USING VULNERABILITY CURVES TO DETERMINE WHETHER TISSUE WATER RELATIONS DIFFER AMONG FRASER FIR CHRISTMAS TREES GROWING AT DIFFERENT ELEVATIONS

by

Jessica Noelle Alizieri Stevens

Honors Thesis
Appalachian State University
Submitted to the Department of Biology
and The Honors College
in partial fulfillment of the requirements for the degree of
Bachelor of Science
May, 2017

Approved by:

Howard S. Neufeld, Ph.D., Thesis Director

Thomas B. Ellis, Ph.D., Second Reader

Lynn Siefferman, Ph.D., Departmental Honors Director

Ted Zerucha, Ph.D., Interim Director, The Honors College

Abstract

The capacity for Fraser fir (*Abies fraseri*) grown for Christmas trees to adjust to the effects of climate change could have a profound effect on the industry and economy of North Carolina, since this species brings in approximately $100 million each year in revenue. This project uses elevation as a surrogate for warming to determine differences in the functioning of the xylem of trees from farms at low and high elevation in the North Carolina mountains. Elevations ranged from 664 m to 1224 m, which would be equivalent to an average temperature difference of 3.6^{o}C as determined by the adiabatic lapse rate.

I hypothesized that Fraser firs growing at low elevation would increase their hydraulic conductivity (K_h) and have a greater vulnerability to cavitation than trees at high elevation. A higher K_h (the ease with which water moves through the xylem per unit pressure) would enhance the ability to move water to the needles during times of water stress and a lower vulnerability would allow water transport during times of drought. To assess this, vulnerability curves (VC) were constructed for trees from low and high elevations. A VC assesses the sensitivity of the xylem to water stress by measuring the loss in K_h as water stress increases. Initial K_h measurements were made on 2-3 year old twigs at a hydraulic pressure of approximately 0.09 MPa using a Sperry conductivity apparatus equipped with an air-injection sleeve to induce embolisms. Cavitation from air-seeding reduces water flow at a given hydraulic pressure because of the development of embolisms and hence lowers K_h. The pressure at which K_h is reduced by 50% (K_{h50}) was determined for all twigs measured. Average baseline K_h and wood density averages were taken for the low and high elevation twigs and compared. Analyses of the vulnerability curves and baseline K_h did not find any statistical differences in K_h among trees from low and high elevations or in their vulnerability to cavitation, suggesting that in this species, adjustment to low elevation conditions probably occurs by other means. Low elevation twigs did show a significantly greater wood density, which presents a possible means of adaptation and opportunity for future study.

Acknowledgements

Thank you to my mentor and advisor, Dr. Howard S. Neufeld, for his patience with me during this process, as well as his wisdom, guidance, support, and advice. Thank you to Dr. Thomas B. Ellis for his counsel as a committee member. Lauren Wood and Scott Cory took me in during my first semester in the lab, and I would like to thank them for showing me the ropes of Fraser fir research. Thank you to Rachel Jordan and Zane Sink for their knowledge and passion regarding plant ecophysiology. Financial assistance was provided by the Office of Student Research and the Honors College at Appalachian State University. Thank you to Dana Greene and Mike Hughes for technical assistance with the experimental design and to Dan Johnson for advice and help with the K_h measurements. A huge thank you to our Christmas tree farmers, Carroll Garland, Thad Taylor, and Jim Bryan for their cooperation.

Dedication

To my wonderful parents, Lori and Jim Stevens, who I owe thanks for their endless support in everything I do. Also, to Will White, Nick Gilliam, Jess Phillips, Michael Tucker, and Jonathan Nichols for making me a better scholar in so many ways.

Table of Contents

Abstract……………………………………………………………………………………1

Acknowledgments………………………………………………………………………..3

Dedication…………………………………………………………………………………4

Introduction……………………………………………………………………………..6

Materials and Methods…………………………………………………………………20

Results………………………………………………………………………………….26

Discussion……………………………………………………………………………….32

Conclusion……………………………………………………………………………….40

References………………………………………………………………………………41

Introduction

This thesis analyzes the relationship between Fraser fir xylem structure and climate conditions that differ from the native environment of wild Fraser fir with the goal of understanding the mechanisms by which Fraser fir may adjust its wood anatomy as climate change occurs. The importance of Fraser fir to North Carolina's economy will be described, followed by an overview of tissue-water relation mechanisms. I used an elevation gradient to simulate environmental stress (warming) that could result in changes in hydraulic conductivity (K_h), the ability of trees to move water through their xylem per unit pressure (i.e., xylem water is under negative pressure, or tension). K_h describes the ease with which water moves through the xylem and the patterns characterizing potential changes in K_h could shed light on the means by which Fraser firs may adjust physiologically and anatomically to a warmer, more drought-prone environment. Consequently, meaningful predictions could be made regarding the future of this industry if substantial warming and/or drying occurs in the mountains of western North Carolina.

Abies fraseri: A North Carolina Specialty Crop

Christmas tree production occupies a significant position in North Carolina's economy, bringing in anywhere from $100 million (wholesale value) to $250 million (retail value) annually (NCSU 2008). The 1,300 growers in Western North Carolina produce approximately 7.5 million Christmas trees annually, second in the nation only to Oregon (Chastagner and Benson 2000). While Pacific Northwest Christmas tree production exceeds that of North Carolina, North Carolina Christmas trees are repeatedly regarded as the best in the nation (Chastagner and Benson 2000). The North Carolina Fraser fir has been recognized as the Nation's Best by the National Christmas Tree Association and is frequently chosen as the White House Christmas tree, contributing to its iconic status alongside tobacco as a symbol of North Carolinian agriculture.

Historically, dozens of tree species have been grown in western North Carolina in addition to Fraser fir for use as Christmas trees, including Douglas fir (*Pseudotsuga menziesii)*, balsam fir (*Abies balsamea)*, black spruce (*Picea mariana*), white spruce (*Picea glauca*) and eastern red cedar (*Juniperus virginiana*) (Chastagner and Benson 2000). Despite this, Fraser fir has always distinguished itself as the preferred species, increasing from 50% in 1970 to its current proportion of 96% of total North Carolina Christmas trees produced. This is in part due to their superior branch rigidity after harvest, and capacity for needle retention, as well as their pleasant aroma (Mitcham-Butler 1988; Chastagner and Benson 2000).

Fraser fir is native to the Appalachian Mountains of western North Carolina, but is also found in high elevation regions of Virginia and Tennessee (Beck 1990; Figure 1). Natural growth typically occurs above 1,372 m and has been recorded at 2,037 m on Mount Mitchell, the highest peak in the eastern United States. However, choose-and-cut farms typically grow them at much lower elevations that are below the native elevational range. Wild Fraser fir are considered mid-sized conifers that grow slowly and can reach a maximum height of approximately 26 m, though cultivated trees are typically cut upon reaching two meters for use as Christmas trees. Fraser fir is slow growing, shade-tolerant, and relatively wind-tolerant, though they thrive with sun exposure and abundant fog as a precipitation source (Beck 1990; Berry 2012).

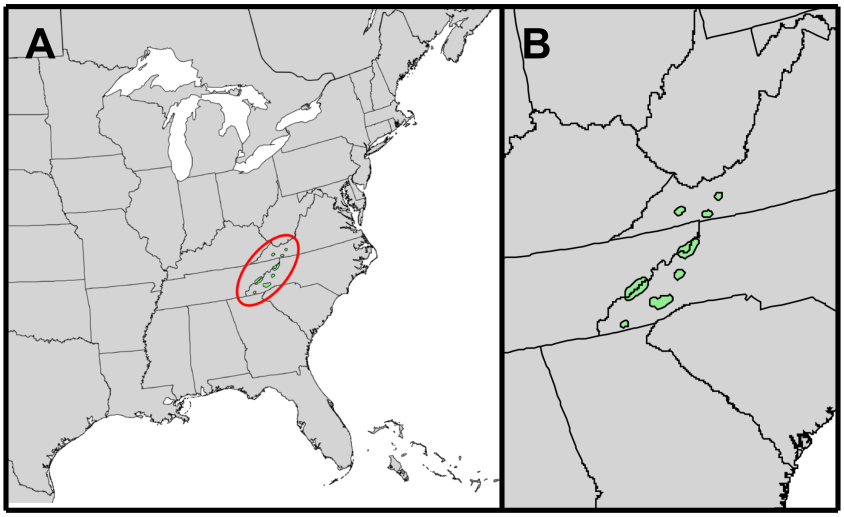

Figure 1. Distribution maps of *Abies fraseri.* (USGS) Green regions indicate the native range of Fraser fir from a national (A) and southeastern (B) perspective.

Fraser fir was classified as endangered by the International Union for Conservation of Nature (IUCN) in 2013 due to destruction by the invasive balsam woolly adelgid (*Adelges piceae*), a small insect that feeds on firs and releases toxic saliva into the sapwood (Farjon 2013). Fraser fir populations are poised to face another threat in the near future—rising temperatures as a result of climate change. The native habitat of Fraser fir is the high elevation, cool, moist, Appalachian temperate rainforest with temperatures ranging from below freezing to approximately 15.6º C and frost presence occurring over two thirds of the year. Climate change projections show an overall temperature increase anywhere from 3º C to 12º C by 2100 (Collins et al. 2013). Fraser firs are already confined to the tops of mountains and would need to continue moving upward to survive, though there is nowhere else to go in their native range. Widespread forest dieback due to temperature and moisture changes has already been observed in other forest species (Allen et al. 2010). Studies of farmed Fraser fir are useful in this respect, as this species is almost always grown below the native range and can be analyzed for adaptations permitting survival in warmer, drier environments.

Xylem Anatomy and Limitations

Xylem is the transport tissue in vascular plants that is responsible for water and nutrient movement from roots to leaves. Water is drawn up from the soil through the roots, travels through the xylem cells in the stem and branches and then evaporates from the needles in the process known as transpiration. This transpiration process is passive, as water in the xylem is under negative hydrostatic pressure (tension) and is carried down the water potential gradient (even though it is physically moving upwards in the plant!) extending from the basal to distal ends of the plant in a process known as the cohesion-tension transport (Dixon and Joly 1894; Tyree and Ewers 1991; Ding et al. 2014). Water potential (Ψ) represents the ability of water to do work—the potential energy of water due to unique properties such as cohesion, capillary action and osmosis (Brodribb 2009). Fully saturated soils have a water potential of zero, while the plant has negative water potential due to higher solute concentrations, in addition to several other components of water potential, including gravitational, matric, and turgor pressure. Increasingly negative water potentials in the pathway through the soil, roots, stems, leaves and the surrounding air characterize a properly functioning soil-plant-atmosphere continuum (SPAC) and a gradient for passive water transport (Figure 2). Equation 1 illustrates the tradeoff as water is lost through open stomata via transpiration and replaced when leaves facilitate a tension force on the water column (Brodribb 2009). E represents the evapotranspiration rate, Ψ_{soil} - Ψ_{leaf} represents the difference in water potentials forming the soil to leaf gradients and K_{plant} represents the hydraulic conductivity capacity of the plant (Brodribb 2009).

$$E = (\Psi_{soil} - \Psi_{leaf})K_{plant} \tag{1}$$

As evaporation creates the tension force pulling water upward, the air-water interface recoils inward toward the water column. Capillary action, cohesion and surface tension, properties that are unique to water, prevent breakage of the water column that would otherwise disrupt the flow of water to the leaves (Tyree and Sperry 1989). Passivity, meaning that the plant does not have to expend metabolic energy to move water in the xylem, is an obligate component of vascular transport, as over 90% of water in the xylem is lost due to transpiration. However, studies suggest that living parenchyma cells interspersed among the dead xylem cells may play a role in refilling xylem elements that become dysfunctional due to embolisms caused by cavitation of stressed cells (McCully 1999). The actual transport process is energetically downhill and thus requires only sunlight to drive the movement of water through the SPAC. If water movement required metabolic energy, it would most likely dominate the physiology of the plant, leaving little energetic reserves for other processes and this would ultimately lead to the death of the plant. (Hacke and Sperry 2001).

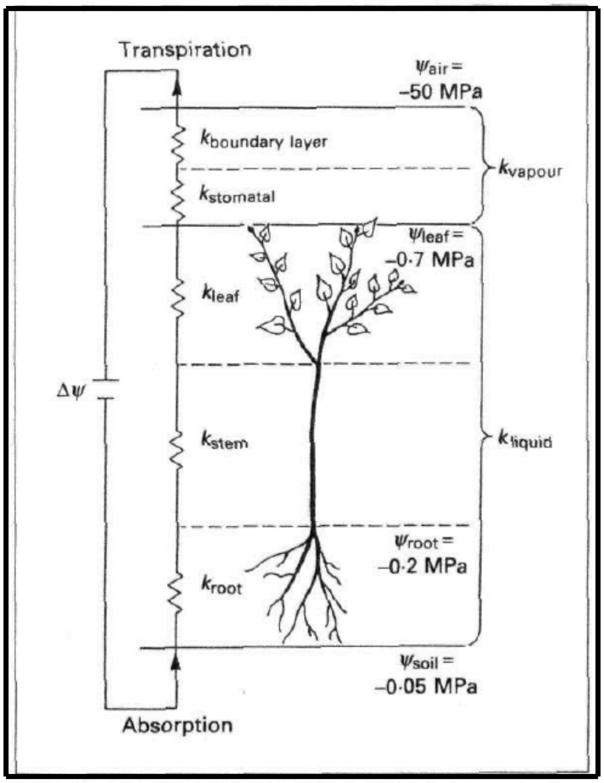

Figure 2. Increasingly negative water potential of the SPAC (Ewers and Cruziat, 1990). Water potential is the least negative in a moist soil and becomes more negative with progression through the roots, stems and leaves to the surrounding atmosphere. Since water moves passively from higher to lower water potentials, it is this process that drives water movement through the xylem in vascular plants. Hydraulic conductivity (K_h) is the rate of movement of water per unit of water potential and describes the ease with which water can move from one plant part to another.

Xylem conduits are composed of tracheids and vessel elements, both of which are non-living, lignified conducting cells (Tyree and Ewers 1991). Angiosperms possess both types of tracheary elements, while conifers such as Fraser fir contain only the more primitive tracheids. Tracheids have an elongated morphology and allow inter-conduit passage via contact between small, bordered pits in each cell wall. In tracheids, unlike in vessel elements, the pits contain a flexible membrane called the margo and a rigid central structure known as the torus that can cover the pit aperture (Bouche et al. 2014; Figure 3A). The torus acts as a safety valve, blocking fluid transport between tracheids if pressure differences are extreme (Hacke and Sperry 2001; Figure 3C). This can prevent an air bubble from aspirating into a stressed tracheid, which would result in cavitation and the development of an embolism, and which would prevent water movement in that cell (Pittermann et al., 2005).

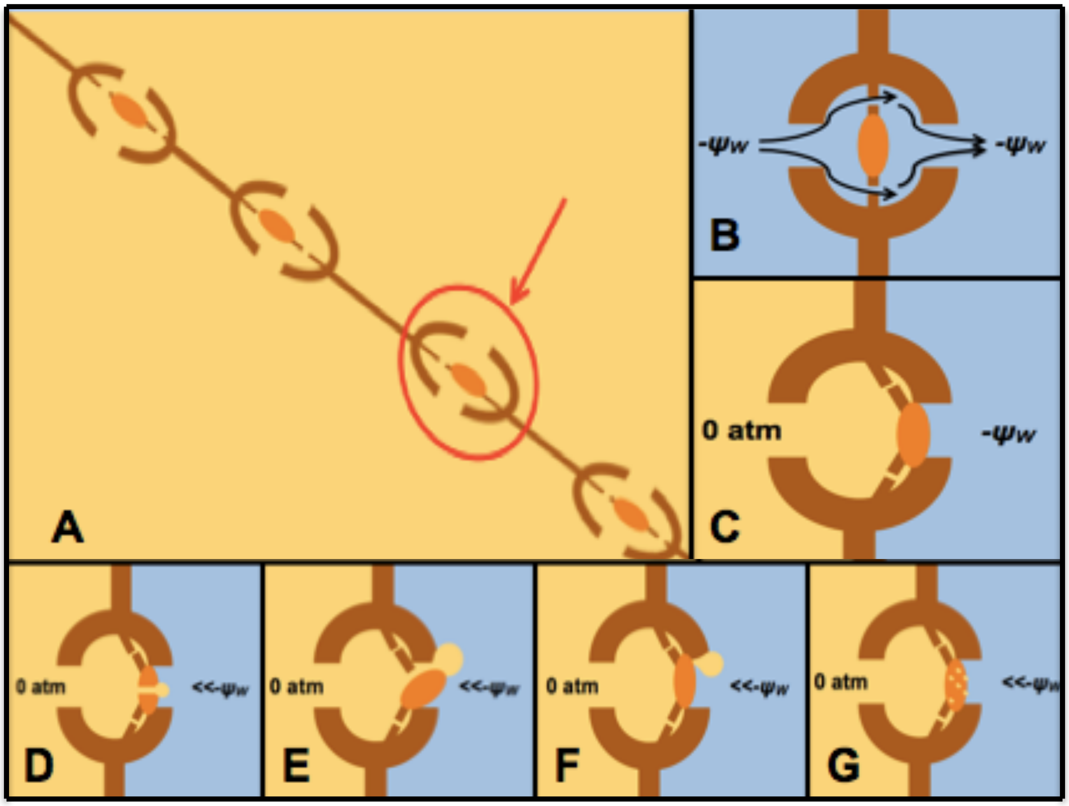

Figure 3. Tracheid pit location, structure, and mechanisms of cavitation (figure by author). (A) Adjacent tracheids make contact at the cell wall to form the xylem conduit when the pit aperture (circled in red) containing a margo (porous membrane) and torus (thickened central region) of one wall lines up with the pit aperture of the second wall. (B) The margo and torus remain in central resting position, allowing water to pass from tracheid to tracheid in cohesion-tension transport when water potentials are increasingly negative. (C) The torus-aperture overlap functions as a check valve when cavitation occurs, as the pressure difference forces the torus over the aperture, preventing the spread of embolism. (D) and (E) Two air-seeding hypotheses explain cavitation when pressure differences between conduits ruptures the torus (D) or the margo (E). (F) Air-seeding may occur when air bubbles travel through the margo and past the torus as a result of a weak seal from an insufficient torus-aperture overlap. (G) The torus may be semi-permeable, allowing air bubbles to escape through microscopic pores when pressure differences are extreme.

Despite the safety valve mechanism of tracheid pits, severe drought and low temperatures can still lead to cavitation events in which air bubbles form within the xylem conduit (Hacke and Sperry 2001). Air bubbles that fail to re-dissolve in xylem sap can potentially expand to fill the lumen of the tracheid, a process known as cavitation, and which results in embolizing the cell. Embolized cells have xylem pressures (P_x) of zero MPa and no longer function in the increasingly negative gradient from soil to atmosphere (Hacke and Sperry 2001; Figure 3). Once a tracheid becomes embolized, the pressure difference between it and the next functional cell becomes so great that the next cell suffers aspiration as well. This process can spread uncontrollably, leading to run-away embolisms. Closure of the stomata can compensate for run-away embolism by reducing transpiration and lowering the pressure between the embolized and functional cells. Meniscus rupture may also be prevented with a lower pressure difference. Embolisms that spread to a critical proportion of other tracheids cause hydraulic failure resulting in desiccation of the needles and eventually death because of the inability to conduct water.

Cavitation events frequently occur in plants without exposure to freezing temperatures. These events are referred to as “drought-induced,” and are generally explained via the air-seeding hypothesis (Zimmerman 1983). Herbivory, fracture, or damage to plants leads to cavitation of injured tracheids and they can fill with air (Hacke and Sperry 2001). In angiosperms, increased evaporative demand or drought may cause unusually negative water potentials in the xylem that can overcome cohesive forces and surface tension at the meniscus of pit pores connecting adjacent vessel elements. The meniscus then ruptures, and an air bubble is pulled into the previously sap-filled and functional vessel element, whereupon because of the lower pressure (tension) in that vessel, it causes that cell to embolize (Tyree and Sperry 1989).

An alternative way that tracheids can become embolized is known as freezing-induced cavitation, which occurs when air bubbles form as xylem water freezes in tracheids. These air bubbles remain isolated in the frozen xylem sap, but expand during thawing due to their higher pressure than the surrounding liquid sap (Charrier et al. 2014). In large tracheids or vessel elements, the air bubbles can expand explosively to embolize the xylem cell. Air bubbles in frozen xylem sap may re-dissolve into solution if the plant produces positive pressure through the process of root pressure (Utsumi et al. 1998), though this becomes less likely with increasingly rapid thawing events and also because root pressure is rarely found in conifers (Hacke and Sperry, 2001).

In conifers such as Fraser fir, tracheids use a xylem safety-valve mechanism to cope with the increased pressure difference between a cavitating cell and the adjacent sap-filled cell. A pressure difference causes the torus to move toward the pit aperture and block further fluid (and air!) passage between the cells (Figure 3B, C). If the pressure difference becomes very large, it may overcome the resistance of the torus, and allow an air bubble to pass from one cell to the next. For example, pressure differences may be great enough to rupture the torus or margo, allowing bubble formation across the air-water interface (Figure 3D, E). Some studies have shown that tori may be smaller in some plants that others (Bouche et al. 2014). A sufficient pressure difference across an insufficient torus-aperture overlap may lead to air bubbles that can escape through the weak seal between the torus and pit aperture (Bouche et al. 2014; Figure 3F). If the torus is semi-permeable, it is also possible that sufficiently large pressure differences may result in air traveling through microscopic pores in the torus and outward expansion once they enter the negative pressure of the adjacent tracheid (Figure 3G) (Jansen et al. 2012).

Non-air seeding cavitation hypotheses state that negative hydrostatic pressure may become great enough to cause implosion of the tracheids leading to hydraulic failure (Hacke and Sperry, 2001; Bouche et al. 2014). Implosion is not likely a common cause of hydraulic failure, as the pressures that exceed lignified tracheid wall resistance tend to cause cavitation by air-seeding first (Choat et al. 2012). In short, the xylem in conifers seems more protected from failure due to implosion than from air-seeding (Domec et al. 2009).

Vulnerability Trends

Previous studies have analyzed the commonalities in tree species that are similarly vulnerable to cavitation based on geographic location and climate. Several safety-efficiency tradeoffs seem to exist, limiting the extent of defense against cavitation and the maximum amount of water a plant can transport per unit time through a xylem conduit. Conduits that conduct more water in higher-volume columns and have larger pit pores also have a much greater risk of cavitation while under tension. Warm weather species, for example, have been found favoring different traits than temperate species. Tropical plants have adapted to conduct more water due to a higher evaporative demand, and can do this because they also have a greater availability of water. Arid species, in contrast, favor xylem safety over efficiency, and have a lower K_h while being more resistant to drought-induced cavitation (Hacke and Sperry, 2001). Hydraulic safety and efficiency were thought to always exist in a state of tradeoff, but recent studies have shown that they frequently correlate weakly. Despite this, no species have been found with high efficiency and high safety, though some species have low efficiency and low safety (Gleason 2015).

Conduit Diameter

Conduit diameter refers to the diameter of the tracheid lumen used for conducting water. Originally, conduit diameter was thought to indicate the safety or efficiency priority of a species, but this does not appear to be the case after further investigation. One study revealed no correlation between conduit diameter and vulnerability to cavitation of angiosperm tracheary elements, and a weakly positive correlation between the two for conifers (Hacke and Sperry, 2001). An additional study on 60 other conifer species found no significant correlation either (Bouche et al., 2014). In an isolated tracheid, greater conduit diameter would be more physically vulnerable to cavitation, but this does not hold across species. Instead, the pit pore diameter is far more responsible for controlling vulnerability to cavitation. Pit pore dimensions do tend to vary with conduit diameter due to the stretching of tracheids as they grow, but size is ultimately more independent than dependent (Hacke and Sperry, 2001).

Pit Pore Variations

Differential pit pore development can have a profound effect on the safety-efficiency tradeoff of conifers through several anatomical mechanisms. The quantity and diameter of pit pores contribute to vulnerability in that greater numbers of pit pores and larger pit pore sizes make a cell more susceptible to cavitation (Neufeld 1992). A larger air-water interface can rupture more easily and the probability of pit pores falling victim to air-seeding increases with every additional pit pore. The porosity of the margo appears to vary as well, with more vulnerable species possessing larger or more numerous pores. Increased margo permeability allows water to pass through tracheids at a greater rate, but leaves greater opportunity for air seeding (Piñol 2000). The torus-aperture overlap of tracheids varies with vulnerability as well. Torus-aperture overlap refers to the ratio of torus diameter to pit aperture diameter. A larger ratio means that the torus covers the aperture to a greater extent, creating a more effective seal. More extensive coverage reduces the likelihood of air-seeding events, as seen in Figure 3F. Trees favoring safety show significantly increased torus-aperture ratios (Bouche et al. 2014).

Wood Density

The density of lignified cell walls in tracheary elements was thought to vary for structural purposes with taller trees in windier or icier environments possessing denser wood. Instead, 48 angiosperm and conifer species had greater wood density when vulnerability to cavitation was lowest, regardless of height or environmental pressures (Hacke and Sperry, 2001). Shorter species in arid environments possessed the densest wood and conducted far less water in favor of reducing the risk of hydraulic failure. This wood density may have adapted to retain xylem integrity and avoid implosion despite higher tensions within the xylem (Hacke and Sperry, 2001). In arid species, this is worth the limitations on growth and storage abilities.

Fraser Fir Hypotheses

Fraser fir trees are farmed at elevations below their native range, which means that they experience higher temperatures, greater evaporative demand, less shading by competitor species and less precipitation and also less interception of fog water. In their native habitat at high elevations, cloud immersion provides 50% of total water intake and the trees are bathed in fog 65% of growing season days (Reinhardt 2008). Despite increased drought risk and higher potential for cavitation, I hypothesized that Fraser fir would have a higher K_h at the lowest elevation farms, due to the higher evaporative demand. A higher K_h would allow the tree to conduct water from the roots to the needles more easily, and be one mechanism to cope with the higher evaporative demand. While arid species have a tendency to prioritize safety against cavitation, low-elevation sites of Christmas tree farms are located on mesic sites with generally adequate rainfall throughout the year (note: farmers rarely if ever irrigate their trees after they are established). A higher K_h implies some sort of anatomical adjustment that allows a higher rate of water flow through the tracheids, such as increased lumen size or larger pit pore sizes, or more abundant pit pores. Other adjustments that a plant might make could include adjustment of biomass allocation such that low elevation trees have fewer needles and/or more roots per unit area of xylem, which would reduce the demand for water by the tree, or an increase in xylem area so as to provide more water for the same number of needles. Conversely, high elevation trees could have a lower $K_{h,}$ as the lower temperature and saturated atmosphere due to cloud immersion would result in a lower evaporative demand. Water potentials from soil to atmosphere would be less negative in the native environment leading to a smaller gradient.

Hypotheses and Objectives

H1: Trees from lower (warmer) elevations may alter xylem structure to enhance water transport to needles, possessing a higher baseline K_h than trees from higher elevations.

H2: Trees from lower elevations will be more susceptible to cavitation due to greater evaporative demand and deprioritizing of xylem safety due to their mesic environment.

Xylem safety and efficiency determined via K_h calculations and vulnerability curves will allow predictions to be made regarding the future of the Christmas tree industry in the North Carolina mountains. As a major export and significant portion of the North Carolina industry, these predictions are vital to determining economic security of the state as climate change progresses.

Materials and Methods

Site Selection

Three Christmas tree farms were chosen to act as high, mid, and low elevation sources of Fraser fir twig samples (Figure 4). The chosen sites were located at 1224 m above sea level in Meat Camp, NC; 1070 m in Foscoe, NC; and 664 m in Eastern Tennessee, respectively. All farms contained relatively uniform spacing of Fraser fir grown from 1.8 to 2.5 m tall in direct sunlight on north-facing slopes. Sites were chosen due to similar tree spacing, lack of irrigation, similar trimming/fertilization agricultural techniques, and similar seed sources to limit genetic variation (Cory, unpublished). No trees were trimmed during 2015 or 2016. Data were obtained using samples from all three sites in 2015 and only from the high and low elevation sites in 2016.

Figure 4. Fraser fir grown on a Christmas tree farm at the high-elevation site (1224 m above sea level, Meat Camp, NC).

Water and Sample Preparation

Before sample collection occurred, 10 liters of microfiltered (0.2 μm pore size) water was acquired and adjusted to a pH of two using 12 M HCl. Low pH discourages microbial growth during collection and storage (McCulloh et al, 2015). The filtered, acidified water was then separated into 10 1 L bottles that were degassed three at a time in a vacuum chamber (~0.073 MPa) for a minimum of 30 minutes. Degassing eliminated any existing bubbles from entering the samples and causing extraneous embolisms that would confound the results. After degassing, bottles were closed and stored until needed.

Samples were collected sporadically throughout late fall/early winter on the afternoon before the experiment was conducted. Trees were numbered such that samples were never taken from the same tree twice. Three twigs from three separate robust, untrimmed Fraser firs were cut with sharp pruning shears immediately after the twig node separating third and fourth-year growth. Smaller, younger twigs were cut off to allow storage in a small cooler filled with 1 L of the previously microfiltered, acidified, and degassed water. Care was taken to ensure that the third-year growth was relatively consistent in length and diameter from twig to twig. Twigs were submerged in water and brought back to the lab within 1 hr of collection.

Once back in the lab, the nodes on either end were clipped off, leaving only the third-year growth portion of the twig. Twigs were then de-needled, debarked, and vacuum infiltrated overnight at (pressure) to remove existing embolisms. Vacuum infiltration occurred in a glass chamber with the twigs submerged in another liter of prepared water (Figure 5A, B).

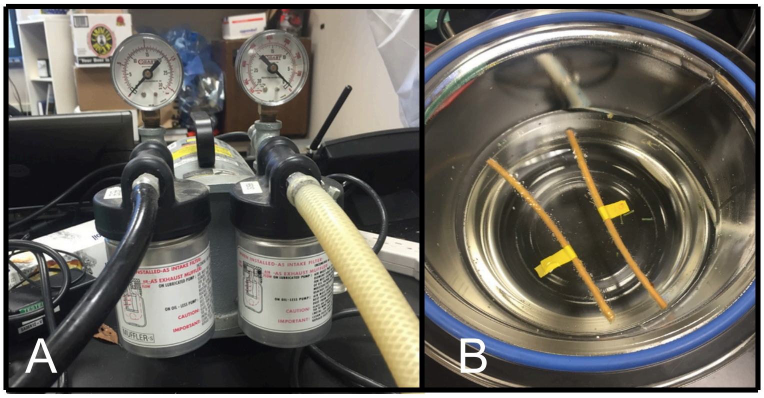

Figure 5. Fraser fir twig preparation. (A) A vacuum container was used to infiltratetwigs and to eliminate existing embolisms. (B) De-needled, debarked twigs were left submerged in a vacuum chamber overnight prior to measuring their K_h the next day.

Experimental Construction

A Sperry Apparatus and air-seeding method were used to simulate the pressure differences while conductivity measurements took place (Sperry and Saliendra 1994). A sealed water reservoir was filled with a 1 L of prepared water and suspended 70-100 cm above the lab bench surface (Figure 6A). The ambient temperature and water height were recorded. A twig was removed from the vacuum chamber, wiped down to eliminate resin or residue, and moved into a glass dish where it was submerged in prepared water. An additional half-centimeter was cut from each end of the submerged sample with clean pruning shears. Any small twigs along the length of the sample twig were cut off with a fresh razor blade as close to the sample twig as possible. The base of the small twig was sealed with super glue and dried with an accelerant to prevent air bubble entry through these larger openings. Tubing extended from the reservoir to a stopcock with additional Tygon Tubing from the stopcock to the end of the twig. Various sizes of tubing and clamps were used to ensure no leakage from the twig contact point (Figure 6B). If air bubbles arose at any point along the tubing, the tubing was manipulated to allow the bubble to rise into the reservoir or a transfer pipette was used to eliminate the air bubble.

Once water was flowing from the reservoir to the twig, the air injection sleeve was secured to a hose that ran through a metal base containing a pressure gauge connected to a tank of nitrogen gas (N_2) (Figure 6C). The air-injection sleeve was assembled around the twig and tightened (Figure 6D). Additional tubing was used to hold a calibrated capillary tube in contact with the distal end of the twig (Figure 6E) with which I could measure the rate of water flow through the twig.

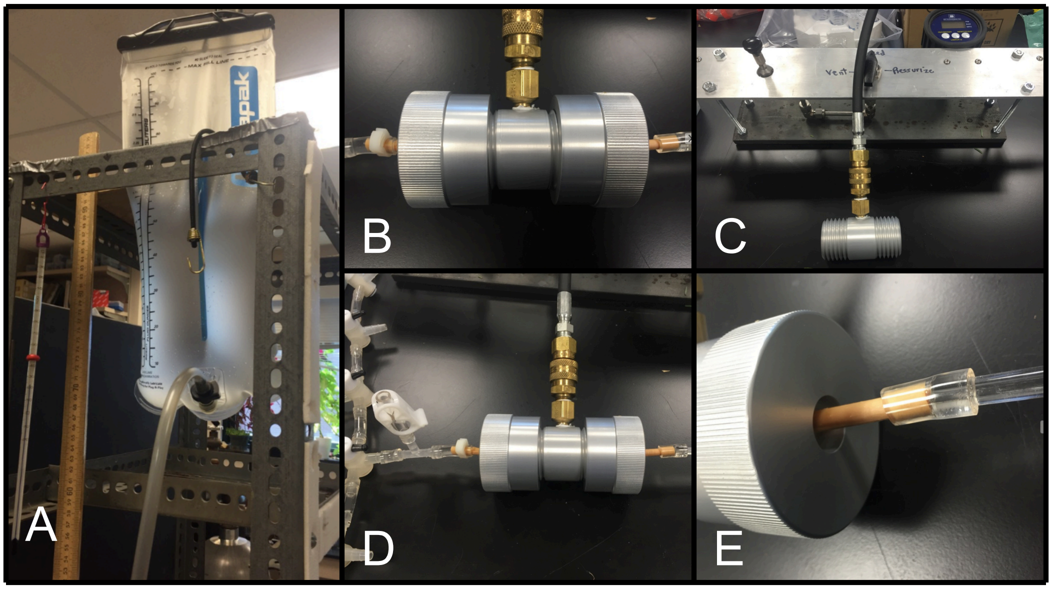

Figure 6. Sperry Apparatus and air-injection method experimental design. (A) The Sperry Apparatus creates a known constant pressure equal and opposite to the tension force created by evaporative demand. (B) Clamps and tubing secure the twig and prevent leakage while water flows. (C) The air-injection sleeve is part of the air-injection method in which positive pressure forces air bubbles into tracheids in the same way that negative pressure pulls air bubbles into tracheids during natural cavitation events (Sperry and Saliendra 1994). (D) The air-injection sleeve contains the middle majority of the twig for pressurization with stoppers at each end. (E) Conducted water exits the twig and enters the capillary tube where it can be measured and quantified.

K_h Data Collection

Three baseline conductivity measurements were made with no pressurization of the air-injection sleeve in order to obtain an estimate of the maximum K_h in twigs that had no embolisms (i.e., they had fully functional xylem). Three baseline conductivity measurements were taken three minutes apart by recording the distance that water traveled from the twig through the capillary tube over a three-minute interval (Figure 7). The tube was calibrated earlier by determining the amount of water contained per cm length of tube. The air injection sleeve was then pressurized to 2.0 MPa (~290 psi) and held there for three minutes. The twig was then given two minutes to relax and three conductivity measurements were recorded, each three minutes in length. Between pressurizations, care was taken to ensure that bubbles were eliminated from the tube and that escaping air from the pressurization was directed out an extra valve, rather than back into the prepared water. This process was repeated at 3.0 MPa, 4.0 MPa, 5.0 MPa, 6.0 MPa and 7.0 MPa, or until all conductivity ceased. The length and average diameter of the twig was recorded, after which it was dried for at least 24 hours at 65° C in a drying oven before obtaining the dry weight. Wood density was calculated as g dry weight divided by volume, where volume was determined by assuming that the twig was a cylinder with a diameter the average of the distal and basal diameters and multiplying the cross sectional area times its length.

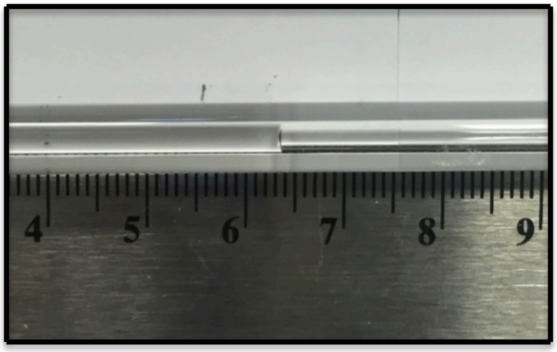

Figure 7. K_h was determined by measuring the distance water traveled in a calibrated capillary tube.

Statistical Analysis

The K_h after each level of pressurization was calculated and the means for twigs at each elevation compared using two-tailed *t*-tests with a level of significance indicated by $p<0.05$. K_h is calculated from the mass of water moving through a unit length of branch in a known length of time under a known pressure (Heine 1970). This value is corrected for sapwood area of the branch and displayed in units Kg m MPa^{-1} s^{-1}. Vulnerability curves were constructed to display change in K_h as pressure decreased. The K_h at each pressure was used to determine percent of the maximum K_h, using the baseline K_h as the maximum. These percentages were graphed against the changing pressure. Comparisons were made of the water potential at which K_{h} was reduced by 50% and also for the baseline K_h in the absence of any water stress; *T*-tests were also used to compare wood density between elevations.

Results

We chose the fall and winter seasons because growth would have ceased during these times and the xylem measured would be mature. Sample site characteristics are shown in Table 1.

Hydraulic Conductivity (K_h)

2015-2016

Low, mid and high-elevations sites were utilized exclusively in this data set. Hydraulic conductivity was highest at the low-elevation site and lowest at the mid-elevation site with baseline K_h values at 4.66 ± 3.97 x 10^{-6} Kg m MPa^{-1} s^{-1} and 1.67 ± 9.20 x 10^{-6} Kg m MPa^{-1} s^{-1}, respectively. Mean high elevation-conductivity K_h was closer to that of the mid-elevation average (Figure 8A). Results were statistically significant between the low and mid-elevation sites, showing a difference in K_h between these data sets ($p = 0.047$). There were no statistically significant differences between the low and high-elevation or mid and high-elevation sites ($p = 0.143$, $p = 0.672$, respectively; Figure 8A).

2016-2017

The most recent K_h data were taken from the low and high-elevation sites only. Unlike the previous year's data, the high-elevation site showed the greatest average baseline K_h at 1.86 ± 5.02 x 10^{-6} Kg m MPa^{-1} s^{-1} compared to the low-elevation site at 1.27 ± 5.79 x 10^{-6} Kg m MPa^{-1} s^{-1} (Figure 8B). There was no statistically significant difference between the mean K_h of these sites ($p = 0.205$) (Figure 8B). Results between years are comparable with the exception of the unusually high value of the low elevation site in 2015-2016 (Figure 8A, 8B).

Table 1. Summary of sample site characteristics. The Johnson, TN lowest elevation site simulates a warmer, drier environment than the natural Fraser fir range in the Southern Appalachians (Cory 2015).

Site Name	Elevation (m)	Elevation Category	Geographical Coordinates	County, State	Planting Density (# trees per ha)
Carroll1	664	Low	36°22'0.95"N 82° 0'54.47"W	Johnson, TN	4499
Thad	1021	Middle	36°10'42.10"N 81°45'37.76"W	Watauga, NC	4026
Jim	1224	High	36°17'23.86"N 81°40'55.92"W	Watauga, NC	4090

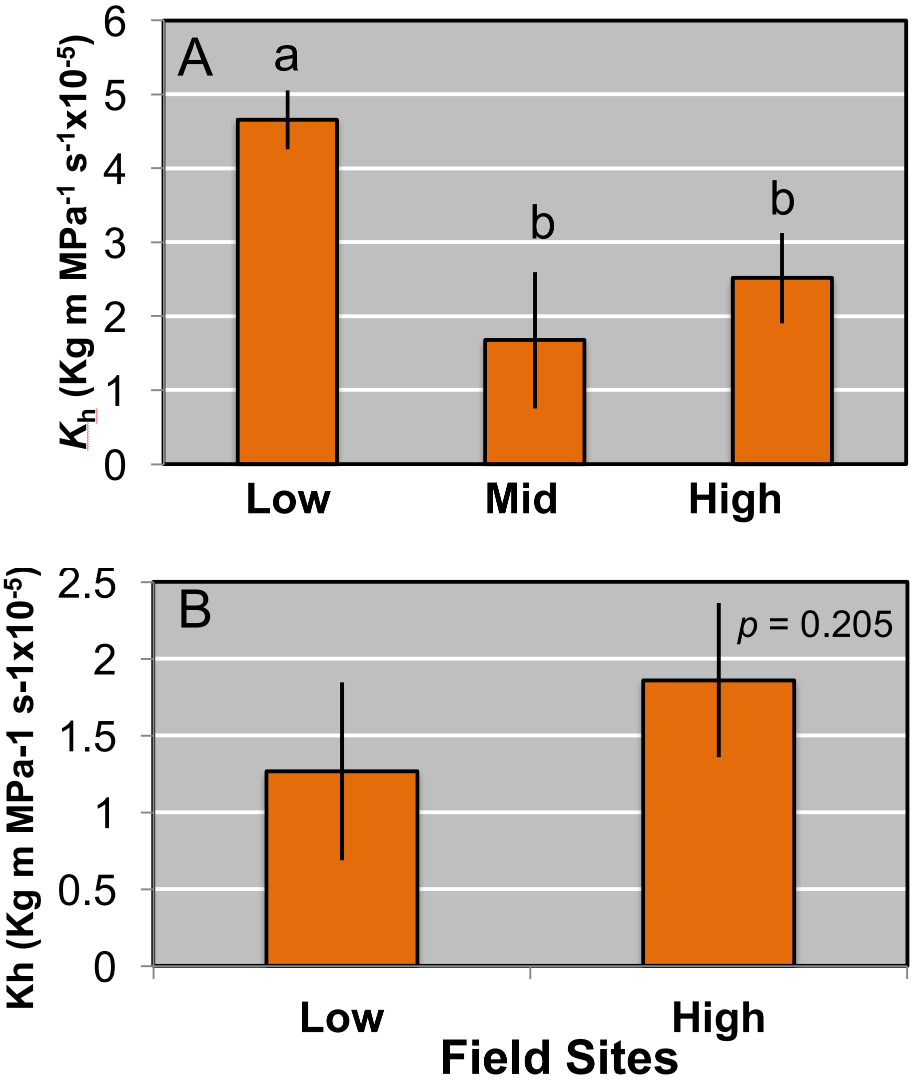

Figure 8. Baseline K_h at various elevations. (A) Baseline K_h was determined for the low (760 m), middle (1070 m), and high (1370 m) twigs in fall 2015-winter 2016. Bars labeled “b” are the same while bars labeled “a” are different. (B) In fall 2016-winter 2017, K_h was determined for twigs from the high and low sites only (n = 2-4).

Vulnerability Curves

2015-2016

Three low-elevation twigs and two high-elevation twigs illustrate the decreasing flow of water occurring as pressurization increases successively by one MPa. P_{50} refers to the pressure at which hydraulic conductivity is 50% of the baseline maximum. P_{50} was induced by pressures ranging from ~3.5-5 MPa for low-elevation twigs and ~5-6 MPa in high-elevation twigs (Figure 9A). Although these data show a greater average pressure for high-elevation twigs when compared to low-elevation twigs (5.27 ± 0.22 MPa and 4.17 ± 0.43 MPa, respectively) there was no statistically significant difference between the data sets, indicating that P_{50} does not differ from low to high elevation twigs ($\alpha = 0.05$, $p = 0.0759$) (Figure 10A).

2016-2017

P_{50} averages are comparable to those of 2015-2016. P_{50} was induced from ~3-5.5 MPa in low-elevation twigs and from ~3.7-5.5 MPa in high-elevation twigs (Figure 9B). Far more overlap is visible in the 2016-2017 data when compared to the 2015-2016 data (Figure 9A). The low-elevation site has an average P_{50} of 4.22 ± 1.04 MPa and the high-elevation site had a P_{50} of 4.16 ± 0.628 (Figure 10B). There was no statistically significant difference between the high and low-elevation sites ($p = 0.584$) (Figure 10B).

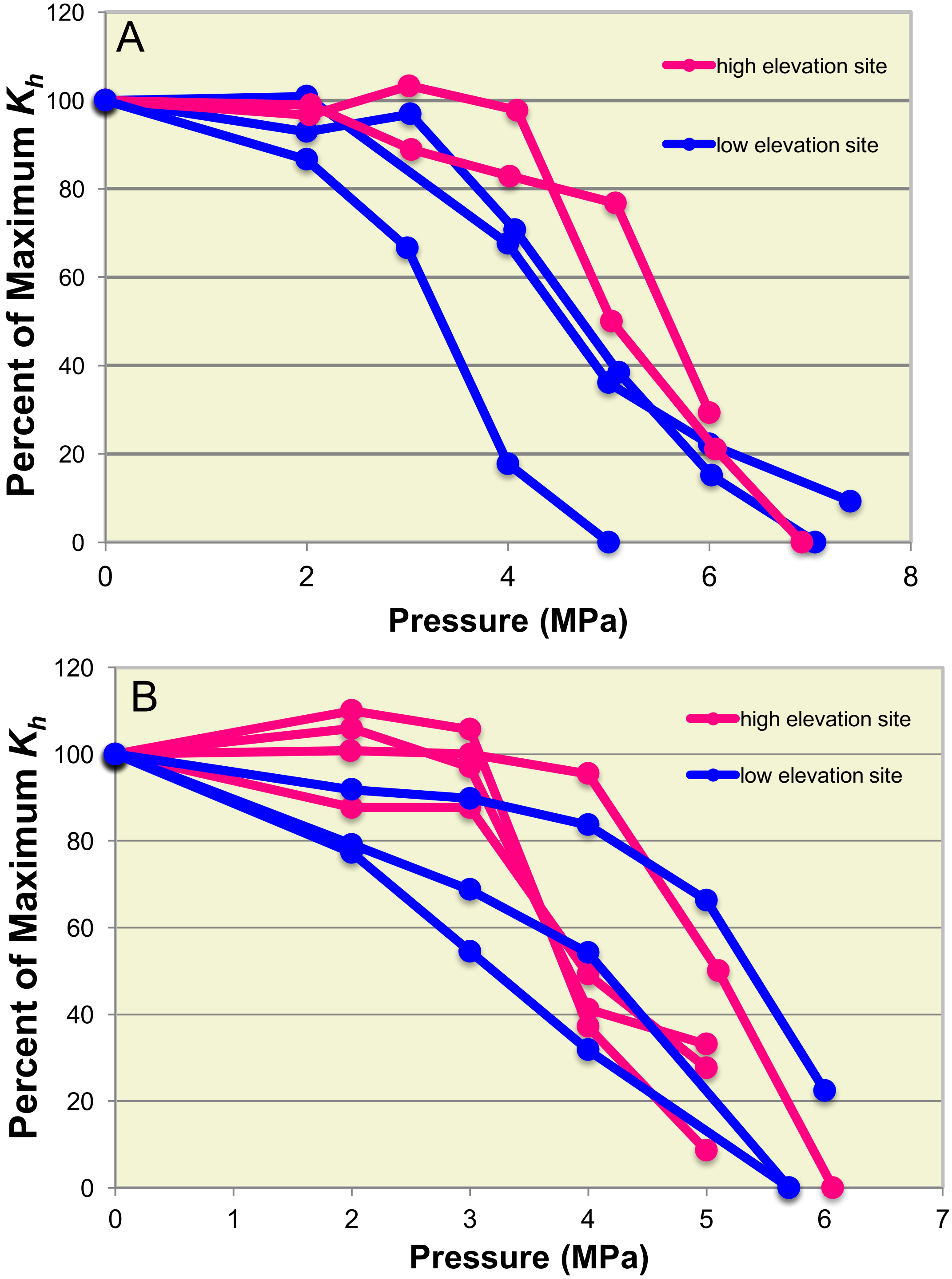

Figure 9. PV Curve showing percent of original flow rate (maximum K_h) as pressurization increases during 2015-2016 (A) and 2016-2017 (B).

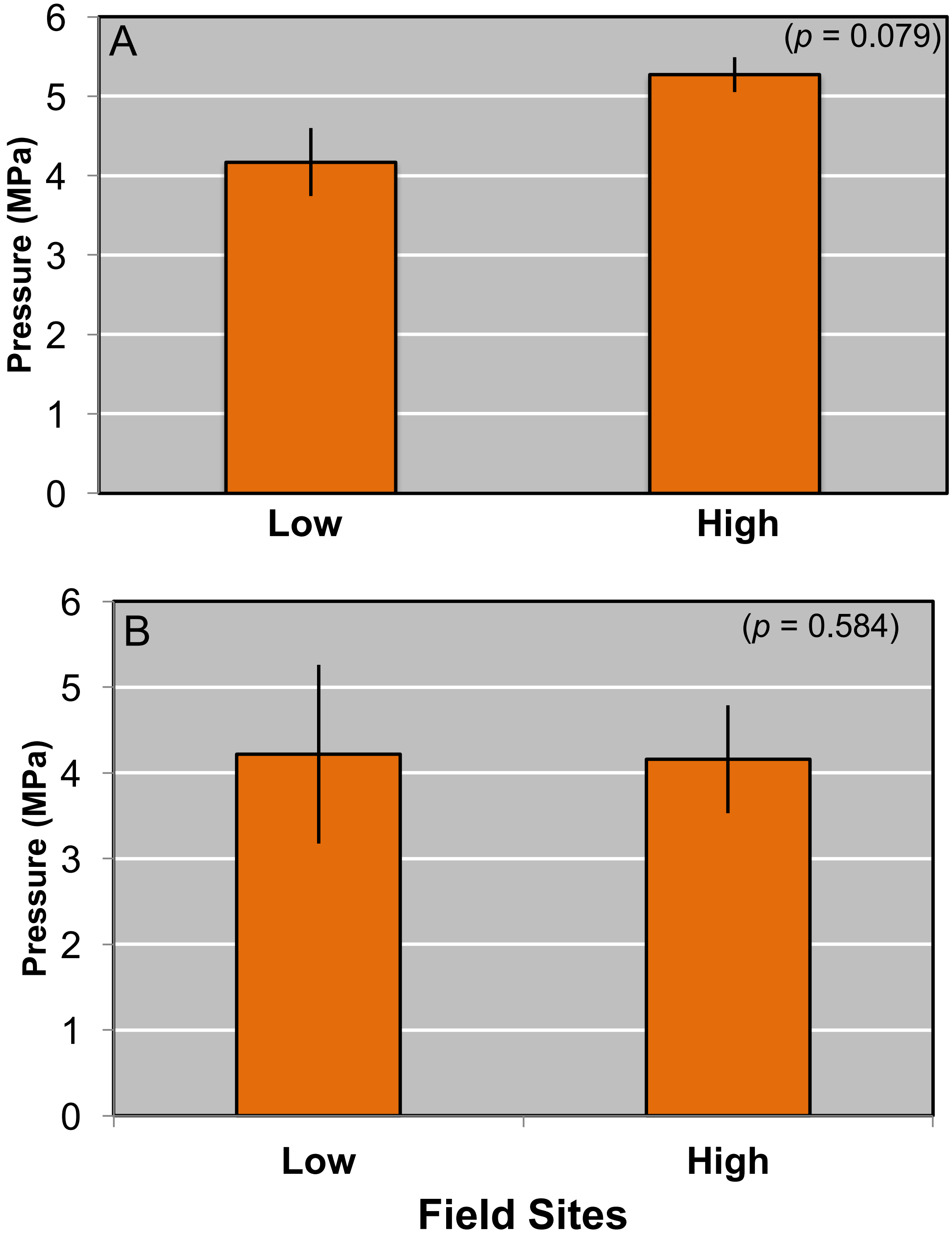

Figure 10. Pressure in MPa required to induce a 50% loss in K_h. Neither (A) 2015-2016 twigs nor 2016-2017 twigs showed significant difference in pressurization to induce K_{h50} between low and high elevation sites.

Wood Density

Wood density was determined for the low and high-elevation twigs from 2016-2017. The low-elevation site had a greater average density of 0.56 ± 0.04 g cm^{-3} compared to the high-elevation site average of 0.48 ± 0.04 g cm^{-3} (Table 1). The wood density of low-elevation twigs was significantly greater than that of high-elevation twigs (p = 0.039) (Figure 11).

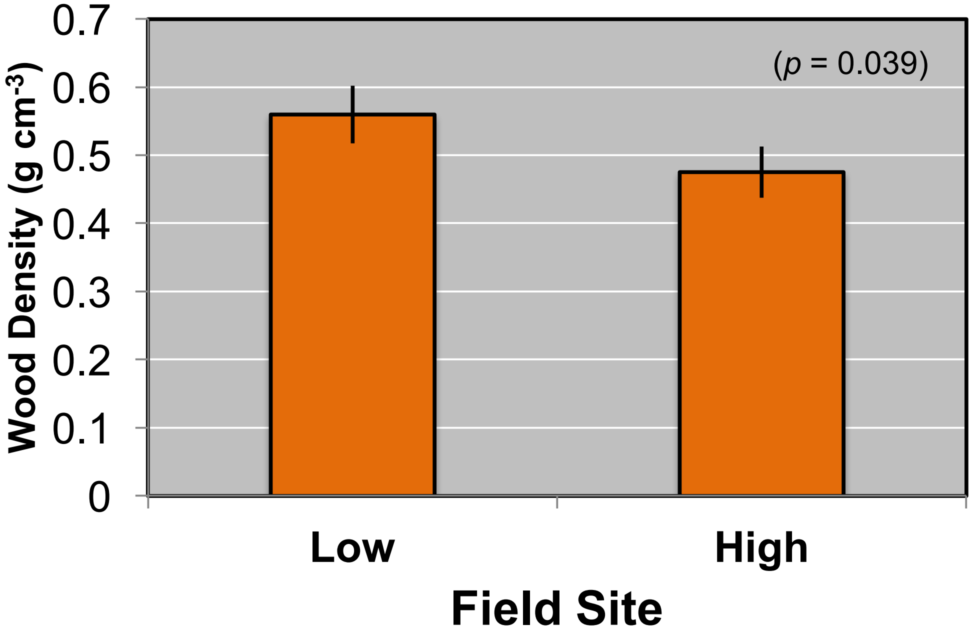

Figure 11. Wood density of 2016-2017 samples at low and high elevation sites. Low elevation sites had significantly greater wood densities than high elevation sites.

Discussion

Across the elevation gradient and over two years of data collection, there were no significant differences for Fraser fir Christmas trees in their vulnerability to cavitation as measured by the response of K_h to air seeding. Additionally, there was no evidence to suggest that maximum K_h differed substantially across the range of elevations covered in this study. This suggests that Fraser fir Christmas trees are relatively insensitive to the magnitude of change in climate from their lowest growing sites to those at the highest elevations. This 560 m difference corresponds to an average adiabatic temperature decline of ~3.6° C over the range. Furthermore, it suggests that mechanisms other than those associated with the vulnerability of xylem to cavitation may be responsible for allowing this species to grow over such a large elevational range.

Fraser firs growing at lower elevations encounter higher temperatures, higher solar radiation levels, greater vapor pressure deficit (VPD) and greater daily evaporative demand (Cory 2015). Trees may cope with these changes using a variety of mechanisms to transport adequate water to the needles and prevent excessive water loss. In lieu of a larger water transport mechanism, Fraser firs at the low-elevation site may be closing their stomata more frequently to reduce water loss and adjust their conductances to water vapor to match that of liquid water in the xylem (Bryukhanova and Fonti, 2012). Alternatively, Fraser firs could alter the ratio of needles to xylem cross-sectional area. Fewer needles would require less water collectively, reducing demand. These trees would have fewer total stomata, functioning similarly to more frequent stomatal closure by reducing the opportunity for water loss by transpiration. Another mechanism, and one focus of this study, is that K_h could increase to match higher stomatal conductance of low-elevation site trees, which would allow Fraser fir trees to thrive in warmer climates with higher evaporative demand.

Hydraulic Conductivity

I had hypothesized that the higher temperatures and higher evaporative demand of the low-elevation site would have resulted in adaptive measures by Fraser firs to increase K_h. Such increases can be effected by larger conduit diameters, more pit pores, larger pit pores or increased pit membrane porosity (Hacke and Sperry, 2001).

In this study, there were no statistically significant differences between low and high-elevation site twigs in 2015-2016 or 2016-2017, indicating that Fraser firs are not modifying their xylem anatomy in any of the aforementioned ways (Figure 8A, B). This is consistent with Wood's study (Wood 2016) showing a lack of differences in xylem conduit diameters and cell wall thicknesses for trees growing at these different elevations.

The only significant result was that the low-elevation K_h average was significantly higher than that of the mid-elevation trees (Figure 8A). This was an unexpected result, as low and high elevation K_h measurements were the same despite greater elevation difference. The use of first-year growth twigs in 2015-2016 rather than third-year twigs as in 2016-2017 may have contributed to this result. Conductivity differences in newer growth may be due to immature wood or unique weather during xylem formation that differed from weather in previous years. Or, cultivation techniques at the mid-elevation site could have differed from those at the other two sites, although we took care to ensure that such practices were as similar as possible. The mid-elevation site did have more rainfall than the other two sites, at least in 2014 (Cory 2015), and early seasonal rain could have resulted in xylem with higher K_h. More research should be done on this topic.

The lack of difference between low and high elevation trees in K_h may indicate that these trees were not be stressed enough by the elevation gradient to change their hydraulic architecture, or, they may be incapable of this plasticity. In this study, the difference in elevation between the low and high elevation sites was 560 m. However, other conifers appear to have the capacity to adjust K_h along an elevational gradient of similar magnitude. For example, K_h decreased over a 500 m rise in elevation in *Pinus cembra*,(Losso et al. 2015), over a 900 m rise in elevation in *Picea abies* (Castagneri et al. 2015), and over a ~1000 m rise in elevation in *Pseudotsuga menziesii* (Panek 1995). In Castagneri et al.'s study, increased K_h at lower elevations was due to increased cell lumen area and more tracheids per unit xylem. It would be interesting to compare the xylem anatomy of Fraser firs growing in their native habitat, at elevations of 1372 m or above with those at the lowest elevation, which would be a 760 m difference in elevation, some 200 m larger than the gradient I used in this study.

Despite a lack of change in the water transport mechanisms, farmed Fraser fir grow to similar heights as wild Fraser fir and appear to thrive in farms over 300 m below their natural range (Wood, unpublished). This may mean that Fraser fir survival at low-elevation is restricted by something other than water relations or that

Fraser fir is particularly drought and temperature tolerant. Competition may be the limiting factor for low-elevation growth. As a slow-growing species, Fraser firs could be easily shaded out by faster growing species, compromising their ability to photosynthesize. Wild Fraser firs may have adapted by moving to high-elevation mountaintops where few other species can thrive and competition is significantly decreased (Kelly and Goulden 2008; Beckage et al. 2008). The shorter and cooler growing seasons at high elevations would favor evergreen species such as the Fraser firs, as they can begin photosynthesizing as soon as weather conditions become favorable, and can continue into the fall when the other species have become dormant. Deciduous trees have to devote a portion of the spring season to forming new leaves, which puts them at a disadvantage when compared to the Fraser firs, and they can't continue to take up carbon in the fall because their leaves have fallen off.

Christmas tree farmers plant their trees in evenly spaced rows in open fields with direct sun exposure. No shade trees or other competitive species are allowed on these farms, so competition is essentially eliminated. Furthermore, they are periodically fertilized, and sprayed for pests, the combination of which allows them to persist in these sites, which are far below their natural habitat.

The results of my study are encouraging for North Carolina Fraser fir farmers, as they imply that impending climate changes won't affect the ability of Fraser firs to flourish in areas with higher evaporative demand so long as they are grown under controlled conditions, as on a Christmas tree farm. Unfortunately, wild Fraser fir populations will remain largely at risk as future temperatures rise and competitive species move to higher elevations. In Vermont, researchers have documented the upward movement of deciduous trees species, and the displacement of red spruce and balsam fir, just since the 1960s, as temperatures have risen significantly in that state (Beckage et al. 2008) and in California, rising temperatures have resulted in many species moving up in elevation also (Kelly and Goulden 2008).

Fraser firs in their native habitat are known to depend on cloud water for up to 50% of their annual water budget, and can directly absorb moisture from fog (Berry 2012), and if the climate warms, the cloud base may rise, reducing the amount of time these trees are bathed in fog, as well as the amount of moisture taken up. Such changes could favor deciduous trees over the evergreen Fraser firs, changing completely this endangered ecosystem found only on 7 peaks in the southern Appalachians (Berry and Smith 2012).

Vulnerability Curves

Figures 9 and 10 indicate that Fraser fir do not appear to adapt their wood anatomy in ways that alter vulnerability to embolism by air-seeding. During the 2015-2016 and 2016-2017 seasons, there was no significant difference between the pressures required to induce a 50% loss in K_{h} in low and high-elevation twigs. Figure 9 shows a significant amount of overlap in PV curves among trees from different elevations as well. Any of the mechanisms that would have increased the baseline K_h would also have left more opportunity for air-seeding to occur. Though these results do not elucidate the adaptive abilities of Fraser fir, they are consistent in that K_h and vulnerability should correlate positively.

Xylem plasticity of Fraser fir has not been extensively studied, yet some studies have examined long-term and short-term structural adaptations of other species. One such study examined *Larix decidua and Picea abies*, finding that adaptations constituting short-term plasticity fell just below the significance level (Bryukhanova and Fonti, 2012). This study hypothesized that a greater environmental gradient may have yielded significant results. Though no significance was present in K_h and P_{50} comparisons, higher temperatures producing a greater amount of stress for Fraser firs may be necessary to see any modifications in wood anatomy.

It is also important to note that while the 2016-2017 results had a p value falling well above the level of significance, the 2015-2016 results had a p value only 0.029 higher than the level of significance (Figure 10). The 2016-2017 collection period fell right within a period of record high temperatures and extended drought that may have influenced the anatomy and adaptive behavior of Fraser fir. Collected twigs, vacuum infiltrated overnight, often had to be wiped down due to a thick layer of a mucilaginous substance that varied substantially in thickness. Some twigs from the same sites had very little, while occasionally, others experienced a layer up to 0.5 cm thick (Figure 12). This layer was not nearly as pronounced in 2015-2016 as in 2016-2017, indicating that the unusually warm weather and drought may have been the cause. If this substance is within the internal wood anatomy, it could have impacted K_h and vulnerability measurements.

Figure 12. Gel-like substance accumulating on twig surface after overnight vacuum infiltration.

Wood Density

Wood density of branches has been measured in previous studies of vulnerability as a feature of wood anatomy that is plastic in response to environmental changes. Some studies have shown wood density to be the only affected xylem feature over an elevation gradient. Bryukhanova and Fonti (2012) found that the lumen and cell walls were the predominant aspect of xylem anatomy adapting to significant environmental changes in other conifer species. Additionally, studies of wood density in angiosperms and conifers have shown that arid species have significantly greater wood density than their temperate counterparts, even if those counterparts are taller or subject to severe wind (Hacke and Sperry, 2001).

In this study, wood density was significantly higher in low-elevation twigs than high-elevation twigs, indicating that Fraser firs grown in a warmer, drier environment develop thicker tracheid walls. Fraser firs in low elevation sites may be subject to greater negative tensions due to greater water stress from higher evaporative demand. This greater negative tension could lead to the collapse of xylem cells. Thicker cell walls, or walls with structural features that prevent implosions, might be selected for, but often at the cost of reducing lumen diameter (Hacke and Sperry 2001). This would likely hinder water flow and reduce K_h. However, the data from Wood (2016) did not find any significant differences in lumen diameters, nor cell wall thickness, which is puzzling. One possibility is that cellulose microfibrils could have been more densely packed in the low elevation twigs without causing a measureable change in cell wall thickness. The issue of environmental impacts on xylem anatomy in Fraser firs deserves more attention in order to resolve these issues.

Wild Fraser Fir

While studies of farmed Fraser fir and *in situ* embolism vulnerability are effective for predicting the implications of climate change on the Christmas tree industry, several limitations exist when applied to Fraser fir in the native range. The high-elevation site in Meat Camp, NC is still lower in elevation than the Fraser fir native range. Additionally, farmed Fraser firs are equally spaced, close together and on mild inclines or flat ground, unlike wild Fraser firs. The twigs used in this study were not exposed to harsh wind speeds and wind chill characteristic of the highest elevation mountain habitats. Further study regarding the conductivity and wood anatomy of wild Fraser fir should be conducted to determine their vulnerability in their native range and observe how their wood anatomy differs from farmed Fraser fir.

Conclusion

As climate change progresses and intensifies, ecosystems and their native species will begin to experience changing temperatures, altered weather events and moisture that may eliminate vulnerable organisms. As far as Fraser fir is concerned, the temperature and moisture changes predicted for the next ~80 years (Allen et al. 2010) are not likely to severely impact the ability of the species to thrive in a farmed environment, though wild trees may seek refuge at higher elevations. Fraser fir did not appear to be experiencing major vulnerability and conductivity changes from a high to low-elevation site. Additionally, noticeable impairments in conductivity were not evident until at least 2 MPa of pressure was applied while P_{50} only sparingly occurred before 3.5 MPa of pressure was applied. A previous study by Lauren Wood (2016) determined that the most negative water potentials experienced in the field did not exceed -1.8 MPa and these are not sufficient to induce embolisms in young twigs. Christmas tree farmers in the Southern Appalachians do not appear to be at risk of losing their crop, nor is North Carolina at risk of losing a sizeable industry in the foreseeable future.

References

A Farjon. 2013. *Abies fraseri*. The IUCN Red List of Threatened Species.

Allen CD, Macalady AK, Chenchouni H, Bachelet D, McDowell N, Vennetier M, Kitzberger T, Rigling A, Breshears DD, Hogg EH, Gonzalez P, Fensham R, Zhang Z, Castro J, Demidova N, Lim JH, Allard G, Running SW, Semerci A, and N Cobb. 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. Forest Ecology and Management 259:660-684.

Beckage B, Osborne B, Gavin DG, Pucko C, Siccama T and T Perkins. 2008. A Rapid Upward Shift of a Forest Ecotone during 40 Years of Warming in the Green Mountains of Vermont. Proceedings of the National Academy of Sciences of the United States of America 105:4197-4202.

Berry ZC and WK Smith. 2012. Cloud pattern and water relations in *Picea rubens* and *Abies fraseri*, southern Appalachian Mountains, USA. Agricultural and Forest Meteorology 163:27-34.

Bouche PS, Larter M, Domec JC, Burlett R, Gasson P, Jansen S and S Delzon. 2014. A broad survey of hydraulic and mechanical safety in the xylem of conifers. Journal of Experimental Botany 65:4419-4431.

Bryukhanova M and P Fonti. 2012. Xylem plasticity allows rapid hydraulic adjustment to annual climatic variability. Trees 27:485-496

Castagneri D, Petit G and M Carrer. 2015. Divergent climate response on hydraulic-related xylem anatomical traits of *Picea abies* along a 900-m altitudinal gradient. Tree Physiology 35:1378-1387.

Charrier G, Charra-Vaskou K, Kasuga J, Cochard H, Mayr S, and T Ameglio. 2014. Freeze-thaw stress: effects of temperature on hydraulic conductivity and ultrasonic activity in ten woody angiosperms. Plant Physiology 164:992-998.

Chastagner, GA, and MD Benson. 2000. The Christmas tree: Traditions, production, and diseases. Plant Health Progress. Plant Health Rev 10:1094

Choat B, Jansen S, Brodribb TJ, Cochard H, Delzon S, Bhaskar R, Bucci SJ, Field TS, Gleason SM, Hacke UG, Jacobsen AL, Lens F, Maherali H, Martinez-Vilalta J, Mayr S, Mencuccini M, Mitchell PJ, Nardini A, Pittermann J, Pratt RB, Sperry JS, Westoby M, Wright IJ, and AE Zanne. 2012. Global convergence in the vulnerability of forests to drought. Nature 491:752-755.

Collins M, Knutti R, Arblaster J, Dufresne JL, Fichefet T, Friedlingstein P, Goa X and WJ Gutowski. 2013. Chapter 12 – Long-term climate change: Projections, commitments and irreversibility. Cambridge University Press 1029-1136.

DE Beck. 1990. Abies fraseri (Pursh) Poir. Fraser fir. Silvics of North America 1:47-51.

Ding Y, Zhang Y, Quan-Shui Z and MT Tyree. 2014. Pressure-volume curves: revisiting the impact of negative turgor during cell collapse by literature review and simulations of cell micromechanics. New Phytologist 203:378-387.

Dixon HH, J Joly. 1894. On the ascent of sap. Philosophical Transactions of the Royal Society of London 186:563-576.

Domec JC, Warren J, Lachenbruch B, and FC Meinzer. 2009. Safety factors from air seeding and cell wall implosion in young and old conifer trees. International Association of Wood Anatomists 30:100-120.

Ewers, FW, and P Cruiziat. 1991. Measuring water transport and storage. Techniques and Approaches in Forest Tree Ecophysiology 91-115.

Hacke UG and JS Sperry. 2001. Functional and ecological xylem anatomy. Perspectives in Plant Ecology, Evolution and Systematics 4:97-115.

JA Panek. 1996. Correlations between stable carbon-isotope abundance and hydraulic conductivity in Douglas-fir across a climate gradient in Oregon, USA. Tree Physiology 16:747-755.

Jansen S, Lamy JB, Burlett R, Cochard H, Gasson P, and S Delzon. 2012. Plasmodesmatal pores in the torus of bordered pit membranes affect cavitation resistance of conifer xylem. Plant, Cell & Environment 35:1109-1120.

Kelly AE and ML Goulden. 2008. Rapid shifts in plant distribution with recent climate change. Proceedings of the National Academy of Sciences 105:11823-11826.

Little EL. Atlas of United States Trees. United States Geoscience Data Catalog.

LK Wood. 2016. Using an elevation gradient as a surrogate for climate warming to understand the effects on wood anatomy and water relations of Fraser fir (*Abies fraseri*) Christmas trees. Masters Thesis. Appalachian State University.

Losso A, Nardini A, Nolf M and M Stefan. 2016. Elevational trends in hydraulic efficiency and safety of *Pinus cembra* roots. Oecologia 180.4:1091.

McCulloh KA, Johnson DM, Petitmermet J, McNellis B, Meinzer FC, B Lachenbruch. 2015. A comparison of hydraulic architecture in three similarly sized woody species differing in their maximum potential height. Tree Physiology 35:723-731.

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

Mitcham-Butler EJ, Hinesley LE, and DM Pharr. 1988. Effects of harvest date, storage temperature, and moisture status on postharvest needle retention of Fraser Fir. Journal of Environmental Horticulture.

NC Cooperative Extension. 2011. North Carolina Christmas Trees by the Numbers. North Carolina State University.

Neufeld HS, Grantz DA, Meinzer FC, Goldstein G, Crisosto GM and C Cristoso. 1992. Genotypic variability in vulnerability of leaf xylem to cavitation in water-stressed and well-irrigated sugarcane. Plant Physiology 100:1020-1028.

Piñol J and A Sala. 2000. Ecological implications of xylem cavitation for several *Pinceae* in the Pacific Northern USA. Functional Ecology 14:538-545.

Pittermann J, Sperry JS, Hacke UG, Wheeler JK and EH Sikkema. 2005. Torus-margo pits help conifers compete with angiosperms. Science 310:1924.

RW Heine. 1970. Hydraulic conductivity in trees. Journal of Experimental Botany 22:503-511.

Reinhardt K and WK Smith. 2008. Leaf gas exchange of understory spruce-fir saplings in relict cloud forests, southern Appalachian Mountains, USA. Tree Physiology 28:133-122.

S Cory. 2015. Growth and ecophysiological responses of Fraser fir (*Abies fraseri)* Christmas trees across an elevational gradient. Masters Thesis. Appalachian State University.

SM Gleason, Westoby M, Jansen S, Choat B, Hacke UG, Pratt RB, Bhaskar R, Brodribb TJ, Bucci SJ, Cao KF, Cochard H, Delzon S, Domec JC, Fan ZX, Field TS, Jacobsen AL, Johnson DM, Lens F, Maherali H, Martinez-Vilalta J, Mayr S, McCulloh KA, Mencuccini M, Mitchell PJ, Morris H, Nardini A, Pitterman J, Plavcova L, Schreiber SG, Sperry JS, Wright IJ and AE Zanne. 2015. Weak tradeoff between xylem safety and xylem-specific hydraulic efficiency across the world's woody plant species. New Phytologist 209:123-136.

Sperry JS and NZ Saliendra. 1994. Intra- and inter-plant variation in xylem cavitation in *Betula occidentalis*. Plant, Cell & Environment 17:1233-1241.

TJ Brodribb. 2009. Xylem hydraulic physiology: The functional backbone of terrestrial plant productivity. Plant Science 177:245-251.

Tyree MT, and JS Sperry. 1989. Vulnerability of xylem to cavitation and embolism. Annual Review of Plant Biology 40: 19-36.

Utsumi Y, Sano Y, Fujikawa S, Funada R and J Ohtani. 1998. Visualization of cavitated vessels in winter and refilled vessels in spring in diffuse-porous trees by cryo-scanning electron microscopy. Plant Physiology 117:1463-1471.