked with water to start all pots at field

capacity after which watering was interrupted for varying lengths of time to generate a range

of water deficits. To better control levels of water deficit, we sampled individuals at each of

the wilt stages shown in Table 2 prior to re-watering.

Table 2. Wilt stages of the most wilted individual leaf that were used to measure the leaf
water potential (adapted from Engelbrecht and Kursar, 2003).

Wilt stage	Visual characteristics
Normal (not wilted)	No signs of wilting
Slightly wilted	Leaves green but leaf angled slightly toward the ground compared to normal seedling
Wilted	Leaves green but leaf angled to 45° with some leaf curling of the leaf blade
Severely wilted	Leaves green but leaf angled to 90° with extensive leaf curling. Some necrosis on the leaf margins and leaf tips.
Nearly dead	Most leaves are necrotic with extensive leaf curling and leaf angled mostly at 90°
Presumed dead	Necrosis on all leaves with extensive leaf curling, leaf blades are brittle and leaf angled mostly at 90°

Leaves of plants at each wilt stage were harvested and leaf water potential was

measured immediately using a pressure chamber (PMS, Corvallis, OR). We sampled 10 to 25

individuals per wilt stage in each species. Subsequently, the plants were re-watered daily after drought exposure. Survival rates, complete stem survival (CSS) and partial stem survival (PSS) were then assessed. Overall survival was defined by the presence of a living shoot or photosynthetically active leaf. Complete stem survival was defined by the presence of a living shoot apex and was inferred by normal leaves or a leaf re-sprout at the shoot apex. Partial stem survival was defined by the presence of re-sprout of new leaves from the middle to the tip of the stem.

Statistical Analysis

Statistical analyses were performed using logistic regression of JMP version 6 (SAS, Cary , NC, USA) to determine whether species type and water potential influenced the overall percent survival, complete stem survival (CSS) and partial stem survival (PSS) of each congener. I used the following logistic regression equation to prove the best fit for the percent survival, CSS and PSS data: $100/(1+\exp(A + B * \Psi))$, where: A and B are the parameters and Ψ is the water potential. I used the likelihood ratio test to test for effects of species type, water potential, and species by water potential interaction. A factor was removed when it did not significantly affect the model and the simpler model is reported.

RESULTS

The leaf water potential of seedlings decreased linearly with wilt stages (Fig. 1).

Manifestations of drought such as bending of the leaves to 45° and 90° from its normal

horizontal position, necrosis of leaf margins, extensive leaf curling, and brittle leaves were

observed to be extensive as the wilting progressed in all species examined (Fig. 2).
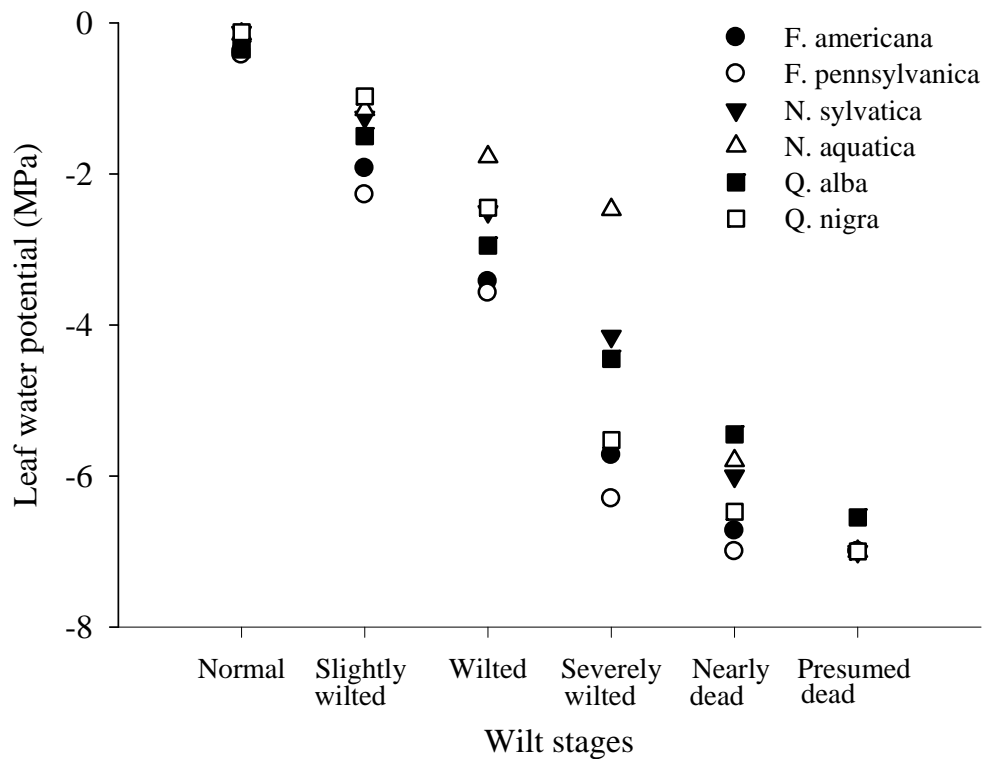
Figure 1. Mean leaf water potential (-MPa) at different wilt stages of the species tested.
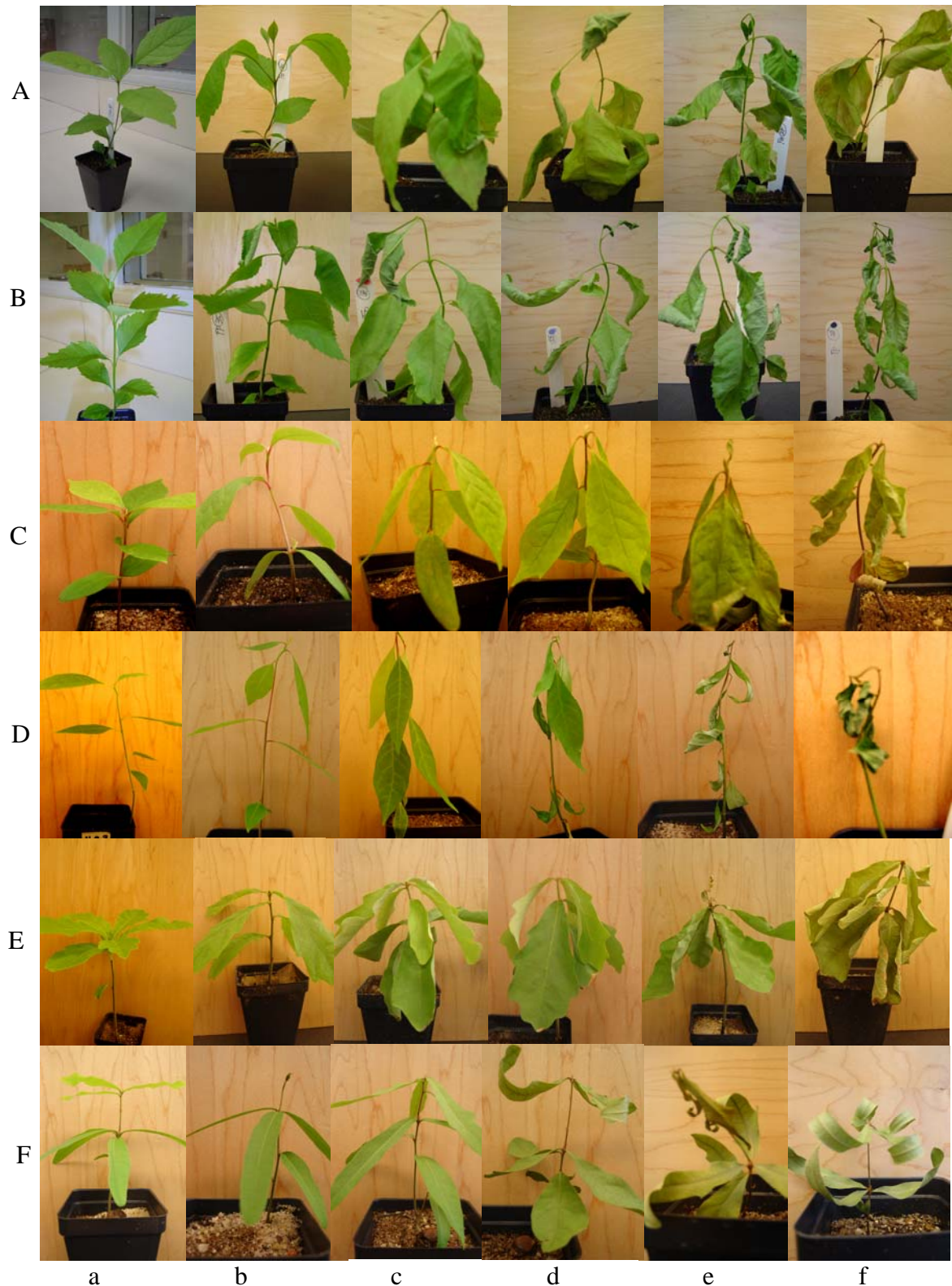
Figure 2. Wilt stages of A) *F. americana,* B) *F. pennsylvanica,* C) *N. sylvatica,* D) *N. aquatica,* E) *Q. alba* and F) *Q. nigra,* respectively: a) normal, b) slightly wilted, c) wilted, d) severely wilted, e) nearly dead, and f) presumed dead.

The xeric and mesic species varied widely in their survival response after drought exposure. When species were slightly wilted to wilted and had complete stem survival (Fig. 3 A-D, F, & H), they re-sprouted new leaves from the shoot apex. Conversely, species which were extremely wilted shed leaves prior to new leaf production at the shoot apex (Fig. 3 G). Partial stem survival after drought exposure was manifested by new growth that occurred at some point below the shoot apex (Fig. 4). Some stems that were presumed to be dead also showed re-sprouting at the base of the stem. Basal re-sprouting indicates that while stems were more vulnerable to death during dry-down, the root systems are still functional. Thus, functional roots are likely able to transport water to newly developed leaves at the base of the stem.
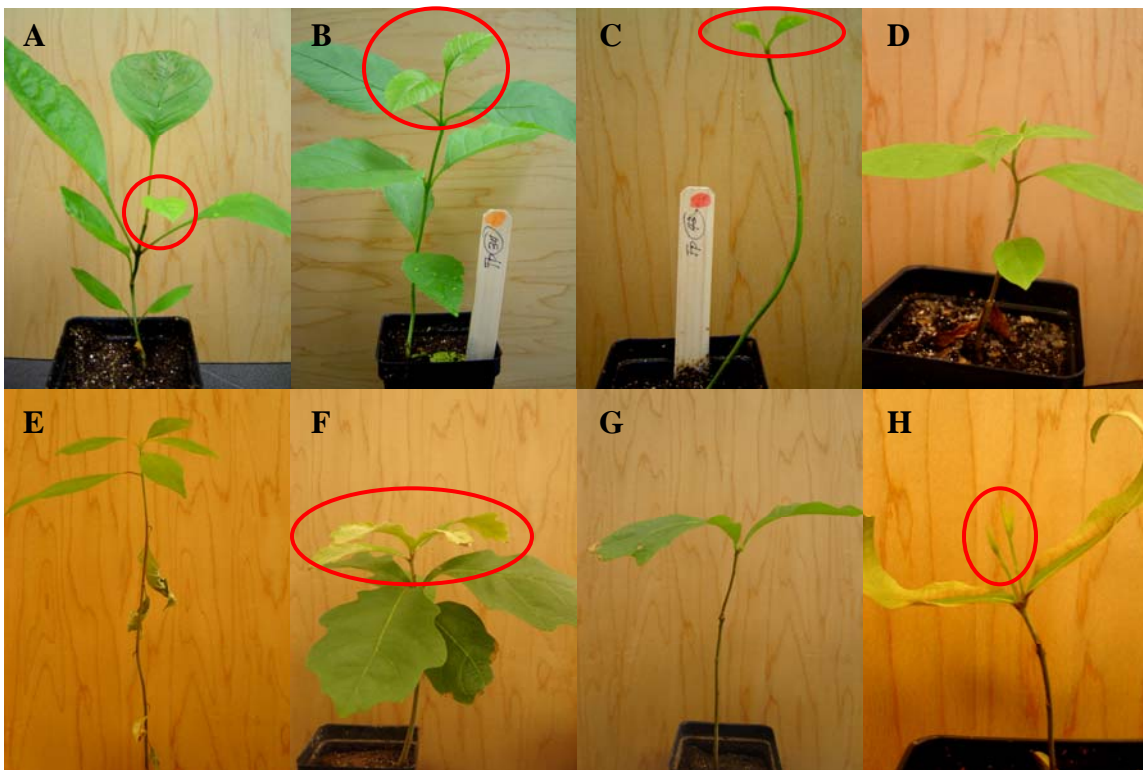
Figure 3. Complete stem survival of A) *F. americana*, B & C) *F. pennsylvanica*, D) *N. sylvatica*, E) *N. aquatica*, F & G) *Q. alba*, and H) *Q. nigra* after re-watering subsequent to drought exposure. Circles indicate new growth that occurred subsequent to re-watering after drought.
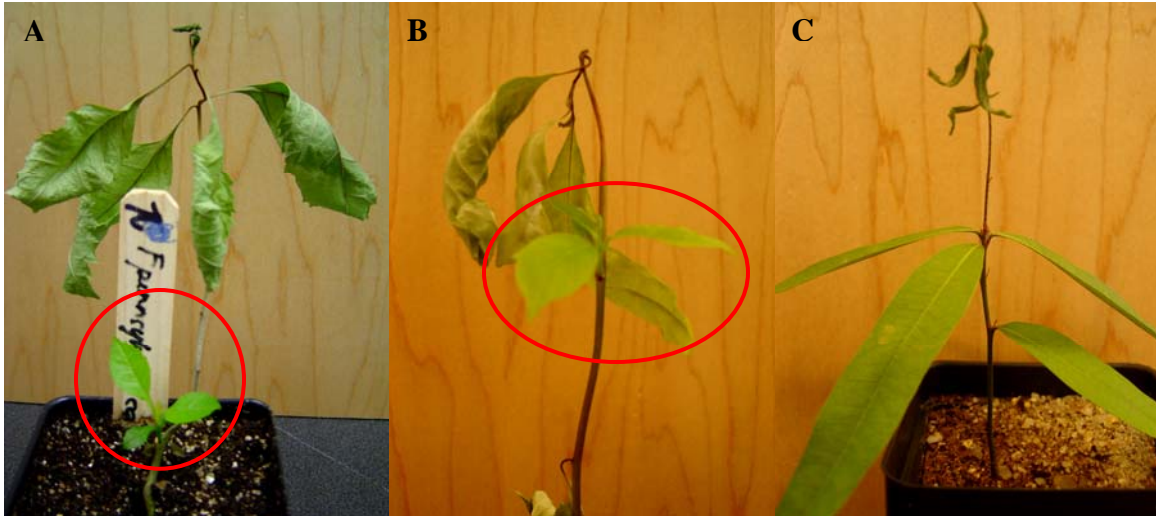
Figure 4. Partial stem survival of A) *F. pennsylvanica*, B) *N. aquatica*, C) *Q. nigra* after re-watering subsequent to drought exposure. Circles indicate new growth that occurred subsequent to re-watering after drought.

Overall, different levels of water stress significantly affected rates of complete stem survival and partial stem survival of all species (Fig. 5 and Fig. 6, p<0.001, for each congener). There was only a significantly higher survival rate in xeric *Fraxinus* sp. relative to mesic *Fraxinus* sp. In all cases, xeric species had a significantly higher complete stem survival than their respective mesic species pair (p<0.001, data not shown) using the simple model of likelihood ratio test. There was a significantly higher partial stem survival of all xeric species than mesic species (p<0.001), except for *Nyssa* spp. (p=0.925), using the simple model of likelihood ratio test. The relatively higher drought survival of species commonly associated with dry sites than those from moist sites was also observed by Engelbrecht et al. (2005) in the tropical moist forest. Either species type or the interaction between species type and leaf water potential showed a significant effect on survival across leaf water potentials. A significant interactive effect of species and water potential on the overall percent survival

was only observed in *Nyssa* (Fig. 5B, p=0.006). There was a significant interaction effect of species by water potential on complete stem survival of *Fraxinus* (Fig. 6A, p<0.001) and *Quercus* (Fig. 6E, p=0.042) congeners but not in *Nyssa* (Fig. 6C, p=0.136). On the other hand, partial stem survival was significantly affected by the interaction of species and water potential, in *Nyssa* and *Quercus* (Fig. 6D, p=0.006; Fig. 6F, p=.050, respectively) congeners and not in *Fraxinus* (Fig. 6B, p=0.066).
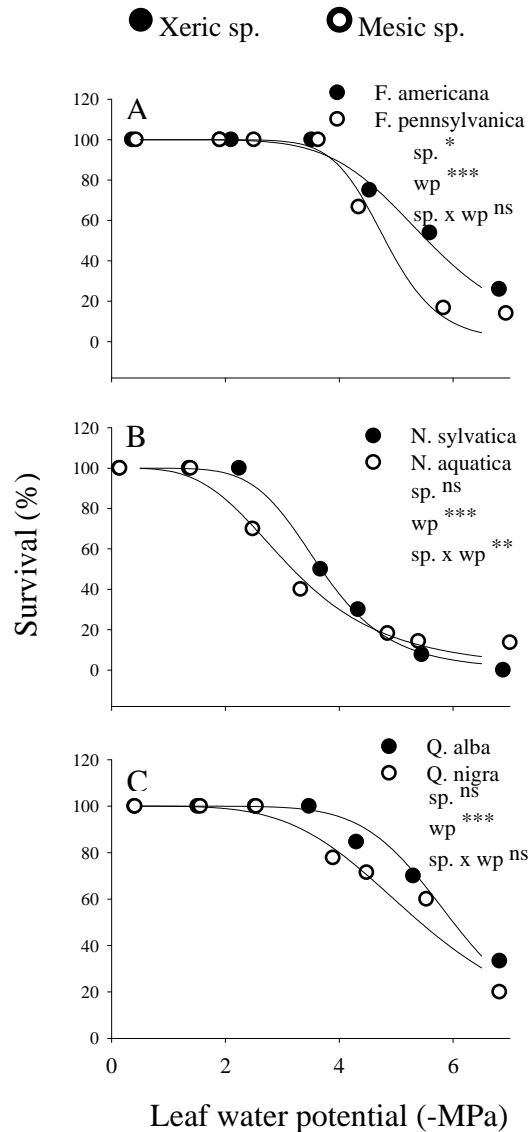
Figure 5. Survival rates of congeneric species pairs of A) *Fraxinus*, B) *Nyssa*, and C) *Quercus*. sp.- species, wp- water potential, ns- not significant, *- p<0.05, **- p<0.01, ***-p<0.005.
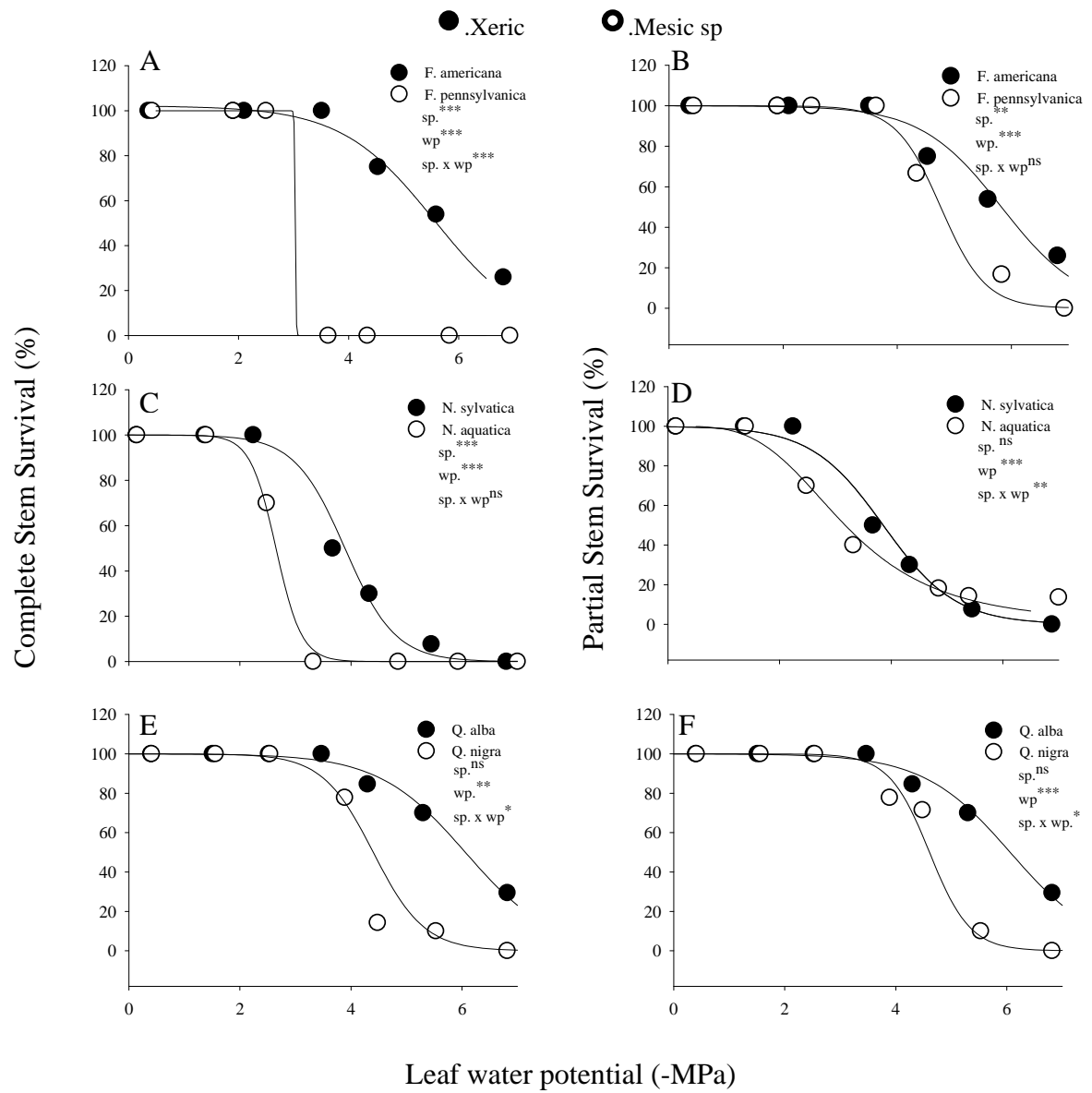
Figure 6. Percent complete stem survival (CSS) and percent partial stem survival (PSS) of congeneric species pairs of A & B) *Fraxinus*, C & D) *Nyssa*, and E & F) *Quercus*. Sp.-species, wp- water potential, ns- not significant, *-p< 0.05, **-p<0.01, ***p<0.005.

DISCUSSION

The ability of a species to tolerate drought often determines the distribution of a species in a landscape (Crawford and Braendle, 1996; Lopez and Kursar, 2003; Van Nieuwstadt and Sheil, 2005). Even if seeds germinate in a particular site, but drought continues to act as a filter to plant survival after germination and initial seedling establishment. I performed a dry-down experiment to evaluate the drought tolerance of three congeneric pairs of xeric and mesic species commonly found in the SE US mixed forest and observed that different wilt stages closely corresponded to the different leaf water potentials across species (Fig.1).

Future seedling establishment might also be negatively impacted by the occurrence of periodic drought because of a decrease in the availability of soil moisture necessary for seedling establishment. The levels of water stress imposed in the experiments significantly reduced the survival of the seedlings of SE US mixed forest species examined. Most of the xeric species had higher drought-tolerance than mesic species as manifested by their higher survival rates especially when both contrasting species were exposed to extreme water stress.

The results of the simple model of likelihood ratio test revealed that the entire stems of the xeric species were less vulnerable to wilting than the mesic species especially at extreme drought stress. This may suggest that xeric seedlings are better able to withstand the low water potentials in well-drained sites (xeric sites). The drought-tolerance strategy that these xeric seedlings exhibited allowed them to have a higher complete stem survival (CSS) under drought.

Having new apical shoots for most of the xeric species that had complete stem

survival (Fig. 3 A, D & F) suggests that these stems possess physiological traits that may

permit continued water transport even at low water potentials, were less vulnerable to

catastrophic xylem cavitation (Tyree et al., 2002), and their meristem tissues may have the

ability to survive low water potentials (Tyree et al., 2003). A significantly higher partial stem

survival (PSS) among the xeric species than the mesic species, except for *Nyssa* spp., may

indicate that portions of the stem are still able to recover from advanced wilting due to

drought stress. Stems that either completely or partially recovered may be able to avoid

cavitation and reverse the loss of hydraulic conductance after re-watering. Since most of the

mesic species had low stem survival, these species may have experienced greater loss of

hydraulic conductance, which may result to greater shoot die-back (Davis et al., 2002; Sperry

and Hacke, 2002; Tyree et al., 2003) relative to the xeric species. The high seedling mortality

rates of the drought-sensitive species (mesic sp.) may be due to a substantial loss of stem

biomass. Loss of stem biomass could potentially reduce growth rate and fitness, which may

make mesic species less competitive than xeric species under drought stress. In addition,

long-term exposure to desiccation may cause loss of turgor pressure resulting to the collapse

of plant cells (Smirnoff, 1993), reduced cell expansion, and inhibition of protein synthesis

(Kozlowski and Pallardy (1997). Desiccation may also cause stomatal closure and reduced

transpiration, which leads to poor water and nutrient absorption because of the lack of

sufficient water potential gradient to drive the mass flow of water. Moreover, stomatal

closure results in reduced photosynthetic capacity due to increased resistance in CO_2

diffusion, inhibition and damage of photosynthetic apparatus (Kozlowski and Pallardy, 1997)

in turn resulting to decreased availability of photosynthates necessary for the maintenance and repair of damaged plant cells (Engelbrecht et al., 2006). Hence, drought sensitive species are more likely to be negatively impacted to drought stress than drought tolerant species.

I can deduce from this study that adaptations to drought allowing high survival rates under drought stress are advantageous in allowing xeric species to successfully colonize relatively well-drained habitats. The low survival of mesic species under drought could explain the exclusion of the drought-sensitive mesic species in drought-stressed habitats. I expect that drought-intolerant mesic species will be more successful in the lowland moist sites, where drought-tolerant xeric species are less abundant. The low water availability in xeric habitats evidently filtered out the mesic species that may have germinated but lacked the appropriate physiological traits to grow and survive under drought conditions. Therefore, drought tolerance strategy of a species could be closely associated with the segregation of xeric and mesic species along a water gradient (Steege, 1994).

The future change in landscape hydrology due to climate change is likely to have a direct impact on species ranges, floristic composition as well as ecosystem function (Engelbrecht et al., 2007). The future shift in species composition of a plant community under climate change may depend on the survival ability of the species. In some sites, displacement of mesic species in their current distribution and a shift in the success of xeric species is possible. A greater survival rate of xeric species in drier sites relative to the mesic species will favor the recruitment of xeric species in habitats with reduced water availability. There may be a decline in the colonization or else exclusion of drought-sensitive mesic species in drying habitats in response to climate change.

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CHAPTER IV

Effect of Changes in Water and Nutrient Availability on Seedling Growth Performance of Xeric and Mesic Southeastern US Mixed Forest Species

INTRODUCTION

Plant species composition in a landscape can be constrained by environmental factors that influence the germination, survival, and seedling performance of individual species. The habitat in which a plant grows influences its growth independent of the influence of its genetic make-up, which changes over longer time scales. Growth performance continues to act as a filter after germination due to differences in environmental requirements between different life-stages, since conditions and traits that are advantageous for one life history stage may not be beneficial for another developmental stage (Broncano et al., 1998). More specifically, the environmental conditions that favor seed germination may not always be favorable for seedling survival and growth (Schupp, 1995). Growth responses to resource availability may play an important role in determining the success of seedling recruitment in an area (Gordon and Rice, 2000; Padilla et al., 2007), species distributions (Dalling et al., 2004), and the confinement of the xeric species and mesic species in their respective edaphic condition. Plant growth performance is an interplay of all physiological processes including water relations, photosynthesis, respiration, and mineral nutrition (Lambers et al., 1998). Therefore, I can expect that the success of plants in a particular area will be determined by the whole suite of traits that govern the uptake of water and nutrients (Park, 1990).

Plants are continuously adjusting their physiological responses and allocation patterns to match changes in the environment. Plants adjust allocation of biomass to shoots and roots in ways that improve acquisition of a limiting resource as well as survival under

environmental stress (Long and Jones, 1996). This phenotypic plastic response is an important strategy that affects carbon allocation in the plant. Some carbon allocation responses in woody plants are reflected in the distribution of plants along a resource gradient (Long and Jones, 1996). For example, plants from xeric sites tend to allocate relatively more biomass to roots than those from mesic sites (Matsuda et al., 1989).

Considerable work has been done to investigate seedling performance of woody plant as a function of light availability (Tyree et al., 1998; Lin et al., 2002; Dalling et al., 2004; Delagrange et al., 2004; Baltzer and Thomas, 2007; Quero et al., 2007) and the interaction between irradiance and water availability on growth performance (Black et al., 2005; Baraloto, 2006; Kobe, 2006; Niva et al., 2006; Pardos et al., 2006; Sanchez-Gomez et al., 2006; de Gouvenain et al., 2007; Feng and Li, 2007; Lavinsky et al., 2007), but in the southeastern United States mixed forest, water availability is one of the primary factors exerting control on vegetation distribution (Bahari et al., 1985; Woodward, 1987), although light is an important influence on seedling growth performance.

The SE US mixed forest is dominated by broad-leaved deciduous trees and is classified by Delcourt and Delcourt (1981) into 3 vegetation types: oak-hickory-southern pine (*Quercus-Carya-Pinus*), southern pine (*Pinus*), and cypress-gum (*Taxodium-Liquidambar*) forests. Deciduous forest in the SE US is composed of tree genera that are diverse and widespread, both geographically and ecologically (Barnes, 1991). For example, the oaks (*Quercus*), many of which are xerophytic (*Q. incana, Q. prinus, Q. marilandica*), are also adapted to mesic conditions (*Q. nigra, Q. palustris,* and *Q. phellos*) whereas *Q. alba, Q. rubra,* and *Q. velutina* are species that are widely distributed (Barnes, 1991) along

gradients of water availability. On the other hand, the hickories (*Carya*), which are typically associated with oaks, are also abundant and widespread on xeric and mesic sites (Barnes, 1991). *Carya glabra* dominates the xeric sites, while *C. aquatica* and *C. cordiformis* dominates the mesic sites. Other genera that dominate the mesic sites are *Acer, Fagus,* and *Tilia* (Barnes, 1991). In the SE US, xeric forest is typically dominated by drought-tolerant species. Conversely, mesic forest is dominated by drought-sensitive hardwood forest species (Braun, 1950; Schafale and Weakley, 1990). Therefore, water availability may play a role in the segregation of xeric and mesic species.

Water plays an essential role in all physiological plant processes. At a whole plant level, water is a medium for the transport of nutrients needed for plant growth and development. While the nutrient level of the soil may not be dependent on soil moisture, the availability of nutrients for plant uptake may be dependent on soil moisture. Nutrient content of the soil is also determined by the leaf litter and organic matter it possesses in addition to other factors such as soil erosion, soil pH, and soil microorganisms (Fitter and Hay, 2002). Plant responses to different nutrient levels can be influenced by water regime (Gusewell, 2003) and may be dependent on the ability of the plant to acquire these resources (Tyree et al., 1998). In a similar habitat nearby, nutrient concentrations decreased from mesic to xeric sites in an Appalachian oak forest in southwest Virginia (Martin et al., 1982). Mesic sites were dominated by mesic hardwood species with relatively higher nutrient concentrations than the xeric pine stands that dominated the xeric sites (Martin et al., 1982). Therefore, I may expect that upland and well-drained sites are more nutrient-limited than those in the bottomland and moist sites. Hence, this study focused on growth performance of xeric and

mesic species as affected by changing water and nutrient availability.

I studied early seedling growth, since this stage is particularly sensitive to resource shortages (Kramer and Kozlowski, 1979) and is also critical in the establishment of any plant species that are significantly influenced by water (Kozlowski and Pallardy, 1997; Turk et al., 2004) and nutrient availability. Forest composition is dictated by establishment success (Ackerly, 2004), which may depend in turn on the tolerance of the species to the limiting resource in a particular area (Valladares, 2003). The differential seedling performance under various water and nutrient levels may potentially cause a separation of xeric and mesic species along gradients of resource availability.

I hypothesized that relative to mesic species, growth performance of xeric species is less sensitive to chronic water stress but is more sensitive to flooding. A two factorial experiment (3 water levels x 2 nutrient levels) was done to: a) determine differences in growth performance, photosynthetic electron transport rate, and stomatal conductance between xeric and mesic species, b) determine biomass allocation patterns in response to changes in resource availability, and c) determine the effects of water and nutrient availability on biomass allocation, electron transport rate, and stomatal conductance.

Study species

Congeneric species pairs of *Fraxinus (F. americana*-xeric sp.; *F. pennsylvanica*-mesic sp.), *Nyssa (N. sylvatica*-xeric sp.; *N. aquatica*-mesic sp), *Quercus (Q. alba*-xeric sp.; *Q. nigra*- mesic sp.), and *Vaccinium (V. stamineum*- xeric sp.; *V. corymbosum*- mesic sp.) were studied. The *Fraxinus, Nyssa*, and *Quercus* seeds were obtained from Sheffield's seed company, who collected seeds from the Southeastern US. *Vaccinium* seeds were obtained from the Department of Horticultural Science, NCSU. Congeners were used to ensure phylogenetic independence, which is an important consideration for making inferences in comparative studies and in improving the statistical power of comparison between two groups (Ackerly, 1999). In each pair, one species is adapted to xeric conditions, while another is adapted to mesic conditions (Radford et al., 1964 and Schafale and Weakley, 1990). We used xeric and mesic in relative terms, wherein xeric species are those common in well-drained sites, while mesic species are those which are more frequent in moist sites.

Table 1 shows the wetland indicator status of the study species. *Fraxinus americana* is a facultative upland species and is typical of rich upland soil (Lance, 2004), which is dry and well-drained (Radford et al., 1964; Burns et al., 1990; Schafale and Weakley, 1990). Natural stands of *Fraxinus pennsylvanica*, a facultative wetland species, are almost completely confined to moist bottomlands (Burns et al., 1990), swamps, and along streams (Lance, 2004). Another facultative upland species is *Quercus alba*, which is found on either sandy plains, gravelly ridges, rich uplands and well-drained soils, but is common in driest shallow soil (Burns et al., 1990; Schafale and Weakley, 1990). Moreover, *Q. alba* grows in

Table 1. Wetland indicator status of study species based on USDA NRCS Plants
Database, USFWS wetland indicator status (http://plants.usda.gov/wetland.html,
accessed May 20, 2008).

Species	Wetland indicator status (Region 2-SEUS)*
Fraxinus americana	FACU
Fraxinus pennsylvanica	FACW
Quercus alba	FACU
Quercus nigra	FAC
Vaccinium stamineum	FACU
Vaccinium corymbosum	FACW
Nyssa sylvatica	FAC
Nyssa aquatica	OBL

* **OBL** (obligate wetland)- Occurs almost always (estimated probability 99%) under natural conditions in
wetlands; **FACW** (facultative wetland)- Usually occurs in wetlands (estimated probability 67%-99%), but
occasionally found in non-wetlands; **FAC** (facultative)- Equally likely to occur in wetlands or non-wetlands
(estimated probability 34%-66%); **FACU** (facultative upland)- Usually occurs in non-wetlands (estimated
probability 67%-99%), but occasionally found on wetlands (estimated probability 1%-33%); **NI** (no indicator)-
Insufficient information was available to determine an indicator status; +- the positive sign indicates a
frequency toward the higher end of the category (more frequently found in wetlands)

association with other tree species such as *Liriodendron tulipifera, F. americana,* and *N.
sylvatica*, which also occurs in well-drained sites. Although *Quercus nigra* appears on a wide
variety of sites, which ranges from wet bottomlands to well-drained uplands, this species is
commonly found and grows very well in moist soils such as those along streams (Radford et
al., 1964 and Burns et al., 1990). *Vaccinium stamineum* is typical in dry upland sites, while
V. corymbosum occurs commonly in moist to boggy soils (Schafale and Weakley, 1990;
Lance, 2004). *Nyssa sylvatica*, which is considered to be facultative, grows very well on
well-drained light textured soils and on drier upper slopes and ridges (Burns et al., 1990;
Schafale and Weakley, 1990). Conversely, *N. aquatica*, which is an obligate wetland species,
can also grow in moist sites, which remain near saturation level (Burns et al., 1990). There
may be an overlap in the distribution of each pair, but relatively, one species is more adapted
to well-drained site and the other to moist site.

Seedling growth experiment

Seeds were germinated in Petri dishes on a blotter paper moistened with deionized water. One week after the radicle and the first leaves emerged, the seedlings were transferred to 4 inch pots with a substrate composed of 50% coarse sand and 50% peat moss and vermiculite. The newly transplanted seedlings were acclimated to the growth chamber environmental conditions for a week before experimental treatments were applied. The seedlings were grown in growth chambers maintained at a particular temperature and day-length periods that mimic environmental requirements for growth of the particular study species (based from USDA forest service handbook, 1974). The alternating day and night temperatures of growth chambers used for this study are as follows: *Fraxinus* and *Nyssa* (30/20 ^{o}C), *Quercus* (26/22 ^{o}C), and *Vaccinium* (22/18 ^{o}C). Growing the seedlings at their optimal temperatures allowed the plants to maximize their growth potential. The main concern was to compare the relative growth performance of each congener, recognizing that differences in the evaporative demand may influence the magnitude of the drought effects across genera. Light intensity at 400 µmol s^{-1} m^{-2} was provided for 9 hours coinciding with the day temperatures in every growth chamber. A long-day period was also provided by using a dark period interruption from 11 pm to 2 am with light from incandescent lamps (NCSU Phytotron, 2008).

In each congener, water and nutrient availability were manipulated in a 3 x 2 factorial design. Nutrients were provided at either high (100%) or low (10% by volume) levels of a complete nutrient solution composed of 106.23 ppm N, 10.41 ppm P, 111.03 ppm K, 54.4 ppm Ca, 12.4 ppm Mg, 5 ppm Fe, 13.19 ppm S, 0.113 ppm Mn, 0.24 ppm B, 0.013 ppm Zn,

0.005 ppm Cu, 0.0003 ppm Co, 0.005 ppm Mo, and 11.04 ppm Na (NCSU phytotron solution analysis, 2008). Water treatments consisted of 3 water regimes: well-watered, dry, and flooded. For the plants assigned to well-watered and flooded treatment, the nutrient solution was applied once per week, followed by a day of no watering to reduce nutrient loss through leaching. Subsequently, plants in these two treatments were provided with 100 ml of deionized water daily until the next nutrient treatment. The watering schedule of the flooded treatment was identical to the well-watered, except that each pot was placed in an individual plastic container that prevented drainage of excess water. The plastic container was as tall as the pots to maintain the water level of the soil surface in each pot (Fig. 1). Each pot was also fitted with a plastic cover to reduce evaporative loss from the soil. Once per week in the dry treatment, plants were given 100 ml of deionized water followed by 100 ml of one of the two nutrient solutions mentioned earlier.

Figure 1. Growth performance experiment set-up. Circle indicates the flooded treatment.

Electron transport rate (ETR) and Stomatal conductance

Electron transport rate (ETR) and stomatal conductance (g_s) were measured after the first and third month from planting and before and after dry-down in each measurement period mentioned beforehand. The term dry-down refers to the withholding of water for 6 days in the dry treatment. I measured ETR and g_s before and after drying the soil substrate to have a baseline measurement for each parameter.

Measurements of electron transport rate (ETR) were made on two fully expanded leaves near the shoot apex of each plant. ETR was measured using a PAM 2100 portable chlorophyll fluorometer (Walz, Effeltrich, Germany). ETR through photosystem II was calculated from fluorescence according to the following equation:

$$J = \Phi_{PSII} \times PFD_a \times 0.84 \times 0.5$$

where: Φ_{PSII}- the quantum yield of PS II
PFD_a- the absorbed light
0.84 – coefficient of absorption of the leaves
0.5- the factor that accounts for the partitioning of energy
between PS II and PS I.

Stomatal conductance (g_s) was measured on the same leaves as ETR, using an AP4 porometer (Delta-T devices, Burwell, Cambridge, England).

Growth performance parameters

All seedlings were harvested when they were 3 months old. The samples were oven-dried at 70 ^{o}C for a week. Individual plants were partitioned into leaves, stems, coarse root, biomass, Root:shoot ratio (root biomass/shoot biomass), leaf mass ratio (leaf biomass/whole

66

plant biomass), stem mass ratio (stem biomass/whole plant biomass), root mass ratio (root biomass/whole plant biomass), coarse root mass ratio (coarse root biomass/whole plant biomass), and fine root mass ratio (fine root biomass/whole plant biomass).

Statistical analyses

Statistical analyses were conducted using JMP version 6 (SAS institute, Cary, NC). I pooled all the data for all congeneric pairs into one data set prior to analysis. The total biomass values were log_{10} transformed to achieve a normal distribution before this parameter was used in the statistical analysis. A full factorial analysis of variance (ANOVA) was used to test the effect of genus, species type, water levels, and nutrient levels on total biomass, Root:Shoot ratio, stem mass ratio (SMR), leaf mass ratio (LMR), root mass ratio (RMR), coarse root mass ratio (CRMR), fine root mass ratio (FRMR), electron transport rate (ETR), and stomatal conductance (g_s). In the factorial ANOVA performed, I treated genus and all other factors used as fixed factors (adapted from Knight and Ackerly, 2002) and photosynthetic photon flux density (PPFD) as covariate in the analysis for ETR.

Effect of water and nutrient availability on biomass allocation

Genera differed significantly in their total plant biomass ($F_{3, 430}$= 810.63, p<0.0001; data not shown), stem mass ratio ($F_{3, 430}$= 144.98, p<0.0001; data not shown), root mass ratio ($F_{3, 430}$= 128.75, p<0.0001; data not shown), root:shoot ratio ($F_{3, 430}$= 117.44, p<0.0001; data not shown), leaf mass ratio ($F_{3, 430}$= 237.47, p<0.0001; data not shown), fine root mass ratio ($F_{3, 430}$= 344.76, p<0.0001; data not shown), and coarse root mass ratio ($F_{3, 430}$= 84.57, p<0.0001; data not shown). Biomass allocation patterns were different between xeric and mesic species and patterns showed changes in response to water and nutrient availability. Whole plant biomass was significantly higher in the mesic species compared to the xeric species (Fig. 2A, $F_{1, 430}$ = 49.55, p<0.0001). There was a significant interaction effect between species type and water levels (Fig. 2B, $F_{2, 430}$=10.60, p<0.0001) on total biomass. This interaction mostly reflects a greater reduction in the whole plant biomass of the xeric species than the mesic species under flooded treatment (Fig. 2B). Neither species type differed between well-watered and dry treatments. The total biomass of xeric and mesic species were highest under well-watered conditions and not significantly different in the droughted condition (Fig. 2B). There was no significant interaction effect between species type and nutrients on whole plant biomass (Fig. 2C, $F_{2, 430}$=2.56, p=0.110).

Root mass ratio (RMR) was higher in xeric species than in mesic species (Fig. 2G, $F_{1, 430}$ = 5.04, p=0.025). RMR increased for both species types when exposed to low nutrients, although changes in RMR between nutrient treatments were greater for xeric species than

Figure 2. Biomass allocation patterns: total plant biomass, stem mass ratio (SMR), root mass ratio (RMR), leaf mass ratio (LMR), fine root mass ratio (FRMR) and coarse root mass ratio (CRMR) of xeric and mesic species; and the effect of different water levels and nutrient levels on the biomass allocation of xeric and mesic species. [ns]-not significant, *$p < 0.05$, **$p < 0.01$, ***< 0.005
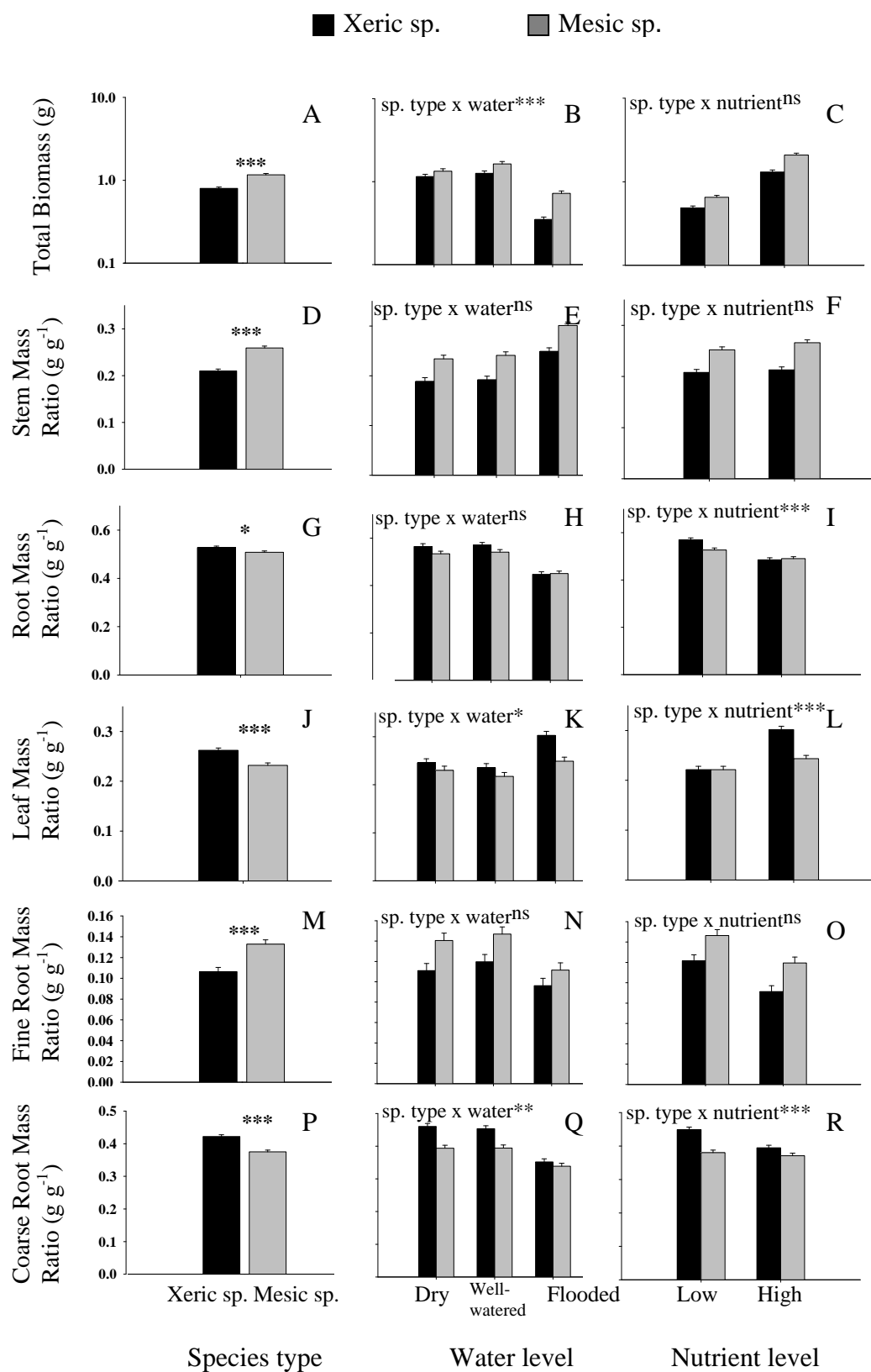
mesic species (Fig. 2I, $F_{1, 430}$ = 7.91, p=0.005). I did not find significant species type by water level interaction effect on RMR (Fig. 2H, $F_{2, 430}$ = 1.72, p=0.181).

Xeric species allocated less to stem (Fig. 2D, $F_{1, 430}$ = 69.93, p<0.0001) and fine roots (Fig. 2M, $F_{1, 430}$ = 20.44, p<0.0001)) and more to leaves (Fig. 2J, $F_{1, 430}$ = 18.22, p<0.0001) and coarse roots (Fig. 2P, $F_{1, 430}$ = 37.48, p<0.0001) than did the mesic species. Leaf mass ratio (LMR) was significantly affected by species type and water level interaction (Fig. 2K, $F_{2, 430}$ = 3.16, p=0.043). Figure 2K shows that both species types responded to flooding by increasing their allocation to leaves relative to well-watered treatment. However, changes in LMR between watering regimes were relatively higher in xeric species than mesic species. LMR in the droughted condition did not differ from the well-watered treatment among species types. On the other hand, species type and nutrient availability had a significant interactive effect on LMR (Fig.2L, $F_{1, 430}$ = 18.13, p<0.0001). Biomass partitioned to leaves increased when plants were exposed to high nutrients, though the increase was higher in xeric species than in mesic species. I found that LMR was positively correlated with FRMR across species (Fig. 4, $F_{1, 6}$= 9.25, p= 0.023).

The fraction of biomass in coarse roots was considerably affected by the interactions of species type and water level (Fig. 2Q, $F_{2, 430}$ = 4.80, p=0.009) and species type x nutrient level (Fig. 2R, $F_{1, 430}$ = 8.47, p=0.003). Both species types had a similar coarse root mass ratio (CRMR) between well-watered and droughted conditions (Fig. 2Q). Flooding, on the other hand, resulted in a reduction in CRMR in both species types, though a greater reduction was observed in the xeric species than in the mesic species (Fig. 2Q). As expected, CRMR was higher under low nutrients, though xeric species reduced partitioning to coarse roots to a greater degree under high nutrients than did the mesic species (Fig. 2R).

Effect of water and nutrient availability on ETR and stomatal conductance

Genera significantly differed in ETR ($F_{3, 387}$ = 3.31, p=0.02) and g_s ($F_{3, 386}$= 59.0, p<0.001). There was no significant difference in the electron transport rate (ETR) between xeric and mesic species (Fig. 3A, $F_{1, 387}$ = 1.00, p = 0.318). The interactive effect between species type and water level also did not significantly affect ETR (Fig. 3B, $F_{2, 387}$ = 2.66, p = 0.072). ETR was affected by the interaction between species type and nutrient availability (Fig. 3C, $F_{1, 387}$= 4.51, p=0.034). ETR increased under high nutrient levels for both species types. The change in the ETR response to nutrient availability was greater in mesic species than in xeric species. Mesic species also had a relatively higher stomatal conductance than xeric species (Fig. 3D, $F_{1, 386}$ = 11.60, p=0.0007). Stomatal conductance was affected by the species type and water level interaction (Fig. 3E, $F_{2, 386}$ = 4.30, p=0.014) and not by species type and nutrient level interaction (Fig. 3F, $F_{1, 386}$ = 3.25, p= 0.072). Both species types reduced stomatal conductance under drought, but a greater reduction was observed in mesic species relative to xeric species. The opposite trend was found under the flooded treatment; stomatal conductance of mesic species was much less reduced than xeric species.
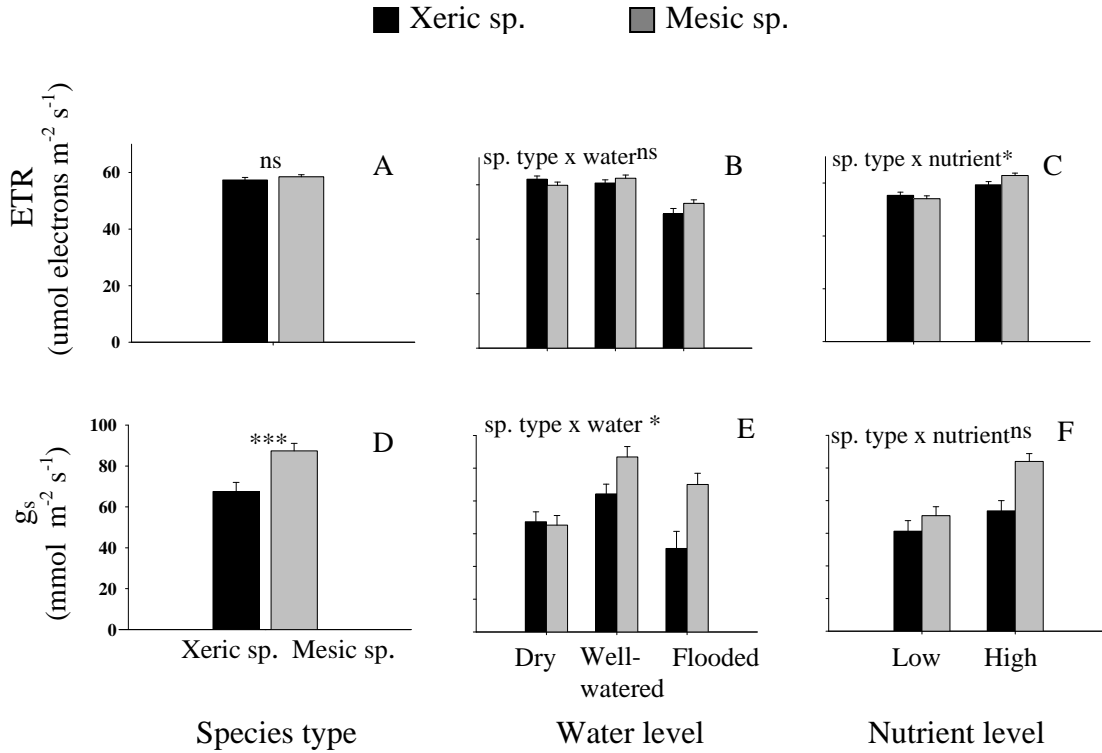
Figure 3. Electron transport rate (ETR) and stomatal conductance (g_s) of xeric and mesic species; and the effect of different water and nutrient levels on ETR and g_s. [ns]-not significant, *p<0.05, **p<0.01, ***<0.005
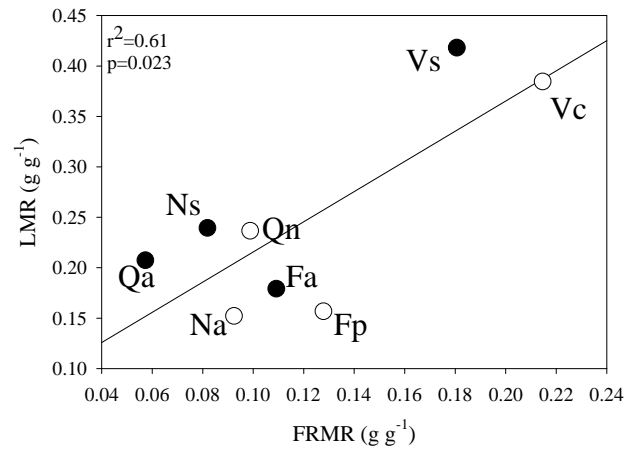
Figure 4. Correlation between leaf mass ratio (LMR) and fine root mass ratio (FRMR) of xeric (●) and mesic (o) woody plant species. Each point is a mean for 60 individual plants. Species codes: Fa, *Fraxinus americana*; Fp, *Fraxinus pennsylvanica*; Ns, *Nyssa sylvatica*; Na, *Nyssa aquatica*; Qa, *Quercus alba*; Qn, *Quercus nigra*; Vs, *Vaccinium stamineum*; Vc, *Vaccinium corymbosum*

73

DISCUSSION

Effect of water and nutrient availability on biomass allocation

There were contrasting biomass allocation patterns between xeric and mesic forest species. Xeric species had a low whole plant biomass when compared to the mesic species (Fig. 2A). Xeric species may employ a conservative water use strategy by a having a lower stomatal conductance (Fig. 3D) and growth performance, in order to overcome the water deficit that is common where these species usually occur. Mesic species appear to exhibit a prodigal water use strategy (Zhang et al., 2005) by having greater stomatal conductance and growth, thereby taking advantage of available water, especially under flooded conditions. Similar to the findings of Neill (1990) and Perez et al. (2007), I observed a significant interactive effect of species type and water availability on whole plant biomass (Fig. 2B) and stomatal conductance (Fig. 3E), but not in the interaction between species type and nutrient availability (Fig. 2C and Fig. 3F). It is apparent that xeric species are more greatly affected by flooding in terms of total biomass accumulation relative to the mesic species. Contrastingly, the total biomass in mesic species was not greatly reduced by flooding if I compare it to the well-watered treatment. Flooding is a stressful condition that can cause drought-like responses (Kozlowski and Pallardy 1997), and stomatal closure is one of the first responses to flooding in both flood-tolerant and flood-intolerant trees (Lopez and Kursar, 1999). The lower stomatal conductance in xeric species relative to mesic species (Fig. 3D) may have resulted in a reduction in their photosynthetic rates thereby producing a lower whole plant biomass. I suspect that a higher rate of photorespiration among xeric species was inhibiting the assimilation of carbon, resulting in their lower biomass

accumulation compared to the mesic species. The potential inhibition of CO_2 assimilation has been associated with photorespiration, since I observed no significant difference in electron transport rate (ETR) between xeric and mesic species (Fig. 3A). The imbalance between ETR and whole plant biomass accumulation that I found was also observed in other C_3 plants such as *Hordeum vulgare*, *Vicia faba* (Lal et al., 1996), and grapevine (Flexas et al., 2000). Photorespiration occurs when the concentration of CO_2 in a leaf becomes low as is expected for species with low stomatal conductance combined with high ETR. Under these conditions, less of the electron transport will contribute to carboxylation, thus resulting in a lower net assimilation of carbon in xeric species relative to mesic species.

A high root mass ratio (RMR) (Fig. 2G) or root:shoot ratio ($F_{1,430} = 11.14$, p=0.0009, data not shown) may allow xeric species to better acquire soil resources, which includes both water and nutrients. A high root:shoot ratio among xeric species relative to mesic species was also observed by Monk (1966) and Matsuda et al. (1989). Root mass ratio and root:shoot ratio generally increases with low water and low nutrient availability (Fitter and Hay, 2002). However, the RMR (Fig. 2I) and root:shoot ratio ($F_{1,430} = 14.17$, p=0.0002, data not shown) observed was only affected by nutrient supply. Both species types showed changes in RMR and root:shoot ratio when grown under different levels of nutrients by increasing RMR (Fig. 2I) or root:shoot ratio (data not shown) under low nutrient level. Changes in RMR and root:shoot ratio were more pronounced in xeric species than in mesic species under low nutrients. RMR and root:shoot ratios were similar for xeric and mesic species under high nutrients. Similarly, RMR and root:shoot ratio were greater under low nutrients on xeric and mesic sandhill tree species (Espelata and Donovan, 2002) and on *Banksia* seedlings

(Vaughton and Ramsey, 2001). Plants growing in substrates with high nutrient availability invest relatively more biomass in shoots, possibly allowing higher leaf areas and higher photosynthetic capacities, and leaving a smaller fraction of photosynthates allocated to roots (Fig. 2I) (Lambers et al., 1998). This trend may have resulted to the high leaf mass ratio (LMR) for both species types under high nutrient levels (Fig. 2L). On the other hand, plants in low nutrient environments may reduce LMR to allow a greater biomass allocation to the roots instead (Lambers et al., 1998), which may allow increased development of the root system (Vaughton and Ramsey, 2001) for better acquisition of nutrients under resource limitation. Plants adapted to mesic conditions, when exposed to low nutrient levels, might be negatively impacted due to their low RMR and root:shoot ratio. The production of more roots over shoot production under resource limitation (i.e. low nutrient) may enhance the ability of the xeric seedlings to cope with nutrient-limited habitats (Grime, 2001).

Regardless of watering regimes or nutrient availability, mesic species had a significantly higher stem mass ratio (SMR) than xeric species (Fig. 2D). Though I did not quantitatively measure plant growth changes (i.e. height over time), I observed that mesic species were relatively taller than did the xeric species. I did not observe an allometric relationship between SMR and whole plant biomass between species types ($F_{1, 5}$ =0.81, p=0.41; data not shown). I could then hypothesize that a higher SMR in mesic species could be a consequence of increasing plant height, which may reflect an adaptation in response for light competition, since light is a limiting resource in the bottomland forest (Long and Jones, 1996).

The greater leaf mass ratio (LMR) in the xeric species as compared to the mesic species (Fig. 2J) may be partly attributed to the low specific leaf area (SLA), a characteristic common to xeric species (Rieger and Duemmel, 1992). According to Lambers et al. (1998), leaf area ratio (LAR) = SLA x LMR, then, LMR = LAR/SLA. Assuming xeric and mesic species have similar LAR, and knowing xeric species tend to have lower SLA than mesic species (Long and Jones, 1996), I would expect a higher LMR among xeric species relative to mesic species (Fig. 2L). I also found an allometric relationship between LMR and fine root mass ratio (FRMR) (Fig. 4). Fine roots are essential for the acquisition and transport of nutrients and water, which could eventually contribute to the photosynthetic capacity of the plants. There could be a coupling relationship between LMR and FRMR. This proposed coupling relationship between LMR and FRMR was only true if I compare across species and not between species types ($F_{1, 5}$= 2.6788, p= 0.162, data not shown).

There could be a compensatory mechanism between the coarse root and fine root production, which creates a balance in the biomass partitioned to the root system of xeric and mesic species. A greater allocation of biomass to coarse roots in xeric species may allow these species to store carbon and nutrients, as well as to explore a greater root depth (Hoffmann and Franco, 2003) that allows these xeric plants access deeper soil water especially in water-stressed (Fig. 2Q) and nutrient-limited habitats (Fig. 2R). These coarse roots act as conduits for water and nutrients (Resh et al., 2003) between fine roots and other plant organs. Fine roots are more effective in water and nutrient absorption (Lambers et al., 1998). Having a higher FRMR among mesic species (Fig. 2M) may enable the mesic species to increase the surface area for water and nutrient absorption. To enhance acquisition and

77

transport of water and nutrients, the mesic species may need to increase their FRMR more than xeric species due to their lower CRMR. On the other hand, the higher CRMR of xeric species (Fig. 2P) was balanced by a lower FRMR of xeric species than mesic species (Fig. 2M), which could help xeric plant species maximize transport of water and nutrients. The compensatory mechanism between CRMR and FRMR may improve the balance in the acquisition, transport and storage of carbon and nutrients among xeric and mesic species.

Effect of water and nutrient availability on ETR and stomatal conductance

I measured electron transport rate (ETR) as a surrogate for photosynthetic capacity of the plants, since ETR is usually correlated with photosynthesis rate (Flexas at al., 1999 and Delagrange et al., 2004). The high nutrient level may have contributed to the higher ETR for both species types (Fig. 3C), since high nutrient availability is expected to yield greater amounts of the photosynthetic apparatus, such as chlorophyll, carboxylation enzymes, and chloroplasts, where electron transport occurs (Rieger and Duemmel, 1992. If I compare the species types under different nutrient levels, the mesic species had a greater increase in ETR under high nutrients. The low stomatal conductance of the xeric species (Fig. 3F) may have contributed to the lower increase in ETR under high nutrients relative to low nutrients (Fig. 3C). This would indicate that photosynthesis of xeric species may have a low capacity for their response to high nutrients due to inherently low stomatal conductance. When there is low stomatal conductance, photosynthesis may be limited by insufficient CO_2. When photosynthesis is reduced, ETR is lower since there would be lower demand for the products of the light reactions. Moreover, it is important to note that species in resource-poor environments tend not to respond strongly to resource availability.

Stomatal closure, a mechanism that reduces transpirational water loss, was apparent in both species types in droughted conditions as manifested by their drop in stomatal conductance (Fig. 3E). A greater drop in stomatal conductance among the mesic species relative to the xeric species may indicate that mesic species are more stressed under such conditions. However, mesic species maintained their stomates open even under flooded conditions, unlike xeric species. The higher stomatal conductance under flooding among the mesic species may be advantageous in their adaptation to moist habitats. On the other hand, low stomatal conductance of xeric species under flooding may result in poor growth performance (Fig. 2B). Thus, flooded habitats may not favor the adaptations of xeric species.

It can be deduced that growth performance of plants is not only controlled by water deficit, but by excessive water as well. In the previous chapters of this thesis, I observed that drought negatively impacted germination and plant survival. Plant survival under drought stress is a determinant in the current distribution of xeric and mesic species. In this study, flooding more markedly influenced the growth performance of both species types than drought. The drought treatment that I used might not be sufficient to impose substantial drought stress.

Growth performances of xeric and mesic species in response to their current habitats are likely due to phenotypic plasticity that will allow them to adjust carbon allocation to acquire resources in their particular habitat. Traits that allow xeric species to perform well in xeric habitats and traits that allow mesic species to perform well in mesic habitats may cause the segregation of xeric and mesic species along a resource gradient. Having a lower plant biomass among xeric species, could be a consequence of their growth response to resource

limitation. The tolerance of xeric species to low water levels by higher RMR, higher root:shoot ratio, higher CRMR and a lower reduction in stomatal conductance under drought may allow the success of xeric species and the exclusion of mesic species in xeric sites. The flood tolerance of mesic species, as manifested by higher stomatal conductance and higher whole plant biomass under flooding, excludes xeric species and allows mesic species in mesic sites. In the context of global warming due to climate change, shortage of resources (i.e. water and nutrients) is likely to become severe in drying habitats. The better growth performance of xeric species than mesic species in drying environments may potentially result in a decline in the mesic species distribution and a success in the xeric species distribution.

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CHAPTER V

Hydraulic Traits and Cavitation Resistance in Xeric and Mesic Southeastern US forest species

INTRODUCTION

Variation in the distribution of vegetation on earth corresponded to past climate change (DeHayes et al., 2000). It is expected that future climate change will have a major impact on the forest composition (Condit, 1998) and cause a shift in vegetation distribution (Hanson and Weltzin, 2000). Climate change is expected to cause an increase in temperature, resulting in greater evaporation and increased soil moisture deficits. An increase in global temperatures is therefore likely to increase the severity or duration of drought conditions, which has the capacity to cause a shift in the vegetation distribution (Graham and Grim, 1990, Hanson and Weltzin, 2000).

It has been projected that a mean temperature increase of about 3-5 ^{o}C is likely to occur in the US within the next 100 years and a 2-3 ^{o}C increase in the Eastern US by 2100 (IPCC, 2001 and NAST, 2001). NAST (2001) also predicted that precipitation is very likely to increase in some areas of the US. However, this is likely to be offset by greater evaporation rates brought about by higher air temperatures (NAST, 2001). Drought is a common occurrence in the longer historical record (Cook et al., 1988, Leuscher et al., 2001). For example, southeastern mixed forests experienced prolonged dry spells and above normal temperatures in 1995 (Baldocchi, 1997) and 2007.

Previous studies associated drought with tree mortality in temperate forests (Allen and Breshears, 1998; Villalba and Veblen, 1998). Extreme drought lead to tree death in mid-western oak forest (Pendersen, 1998), Northern Arizona forest (IPCC, 2001), mixed conifer

forest of California (Guarin and Taylor, 2005), forest of Colorado (Savage, 1997), and in mixed hardwood forest of Southern United States (Elliot and Swank, 1994). Moreover, extended periods of drought have been directly linked to tree death in the tropics (Condit et al., 1995; Fensham and Holman, 1999; Williamson et al., 2000; and Gilbert et al., 2001). Drought was the main factor influencing tree mortality of seedlings in Panama's tropical moist forest and survival rates in water-stressed conditions were higher in seedlings that were associated with xeric habitats relative to those from mesic habitats (Engelbrecht et al., 2005). Drought-induced mortality can cause shifts in vegetation distribution. Prolonged droughts in New Mexico resulted to an upward shift in the pinyon-juniper/ponderosa ecotone as caused by high tree mortality of ponderosa pine (Allen and Breshears, 1998). Similarly, Meuller et al. (2005) found that pinyon-juniper woodland was replaced by juniper dominated woodland as a result of high mortality rates of pinyon. Several models simulate a break-up of pine-dominant forest in some areas of the Southeastern US (NAST, 2001) and projected this forest to be replaced by savannas and grasslands as a result of soil moisture deficits (Bachelet et al., 2001).

Tree die-back and tree mortality can be caused by drought-induced cavitation (Rice et al., 2004 and Mueller et al., 2005), which leads to tissue desiccation and death. Cavitation is the abrupt transition of xylem water from metastable liquid state to vapor, resulting from the large negative pressures induced by water stress (Tyree and Dixon, 1986; Tyree et al., 1994). One mechanism by which cavitation occurs during drought (Tyree and Dixon, 1986) is by air-seeding (Zimmerman, 1983). Because of the high tension in water-filled vessels, air is pulled from neighboring vessels through the pores located in the pit membrane of the primary

cell walls. Once air enters the vessel, it disrupts the cohesion of water molecules and the water column breaks and retracts, filling the vessel first with water vapor. Ultimately, as air comes out of solution from the surrounding water, the vessel completely fills with air (Kozlowski and Pallardy, 1997). Xylem cavitation is a major potential problem in plants (Dickison, 2000), causing xylem dysfunction (Tyree and Sperry, 1989) and disrupting the hydraulic pathway from the soil to the leaf (Zimmerman, 1983). The resulting increased resistance to water flow in the sapwood can eventually limit plant growth and lead to death of the plant (Tyree and Sperry, 1988) since cavitation reduces the ability of plants to transport water to the leaves and induces greater water stress, and reduces transpiration and photosynthesis (Pockman et al., 1995 and Sperry, 1995).

Drought, as a form of water stress, is one of the environmental factors that influence growth and distribution of plants because it affects the physiological processes such as water transport. Plants surviving in habitats with low water availability have numerous traits for coping with water stress. A trait of particular importance for drought tolerance is cavitation resistant xylem, which allows water transport under condition of water deficit.

Plant survival in water-stressed conditions depends on the ability of the plants to conduct water, which requires the ability to either resist or repair cavitation. In addition, the ability of xylem conduits to maintain the high xylem tensions required for water transport in plants is limited by their tendency to become embolized (Sperry and Tyree, 1990). Hence, drought-induced cavitation can limit the distribution of woody plants.

This study focused on woody plant species found in the SE US mixed forest dominated by broad-leaved deciduous trees and is classified by Delcourt and Delcourt (1981)

into 3 vegetation types: oak-hickory-southern pine (*Quercus-Carya-Pinus*), southern pine (*Pinus*), and cypress-gum (*Taxodium-Liquidambar*) forests. Deciduous forest in the SE US is composed of tree genera that are diverse and widespread, both geographically and ecologically (Barnes, 1991). For example, the oaks (*Quercus*), many of which are xerophytic (*Q. incana, Q. prinus, Q. marilandica*), are also adapted to mesic conditions (*Q. nigra, Q. palustris,* and *Q. phellos*), whereas *Q. alba, Q. rubra,* and *Q. velutina* are species that are widely distributed (Barnes, 1991) along gradients of water availability. On the other hand, the hickories (*Carya*), which are typically associated with oaks, are also abundant and widespread on xeric and mesic sites (Barnes, 1991). *Carya glabra* dominates the xeric sites, while *C. aquatica* and *C. cordiformis* dominates the mesic sites. Other genera that dominate the mesic sites are *Acer, Fagus,* and *Tilia* (Barnes, 1991). Although the SE US mixed forest is not considered to be a water-limited ecosystem, such distribution of species suggests that water availability does exert a strong control on the success of the woody plants in their current distribution.

   This study could be used to inform modeling of the future landscape of the SE US mixed forest to predict how the global distribution of vegetation is likely to respond to climate change. Understanding what factors restrict species distributions, the consequences for plant species survival and the associated physiological adjustments could help explain natural patterns in productivity of natural ecosystems.

   I hypothesized that xeric species are better able to withstand drought conditions than mesic species by having greater cavitation resistance. I aimed to: 1) compare the water potential at 50% loss of conductivity (PLC_{50}) of xeric and mesic species, 2) test for

correlations among physiological traits such as specific conductivity, leaf specific

conductivity, Huber value, SLA and PLC_{50} with wood density, and 3) determine if there is a

trade-off between water transport efficiency and cavitation resistance.

MATERIALS AND METHODS

Study Species and Site

We studied seven pairs of woody plants species from the piedmont and coastal plain

of North Carolina, with each pair containing a xeric species and a closely related mesic

species (Table 1). In this study, the categorization into mesic and xeric species are used on a

relative basis and some of the congeneric species pair exhibit greater differences in habitat

requirements than the other pairs. In most cases, the two species were from the same genus

except for *Liriodendron tulipifera* and *Magnolia tripetala*, both belonging to family

Magnoliaceae. These pairs were chosen to ensure phylogenetic independence, which is an

important consideration for making inferences in comparative studies and in improving the

statistical power of comparison between two groups (Ackerly, 1999).

Table 1. Pairs of woody plants used in the study.

Xeric species	Mesic species
Carya tomentosa	*Carya glabra*
Quercus alba	*Quercus nigra*
Fraxinus americana	*Fraxinus pennsylvanica*
Ilex opaca	*Ilex glabra*
Liriodendron tulipifera	*Magnolia tripetala*
Vaccinium pallidum	*Vaccinium fuscatum*
Nyssa sylvatica	*Nyssa aquatica*

Based on Radford et al. (1964) and Schafale and Weakley (1990)

Table 2 shows the wetland indicator status of the study species listed above. *Fraxinus*

americana is a facultative upland species and is typical of rich upland soil (Lance, 2004),

which is dry and well-drained (Radford et al., 1964; Burns et al., 1990; Schafale and

Weakley, 1990). Natural stands of *Fraxinus pennsylvanica*, a facultative wetland species, are

Table 2. Wetland indicator status of study species based on USDA NRCS Plants
Database, USFWS wetland indicator status (http://plants.usda.gov/wetland.html,
accessed May 20, 2008).

Species	Wetland indicator status (Region 2-SEUS)*
Fraxinus americana	FACU
Fraxinus pennsylvanica	FACW
Quercus alba	FACU
Quercus nigra	FAC
Vaccinium pallidum	NI
Vaccinium fuscatum	FAC+
Ilex opaca	FAC
Ilex glabra	NI
Nyssa sylvatica	FAC
Nyssa aquatica	OBL
Carya tomentosa	NI
Carya glabra	FACU
Liriodendron tulipifera	FAC
Magnolia tripetala	FAC

* OBL (obligate wetland)- Occurs almost always (estimated probability 99%) under natural conditions in wetlands; **FACW** (facultative wetland)- Usually occurs in wetlands (estimated probability 67%-99%), but occasionally found in non-wetlands; **FAC** (facultative)- Equally likely to occur in wetlands or non-wetlands (estimated probability 34%-66%); **FACU** (facultative upland)- Usually occurs in non-wetlands (estimated probability 67%-99%), but occasionally found on wetlands (estimated probability 1%-33%); **NI** (no indicator)- Insufficient information was available to determine an indicator status; +- the positive sign indicates a frequency toward the higher end of the category (more frequently found in wetlands)

almost completely confined to moist bottomlands (Burns et al., 1990), swamps, and along

streams (Lance, 2004). Another facultative upland species is *Quercus alba*, which is found

on either sandy plains, gravelly ridges, rich upland and well-drained soils, but is common in

driest shallow soil (Burns et al., 1990; Schafale and Weakley, 1990). Moreover, *Q. alba*

grows in association with other tree species such as *Liriodendron tulipifera, F. americana,*

and *N. sylvatica*, which also occur in well-drained sites. Although *Quercus nigra* appears on

a wide variety of sites, which ranges from wet bottomlands to well-drained uplands, this

species is commonly found and grows very well in moist soils such as those along streams

(Radford et al., 1964 and Burns et al., 1990). *Vaccinium pallidum* usually grows in understories of acidic upland woods with mostly dry soils (Schafale and Weakley, 1990; Lance, 2004). Even if *Vaccinium fuscatum* is likely to occur in wetland and non-wetland habitats, this species is more frequently associated with moist soils near streams (Radford et al., 1964 and Lance, 2004). Although *Ilex opaca* occurs from dry soils to bottomlands (Lance, 2004), this species is also very common in well-drained sites such as the upland pine sites and hammocks and will not survive saturated soil (Burns et al., 1990). In contrast, *I. glabra* is commonly found in bays, bogs and pocosins (Lance, 2004). *Nyssa sylvatica*, which is considered to be facultative, grows very well on well-drained light textured soils and on drier upper slopes and ridges (Burns et al., 1990; Schafale and Weakley, 1990). Conversely, *N. aquatica*, which is an obligate wetland species, can also grow in moist sites, which remain near saturation level (Burns et al., 1990). *Carya tomentosa* is found on drier soils of ridges and hillsides as well as sandy soils and is less frequently found on moist woodlands and alluvial bottomlands (Nelson, 1965; Schafale and Weakley, 1990). Despite the fact that *Carya glabra* is facultative upland, this species is usually associated with moist sites particularly in mountain and piedmont regions (Burns et al., 1990). In addition, Whittaker (1956) classified *C. glabra* in the sub-mesic class. *Liriodendron tulipifera* and *Magnolia tripetala*, both belonging to Magnoliacea, are both facultative. Nonetheless, *L. tulipifera* generally grows in moist but well-drained and loosely textured soil and very rarely grows in wet soil conditions (Burns et al., 1990) while *M. tripetala* occurs in moist rich woodlands near the streams (Lance, 2004). There may be an overlap in the distribution of each pair, but relatively, one species is more adapted to well-drained site and the other to moist site.

Xylem Hydraulic Conductivity

In the summer of 2004 and 2005, six to eight stems of the study species (Table 1) were collected from the understory saplings with heights of 1-2 m. Each plant was cut at its base and re-cut under water before placing the cut ends in water to prevent cavitation and the entire stem was covered with black plastic to prevent transpiration and brought to the laboratory. Prior to hydraulic conductivity measurement, the stems were again re-cut under water. Native hydraulic conductivity was determined by measuring the rate of flow of distilled water through the stem under a pressure of 0.0046 MPa applied at the basal end of the stem. Following measurements, both cut ends of the stem were perfused with Methylene blue stain. Subsequently, a thin cross-section of the wood was made on both ends of the stem segment. The major and minor axis of the xylem and of the inner, non-conducting area was measured. The area was calculated using the formula for the area of an ellipse, and sapwood area (SA) was determined by subtracting the non-conducting area from the total area. Leaf area (LA) distal to the stem segment was determined with a CI-202 leaf area meter (CID, Inc.).

Hydraulic conductivity was calculated as $K_h = F/(dP/dx)$, where F is the water flux through an excised stem segment and dP/dx is pressure gradient causing the flow. Specific conductivity was calculated as $K_s = k_h/SA$, and leaf-specific conductivity was calculated as $K_l = k_h/LA$. Huber value, the ratio of sapwood area to leaf area was calculated as HV= SA/LA.

Wood density and specific leaf area

A segment of each stem was excised for the determination of wood density. The bark and pith of the stems were removed and the volume of the remaining sapwood was determined with the water displacement method. The sample was then oven-dried at 60 $^{\circ}$C and weighed to obtain the dry mass of the sapwood.

Ten leaf discs were collected from each individual, after which their areas were measured. The leaves were then oven-dried at 60 $^{\circ}$C and weighed. The specific leaf area was calculated as SLA=leaf area/dry mass.

Vulnerability to cavitation

Six branches of each pair of xeric and mesic species were cut from the plants grown in their natural habitat in the summer of 2004 and 2005. The stems were re-cut from the base under water to prevent air from entering the cut portion of the stem after the initial cut. The air-injection method (Cochard et al., 1992) was used to determine vulnerability to cavitation. Each stem was placed within the cavitation chamber (PMS, Corvallis, OR) and external pressures ranging from 0 to 7.0 MPa were applied. Each pressure was applied for 15 minutes, after which, hydraulic conductivity was measured. The percent loss of specific conductivity (PLC) for each pressure was calculated as:

$$\text{PLC} = 100 \left(1 - \frac{K_s}{K_{max}} \right)$$

where K_s is the specific conductivity measured at a given pressure and K_{max} is the maximum conductivity at 0 MPa.

Statistical Analysis

        Statistical analyses were performed with JMP version 6.0 (SAS Institute, Cary, NC, USA). Vulnerability curves were fitted to a logistic curve using non-linear least squares regression. The logistic curves were used to determine PLC_{50}, the pressure at which PLC is predicted to reach 50%. Relationships between physiological traits and water transport and trade-offs between specific conductivity and vulnerability to cavitation were evaluated with analysis of covariance (ANCOVA) using species type (xeric vs. mesic) as the categorical variable. The t-test was performed to test for trait differences between xeric and mesic species.

<div align="center">**RESULTS**</div>

Conductivity and other physiological traits

When tested over all species, there was no significant difference between xeric and mesic species in wood density, specific conductivity (K_s), leaf-specific conductivity (K_l), Huber value (HV) or specific leaf area (SLA). Specific conductivity (K_s) decreased linearly with increasing wood density across all species (Fig. 1A, p= 0.007). Although there was a significant negative correlation between specific conductivity and wood density across all species, neither of these traits differed significantly between xeric and mesic species ($r^2=$ 0.467, p= 0.966). There was no significant trend between wood density and leaf specific conductivity (K_l), Huber value (HV) and specific leaf area (SLA) across all species ($r^2<0.256$, p>0.0647). Similarly, none of these correlations were found to be significantly different between xeric and mesic species ($r^2<0.467$, p>0.2783).

Vulnerability to cavitation

As expected, xeric species were more resistant to drought-induced cavitation than mesic species. The xeric species of *Carya, Fraxinus, Ilex, Liriodendron, Quercus* and *Nyssa* (Fig. 2 A-F) were found to be less vulnerable to cavitation. The only exception to this trend was the genus *Vaccinium*, where *V. pallidum* (xeric sp.) was more vulnerable to cavitation than its congener (Fig. 2G). The mean water potential corresponding to 50% loss of conductivity was significantly higher in all xeric species except for *Vaccinium* relative to the mesic species (Fig. 3, p=0.011 and Fig.4 A, p=0.016 and B, p=0.013).

Trade-off between conductivity and vulnerability to cavitation

We found no evidence of a trade-off between specific conductivity (Ks) and

vulnerability to cavitation among the woody plants examined (Fig. 4A, p=0.200). There

was also no significant effect of wood density on cavitation resistance (Fig. 4B, p=0.113).

● Xeric sp.   ○ Mesic sp.
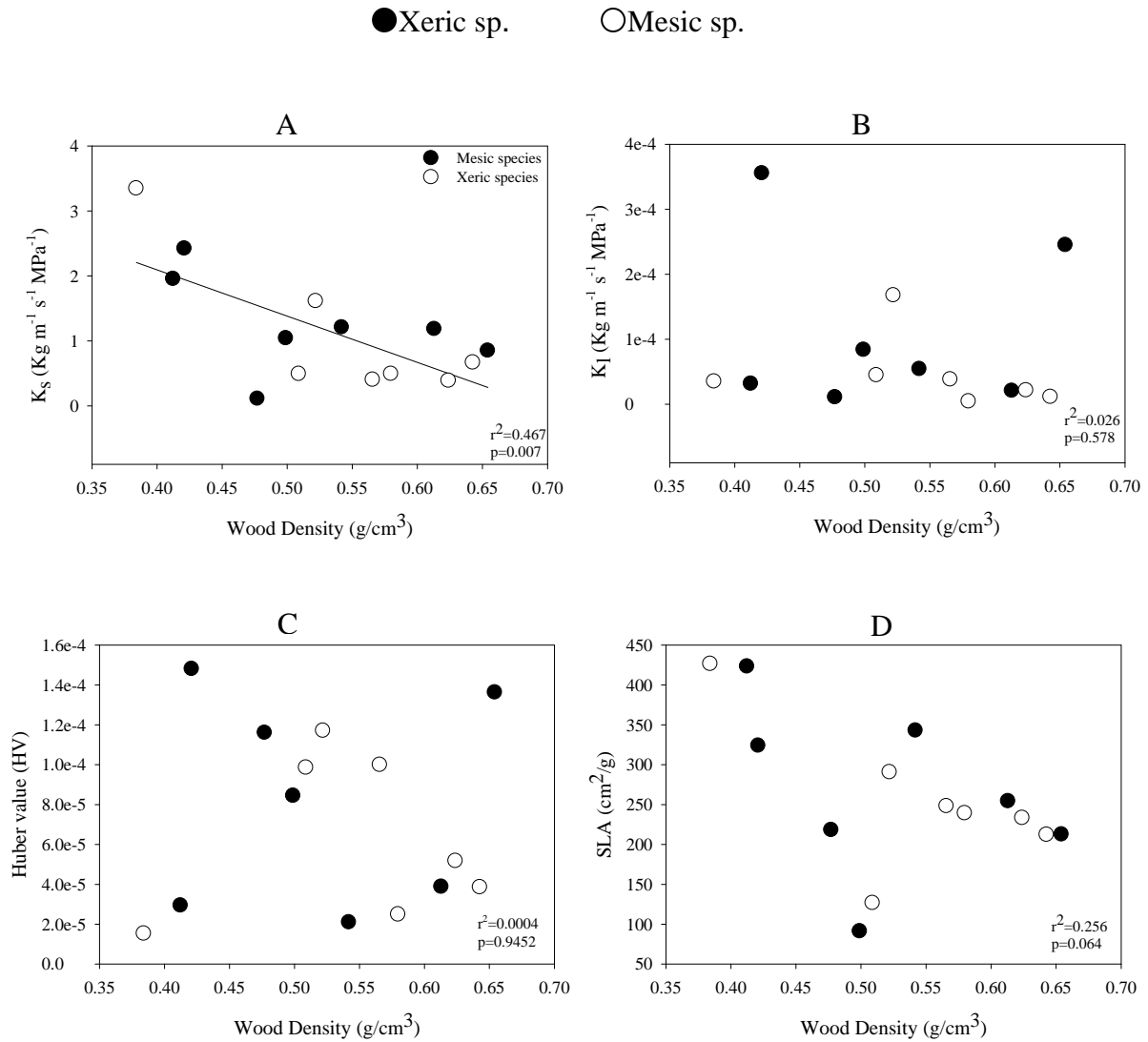
Figure 1. Regression plot showing the relationship between wood density and A) specific conductivity (K_s), B) leaf specific conductivity (K_l), C) Huber value (HV) and D) specific leaf area (SLA) for xeric (•) and mesic (o) woody plant species.
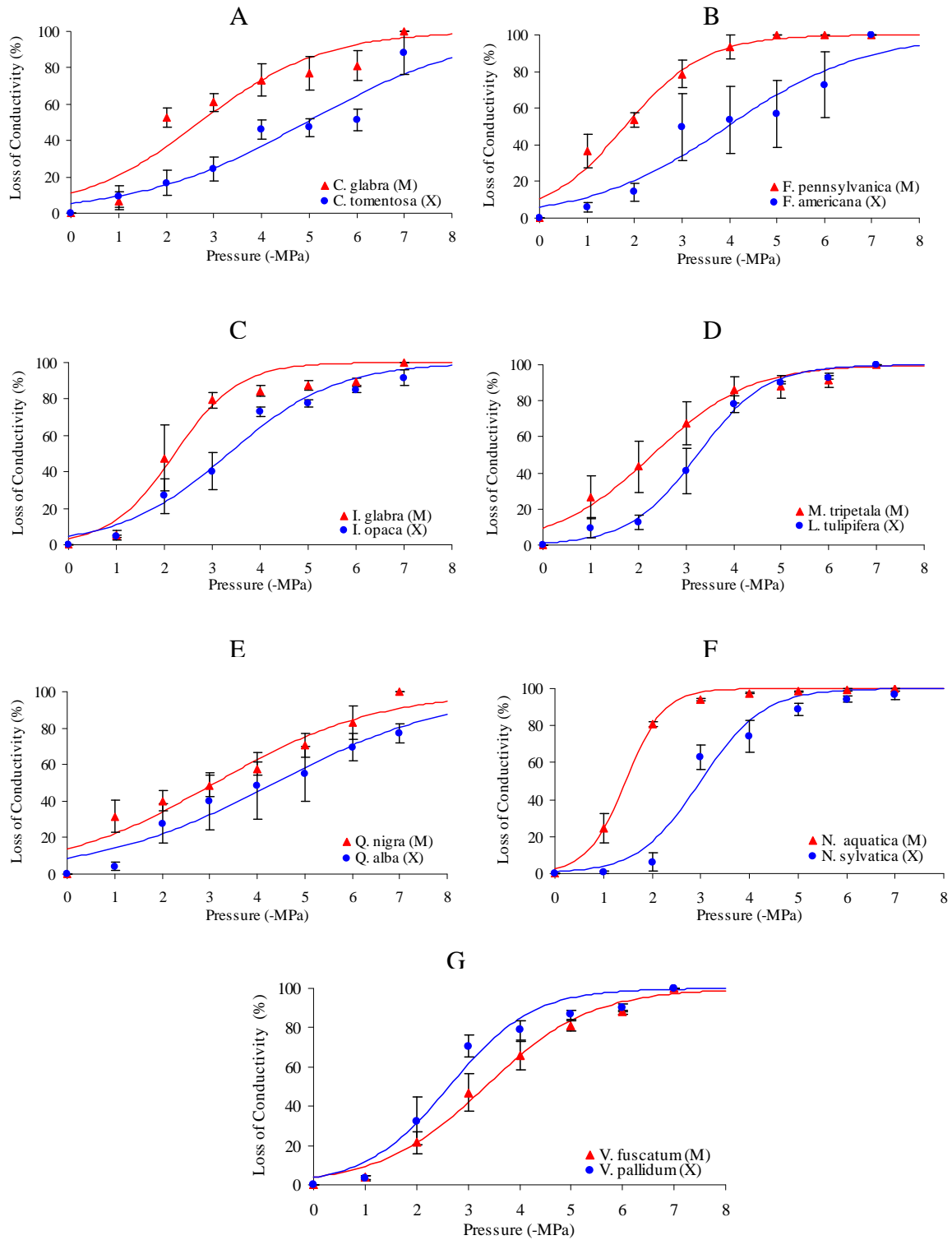
Figure 2. Vulnerability curves in closely related pairs of woody plants. Species name with (X) is from xeric sites while species name with (M) are from the mesic sites.
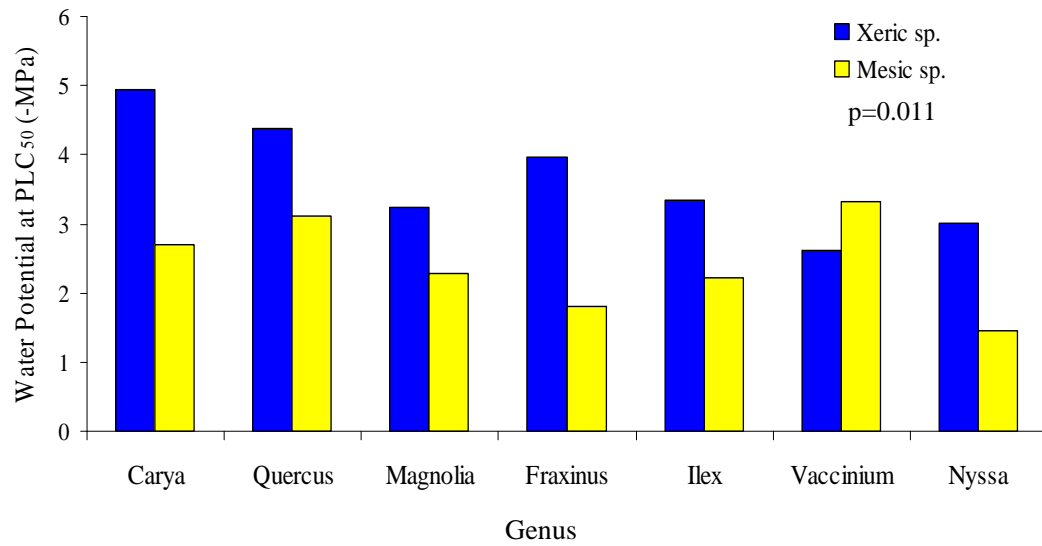
Figure 3. Water potential at 50% loss of conductivity (Ψ PLC$_{50}$) of xeric and mesic species pair.
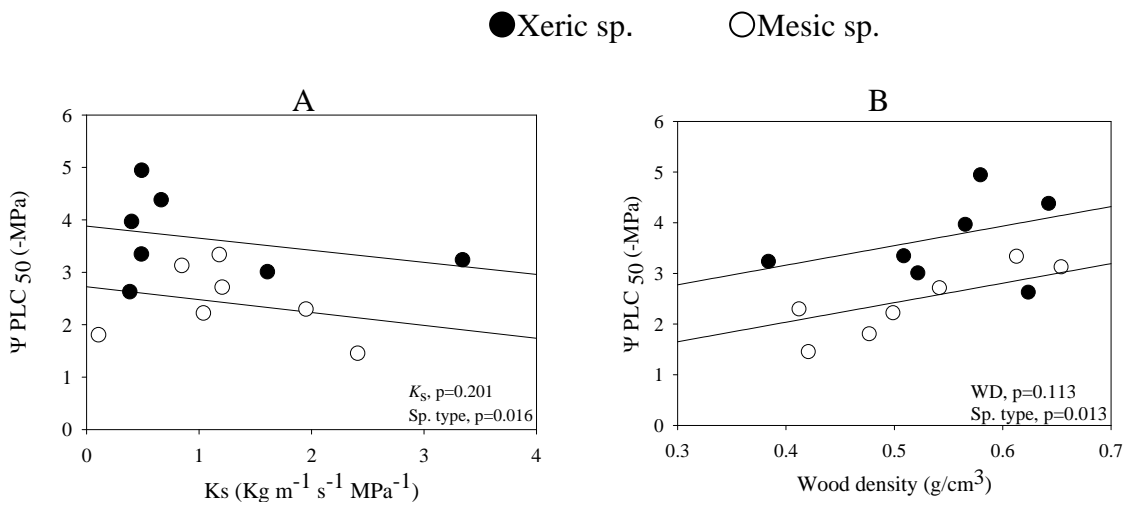
Figure 4. Relationship between A) Specific conductivity (K$_s$) and B) Wood density and the xylem water potential inducing the 50% loss of conductivity (Ψ PLC$_{50}$) in the xeric and woody plants. The regression lines correspond to the significant difference of Ψ PLC$_{50}$ between species types.

DISCUSSION

As hypothesized, there was a strong tendency for xeric species to be more resistant to cavitation than closely-related mesic species. In most genera, resistance to drought-induced cavitation was greater in species from xeric sites than in species from mesic sites (Fig. 2A-F). The only exception to this finding was *Vaccinium* (Fig. 2G). Xeric species attained 50% loss in hydraulic conductivity at a lower (more negative) water potential than the mesic species (Figure 3). All xeric species examined, except *Vaccinium*, are better able to withstand drought conditions by having vessels that are less vulnerable to cavitation. Previous studies have shown greater cavitation resistance of xeric than mesic species and populations. Differences were found between populations of *Pseudotsuga menziesii* (Kavanagh, 1999), *Pinus halepensis* (Tognetti et al., 1997), and *Cordia alliodora* (Choat et al., 2007). Differences were also found between species of *Eucalyptus* in Australia (Franks et al., 1995), *Acer* in French Alps (Tissier et al., 1997), *Juniperus* and *Pinus* in Utah (Linton et al., 1998), *Pinus, Cedrus,* and *Cupressus* in France (Froux et al., 2002), *Cordia* in Costa Rica and Panama (Choat et al., 2007), *Quercus* in United Kingdom (Higgs and Wood, 1995) and in Florida (Cavander-bares and Holbrook, 2001), and subspecies of *Artemesia tridentata* in Utah (Kolb and Sperry, 1999).

Xeric species are able to withstand a more negative water potential prior to catastrophic xylem cavitation than the mesic species. Having a higher water potential threshold may also allow the xeric species to continue water transport even in drier sites. Physiological processes necessary for growth such as transpiration and photosynthesis are dependent on water transport capacity. Since xeric species are still able to conduct water

101

even in drier sites, I expect them to maintain their normal physiological processes as well, which will also aid in the survival of these species. Mesic species may lose some of their physiological function due to their vulnerability to cavitation, which results to inability to transport water, and this would reduce their chance of surviving drier sites (xeric sites). I would then expect a higher mortality in the mesic species relative to the xeric species in drier conditions. The greater vulnerability to drought-induced cavitation is therefore likely to restrict the mesic species from occurring in habitats where water deficit is common. If drought occurrence increases in the Southeast US, as predicted, brought about by climate change, a drier climate will favor the xeric species over the mesic species.

Xeric species would be more resistant to cavitation when compared to mesic species because of investment in more dense wood. However, we found that xeric species did not have a significantly greater wood density to enhance cavitation resistance relative to the mesic species (Fig. 4B, p=0.110). This suggests that there is no cost to producing more resistant stems. Similarly, we did not find a relationship between wood density and vulnerability to cavitation. Our finding contrasts with that of Hacke et al. (2000 and 2001) and Pockman and Sperry (2000), who reported that there was a strong correlation between of xylem cavitation and wood density across species.

I only found a significant negative correlation between specific conductivity (K_s) and wood density (Fig. 1A). There was a reduction in the transport capacity among species with high wood density. Species with high wood density have been demonstrated to reduce water transport and be less vulnerable to cavitation. This trend is thought to be related to safety mechanisms that prevent cell wall collapse in xylem vessels, whereby greater conduit

reinforcement (smaller vessel lumen and thicker cell walls) leads to higher cavitation resistance (Hacke et al., 2001).

I also did not find evidence to support our initial hypothesis that there is a trade-off between hydraulic efficiency and safety (Fig. 4A, p=0.201) and is comparable to the results of Cochard (1992) and Kavanagh et al. (1999). In contrast, Hacke et al. (2001) and Mayr et al. (2006) found a significant correlation between wood density and PLC_{50}. Neither leaf specific conductivity (K_l), Huber value (HV) nor specific leaf area (SLA) was correlated with wood density. This is in contrast to the findings of Bucci et al. (2004), Santiago et al. (2004) and Pickup et al. (2005), who observed a decline in K_l and increase in HV with increasing wood density across species. Even traits that are associated with greater cavitation resistance, such as low SLA and high wood density (Pockman and Sperry, 2000 and Hacke et al., 2001) were not significantly correlated in this study (Fig. 1D, p=0.064). The lack of strong correlation between SLA and wood density may reflect natural selection of individual traits. These traits may have evolved independently of each other in response to similar habitats and do not indicate that there is a direct functional relationship between these traits. For instance, xeric habitats may have selected for high wood density and low SLA, while mesic habitats would have favored lower wood density and higher SLA.

Greater cavitation resistance in xeric species, relative to their closely-related mesic species, appears to be independent of hydraulic conductivity and wood density. Other physiological traits such stomatal control of water loss can play an equally important role in avoiding cavitation of the study species growing in contrasting habitats.

Cavitation resistance may play an important role in determining the species

distribution in the near future. A greater resistance to catastrophic xylem cavitation among xeric species may result to their higher survival rates relative to mesic species. A greater survival rate of xeric species than mesic species in drier habitats may favor the recruitment of xeric species in habitats with low water availability. In some sites, there may be a decline or displacement in mesic species, especially if their migration rate is so slow that it cannot cope with rapid changes in forest climate zones. Therefore, these results will help us understand the future shifts in the species distribution of SE US mixed forest in the face of future climate change.

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CHAPTER VI

SUMMARY AND CONCLUSION

Vegetation distribution in a landscape can be constrained by ecological and physiological factors that influence the different life stages of a plant species. Environmental conditions that are favorable for one life history stage may not always be advantageous for another developmental stage (Broncano et al., 1998). Similarly, differential responses of species to the ever-changing environment will ultimately determine their success at a site. The successful establishment of a species in a certain habitat will depend on its entire suite of traits that allows them to perform well in a particular habitat (Park, 1990).

Water availability is one of the most important factors that influence seed germination, plant growth and survival, overall plant performance, and plant species distribution. This study focused on woody plants common in southeastern United States mixed forest. Although this forest is not considered to be a water-limited ecosystem, the distribution of species suggests that water availability does exert a control on the success of woody plants. Xeric forest is dominated by drought-tolerant mixed hardwood forest species, while mesic forest is dominated by drought-sensitive forest species. The study aimed to determine the ecological and physiological basis underlying the distribution of woody plants along gradients of water availability in the southeastern US mixed forest. This was done by determining: a) differences in the hydraulic properties and water relations between xeric and mesic species, b) differences in germination ability, growth, and survival of established xeric and mesic species, and c) differential responses to water and nutrient availability between xeric and mesic species.

Although it was not possible to collect the full complement of data for all species, the most consistent results are those that relate to the ability of the stems to tolerate low water potentials, which includes stem survival and PLC_{50}. Table 1 summarizes our results and shows that xeric species did not always have a greater ability to germinate under lower water potentials. In some pairs, the xeric species was better able to germinate under osmotic stress and undergo osmotic adjustment, but in other pairs, this was not the case. Entire stems of xeric species were less vulnerable to wilting than mesic species under drought stress. The same trend was also found when we looked at partial stem survival, except for *Nyssa*. Stem survival is probably related to PLC_{50}. In the growth performance experiment, we found that contrasting biomass allocation patterns and tolerance to resource limitations between xeric and mesic species may have influenced the segregation of xeric and mesic species in their current distribution ranges (Table 1). A lower total biomass among xeric species may just be a consequence of resource limitation in xeric habitats. The tolerance of xeric species to low water levels by having higher root:shoot ratio (R/S), higher root mass ratio (RMR), and coarse root mass ratio (CRMR) may allow the success of xeric species and the exclusion of mesic species in xeric sites. The flood tolerance of mesic species, as manifested by higher stomatal conductance and higher whole plant biomass under flooding, may allow mesic species and excludes xeric species in mesic habitats. In an experiment done on saplings of xeric and mesic species to test vulnerability to cavitation, I observed that the xylem of xeric species is less vulnerable to catastrophic xylem cavitation relative to mesic species, except *Vaccinium* (Table 1). A greater cavitation resistance may have influenced the higher survival rates of xeric species in drier sites relative to mesic species (see PLC_{50}, Table 1).

Table 1. Summary of ecophysiological factors that favor the success of xeric species versus mesic species.

Genus	Germination ability under osmotic stress	Root OA	CSS	PSS	Ψ PLC$_{50}$	Total Biomass	R/S	SMR	RMR	LMR	CRMR	FRMR
Carya	NA	NA	NA	NA	[xeric]	NA	NA	NA	NA	NA	NA	NA
Fraxinus	[mesic]	[neither]	[xeric]	[xeric]	[xeric]	[mesic]	[xeric]	[mesic]	[xeric]	[xeric]	[xeric]	[mesic]
Ilex	NA	NA	NA	NA	[xeric]	NA	NA	NA	NA	NA	NA	NA
Magnolia/Liriodendron	NA	NA	NA	NA	[xeric]	NA	NA	NA	NA	NA	NA	NA
Nyssa	[neither]	[neither]	[xeric]	[neither]	[xeric]	[mesic]	[xeric]	[mesic]	[xeric]	[xeric]	[xeric]	[mesic]
Quercus	[xeric]	[xeric]	[xeric]	[xeric]	[xeric]	[xeric]	[mesic]	[xeric]	[mesic]	[mesic]	[neither]	[mesic]
Vaccinium	[xeric]	NA	NA	NA	[mesic]	[mesic]	[neither]	[mesic]	[neither]	[neither]	[xeric]	[neither]

[blue] - indicates that <u>xeric sp.</u> are favored over mesic sp. for a particular trait.

[yellow] - indicates that <u>mesic sp.</u> are favored over xeric sp. for a particular trait.

[hatched] - indicates that neither <u>xeric sp.</u> nor mesic sp. is favored for a particular trait.

NA- not applicable; OA-osmotic adjustment; CSS- complete stem survival; PSS- partial stem survival; Ψ PLC$_{50}$- water potential at which 50% loss of conductivity; R/S- root:shoot ratio; SMR- shoot root mass ratio; RMR- root mass ratio; LMR- leaf mass ratio, CRMR- coarse root mass ratio; FRMR- fine root mass ratio

Seeds may germinate in an area, however, drought may continue to act as filter to plant survival after germination and initial seedling establishment. In this study, the ability of a species to tolerate and survive drought stress appears to be the most critical determinant in the current distribution of xeric and mesic species. Current species distributions along water availability gradients appears to be better explained by the ability of seedlings to resist cavitation, to tolerate and survive water stress, and by their biomass allocation patterns, rather than by their ability to germinate and osmotically adjust under drought. For example, the physiological traits that allow greater cavitation resistance, higher stem survival, higher root mass ratio or root:shoot ratio, and higher coarse root mass ratio under drought stress, may have allowed *F. americana* (xeric sp.) to successfully establish in well-drained sites. On the other hand, the higher stomatal conductance and greater stem mass ratio under flooded conditions may have allowed *F. pennsylvanica* (mesic sp.) to successfully colonize mesic habitats.

These results could be used to inform modeling of the future landscape of the SE US mixed forest in the face of future climate change. Understanding what factors restrict species distributions, the consequences for plant species survival, and the associated physiological modifications could help us explain natural patterns in productivity of natural ecosystems.

Variation in vegetation distribution corresponds to past climate change (DeHayes et al., 2000) and is expected that future climate change will have a major impact on the floristic composition of forest (Condit, 1998). Climate change is expected to cause increases in temperatures resulting in greater evaporation and increased soil moisture deficits. Overall, increase in drought conditions due to global climate change can potentially cause a shift in

vegetation distribution (Hanson and Weltzin, 2000). It has been projected that a mean temperature increase of about 2-3 ^{o}C is likely to occur in the Eastern US by 2100 (IPCC, 2001 and NAST, 2001). Canadian climate model suggests that most part of the SE US will experience a decrease in the average yearly precipitation while the Hadley model projected increases in precipitation (IPCC, 2007). Although these climate models differ in their projections on regional precipitation patterns, both models consistently projected temperature increases (IPCC, 2001), so, it is more likely that precipitation will be offset by greater evaporation rates brought about by higher air temperatures (NAST, 2001). Therefore, drought will have a major impact on the floristic composition in the SE US. In the context of climate change, displacement of mesic species in their current distribution and shifts in the success of xeric species are possible. The greater survival rate of xeric species relative to mesic species in drier sites will favor the recruitment of xeric species in habitats with minimal water availability. There will be a potential decline in the colonization or a possible exclusion of mesic species in drying habitats, especially if their migration rate is so slow that it cannot cope with the future rapid change in the forest climate zones.

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