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

A Thesis
by
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Department of Biology

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Abstract

Fraser fir (*Abies fraseri)* Christmas trees are an important part of the agro-economy of Western North Carolina, accounting for $100 million dollars in annual sales. In order to assess how warming-driven stressors of climate change could affect these trees, I examined water relations and wood anatomy of Fraser fir Christmas trees along an elevation gradient from 664 m to 1228 m. Low elevations exhibited the highest daytime maximum temperatures and higher associated evaporative demand (greater vapor pressure deficit, VPD) than the middle and high elevations, while high elevations experienced greater cloud cover and immersion than the lower elevations, as expected. Stomatal conductance varied over site and season, showing a strong response to VPD and temperature changes. Diurnal water potentials likewise displayed a change over the season and with elevation, with the lowest water potential at the low elevation in the early growing season. In the mid-season the high elevation trees exhibited the lowest water potential because of a high VPD. Trees did not experience enough water stress to induce embolism in the current field sites and conditions. Hydraulic conductivity did not exhibit a change across elevations. From an anatomical perspective, xylem lumen area was similar among all sites. The tracheid count, however, differed among sites with the fewest cells/mm^2 at the low elevation. Middle elevations had the greatest cell wall thickness but neither differences in the tracheid anatomy was great enough to change alter the hydraulic conductivity. Trees across elevations tended to compensate for warming by

physiology, namely stomatal control, rather than adjust anatomy. With climate warming, dryer soil, and lower relative humidity, physiological functioning will decrease and low elevation farms may experience longer periods of stress during the growing season.

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Dedication

Thank you to my sweet, supportive parents, Mary Beth and Bert Wood, who continue to encourage me to follow my passions and to my friends, particularly Scott Satterwhite, for being sounding boards and making me a better scientis

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Foreword

This project was done in close collaboration with Scott Cory in a broader physiological study focused on water and carbon relations, phenology, and wood anatomy. Our results will be combined for publications. The citations are done in the style of the journal *Tree Physiology*.

Introduction

Fraser fir (*Abies fraseri* [Pursh] Poir.) is a relict species from the last glaciation and is endemic to the highest seven peaks (> 1500 m) in the southern Appalachian Mountains (Oosting and Billing 1951, Cogbill and White 1991). It benefits from cooler temperatures and frequent cloud immersion, with up to 30% of whole-plant water supplied by cloud fog (Johnson and Smith 2006, Berry and Smith 2012, Berry et. al. 2014a, Berry et. al. 2014b). Evidence suggests that cloud immersion provides sufficient light saturation for optimum photosynthesis, via diffusion to the 3 dimensional needles, and enhances photosynthetic rates and water status, particularly for areas with a more closed canopy (Johnson and Smith 2006, Berry et. al. 2014b). Cloud immersion also lowers the vapor pressure deficit (VPD), which can increase stomatal conductance (g_s), and allow for more CO_2 uptake (Reinhardt and Smith 2007). Foliar uptake of fog water (Johnson and Smith 2006, Berry et. al. 2014) can also occur during cloud immersion events, further improving the water status of the trees.

Commercially grown Fraser firs are the primary tree species grown by North Carolina Christmas tree farmers. All of the farms are well below the native occurrence of this species, ranging from as low as 600 m to over 1,250 m in elevation. Trees at lower elevation sites are quite susceptible to greater numbers of pests, which may be one reason this species does not occur in these habitats naturally. Competition with hardwoods and herbaceous plants may also prevent successful

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establishment of this species below its natural elevation limit. In addition, populations at lower elevations are exposed to warmer temperatures, fewer instances of cloud immersion and cloud cover, and higher evaporative demand. These elevation-imposed stressors also correlate with the expected environmental changes that would be associated with climate change-induced warming (IPCC 2013). Because of this, I am using elevation as a surrogate to better understand how this species may react to future warming.

Christmas tree farmers plant their trees from a limited source pool, thus making them genetically similar. They employ similar agricultural practices among the farms, such as no irrigation and only occasional fertilization, as well as planting their trees at similar densities. These similarities that the effects of climate warming could be studied in this species by measuring growth and physiological responses of trees planted at different elevations, with elevation serving as a surrogate for climate warming. Given the highest farms are at ~ 1,276 m and the lowest are ~700 m, the resulting temperature difference, assuming an adiabatic lapse moist lapse rate of 7 ^{o}C/1000 m, is 3.6 ^{o}C difference in daily high temperature between the highest and lowest sites.

Climate change models predict a rise in temperature of 1-4 °C by 2100 (IPCC 2013), which corresponds closely to the differences expected between the lowest and highest elevation sites. Higher temperatures lead to a greater evaporative demand from the trees and increased evaporation of water from soil. Without a substantial increase in precipitation, an increase in temperature will likely lead to increased water usage and quicker evaporative losses from soils, all of which can lead to drought

stress (Huntington 2006). Along with temperature changes, there will also be elevated cloud ceilings and, thus, decreases in instances of cloud immersion (IPCC 2013), which will further dry these agro-habitats of Fraser fir Christmas trees, particularly at the lower elevations. The greater VPD that would result from warmer temperatures could also potentially decrease gas exchange because of the VPD-dependence of stomatal conductance (g_s) (Meinzer 1982, Meinzer et al. 1984, Bowling et al. 2002). Continued reduced g_s as a result of greater VPD at low elevations could lower the seasonal carbon gain and reduce growth (Meinzer et al. 1984).

If Fraser fir trees do not reduce g_s by a substantial amount, the increased evaporative demand could result in excessive water stress. Trees have a variety of mechanisms by which they can adjust to greater demand for water and to cope with greater water loss from needles. These mechanism include allometric adjustments such as increased fine root growth to explore a greater soil volume for water, and varying the ratio of needle biomass and area to sapwood conducting area in order to match water loss from the needles with the ability of the trunk to supply those needles with water (Watt et al. 2003). Changes in xylem anatomy such as larger lumens and larger pit pores, and/or greater density of pits, another allometric compensation, would increase the hydraulic conductivity (K_d) and ability of trees to move water from the soil to the needles. Fraser firs may be particularly sensitive to water stress due to low K_d and this may be one reason why they are presently confined to high elevations with low VPD, high amounts of cloud immersion, and cool temperatures, all of which would contribute to a favorable annual carbon and water balance (Kulaç et al. 2012).

Previous Christmas tree research has focused primarily on agricultural practices such as pest control, fertilization, and shearing (Hinesley and Wright 1989; Hinesley and Snelling 1997; Hinesley and Derby 2004; Richter et al. 2011). Genetic variation has received a lot of focus as well (Arnold et al. 1994; Emerson et al. 2008; Potter et al. 2008). A significant amount of research has investigated fog-water usage by natural stands of Fraser fir (Berry et al. 2012; Berry et al. 2014a; Berry et al. 2014b) and the drought-stress associated changes in photosynthesis and gas exchange (Johnson and Smith 2006; Kulaç et al. 2012). These studies found a marked decrease in photosynthetic pigments in water stressed Fraser firs, particularly in the mid to late growing season and irrigated individuals exhibited associated greater biomass. However, studies on warming and the resulting changes in physiology in mature, cultivated Fraser firs have yet to be pursued.

Recent studies on wood anatomy changes and water relations have shown a significant decrease in K_d with warming-associated drought as well as a decrease in number and lumen diameter of tracheids, the cells that conduct water up the trunk (Eldhuset et al. 2012, Anderegg and Anderegg 2013). Such changes may increase the resistance of the xylem to cavitation and prevent the formation of embolism during severe water stress, but they limit the efficiency of water movement. This tradeoff between efficiency and safety has been the subject of a large number of studies, but a recent meta-analysis with Ponderosa pine suggests that there is little support for the idea that an increase in efficiency has to come at the expense of safety (Castagneri et al. 2015).

A common garden experiment with Ponderosa pine (*Pinus ponderosa*) suggested that changes in xylem vulnerability to cavitation and K_d are mainly a result of phenotypic plasticity, rather than ecotypic differentiation (Maherali et al. 2002). Xylem K_d and Ψ_{leaf} are strongly associated with drought recovery (Brodribb and Cochard 2009), probably as a result of changes in xylem structure associated with frequent water stress. Scots pine (*P. sylvestris*) revealed a marked decrease in number of tracheids in addition to smaller lumen areas in now-dead trees compared to living trees within a stand growing in more xeric environments, implying that both temperature and changes in water availability can alter wood formation (Hereş et al. 2014). Four different conifer species were found fewer tracheids, smaller lumens, and thinner cell walls associated with warmer, drier growing conditions (Ziaco et al. 2014). Norway spruce (*Picea abies*) showed fewer and smaller tracheids, compared to non-water stressed trees, as a result of severe drought, as well as less root mass, shorter root lengths, and smaller diameters (Eldhuset et al. 2012). Three conifer species under increased warming-associated evaporative demand revealed increased stem water deficit (ΔW) (Oberhuber et al. 2015). All of these studies suggest that warm and/or drought conditions may lead to lower K_d and a reduced ability to transport water to the needles. Interestingly, none of these studies seemed to indicate an ability of conifers to improve K_d when grown under warmer or drier conditions. While lumen size of tracheid cells is most strongly related to K_d, torus depth and depth of pit pores also correlate positively with K_d and cavitation resistance (Hacke and Janesen 2009), but these latter changes have not received as much attention as has lumen diameter.

Although Castagneri et al. (2015) questions whether there is a tradeoff between K_d and safety (i.e., resistance to cavitation and the introduction of embolisms in the xylem), it is true that larger lumen areas increase efficiency (higher K_d). This could also increase the risk of embolism, particularly for freezing-induced embolisms (Castagneri et al. 2015). It is possible that increasing lumen area to cope with greater evaporative demand in warmer climates would put Fraser firs at a greater risk to cold-induced embolisms, which would limit their ability to acclimate to warmer conditions during the early part of the growing season.

Gleason et al. (2015) found that no plants exhibited both high safety and efficiency; the majority of plants fell along a predicted tradeoff line between these two mechanisms. Thus, Fraser firs may be constrained to only one corner of the efficiency/safety spectrum (low K_d, high safety) as a result of their need to tolerate low winter temperatures and avoid freezing-induced embolisms.

Plants that are conservative with respect to water stress and close their stomata to prevent cavitation are referred to as isohydric, while plants that maintain open stomata and experience lower water potentials are at the opposite end of the spectrum of drought strategies and referred to as anisohydric (McDowell et al. 2008). Stomatal closure at low water potentials in isohydric species prevents severe water stress but the plant risks becoming carbon-starved during drought because stomatal closure prevents the uptake of CO_2. Anisohydric species favor carbon gain over preventing water stress and, as a result, may risk the formation of embolisms via cavitation events (McDowell et al. 2008). Negret et al. (2013) found that species in an area frequented by fogs tended to exhibit higher hydraulic efficiency while those in drier,

marginal sites favored a more conservative, isohydric strategy. European beech exhibits a stronger morphological and anatomical response to water stress than does physiology (Knutzen et al. 2015). They exhibit a decrease in cell densities and lumen areas, which led to a higher risk of hydraulic failure in trees from moist habitats exposed to greater water stress (Knutzen et al. 2015). The higher K_d may be necessary to maintain high rates of aboveground growth (Hajek et al. 2014) when water is not limiting.

In an analysis of Norway spruce, tracheid anatomy was studied as a function of light acquisition (Gebauer et al. 2013). After thinning, and allowing more light to penetrate the canopy, tracheids tended to have larger lumen areas than when experiencing more shade, suggesting that a long term increase in light, as may occur if cloud ceilings are lifted due to warming, will lead to an increase in tracheid conducting area (Gebauer et al. 2013). Increased lumen diameter would, in turn, help such trees cope with the greater evaporative demand.

Few studies have attempted to link elevation to changes in xylem anatomy in conifers. In Norway spruce, there was a strong negative correlation between the temperature of the previous year and lumen area and cell count at high elevation; low elevation was more influenced by precipitation of the previous year (Castagneri et al. 2015). The current growing season showed a similar trend for the low elevation trees whereas high elevation trees had a positive correlation between early temperatures and cell numbers (Castagneri et al. 2015). Mid-season precipitation at the high elevation was particularly important for cell enlargement and, as a result, K_d increased at the highest elevation (Castagneri et al. 2015). These results suggest that changes in

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xylem anatomy and K_d in conifers will depend in complex ways on both warming and water status.

The purpose of my study was to examine the water relations and wood anatomy of Fraser fir Christmas trees along an elevation gradient, from 664 m to 1228 m. The elevation gradient represents an ideal *in situ* setting to assess physiological plasticity among genetically similar populations of Fraser fir (Chevin et al. 2013). The temperature differences among the sites can act as a surrogate for climate warming, allowing for a study of *in situ* warming effects. Elevation gradients have been used in other warming experiments for observing phenology (Crimmins et al. 2010; Ranjitkar et al. 2013; Vitasse et al. 2013; Louw et al. 2015), transpiration (Matyssec et al. 2009), wood anatomy (Ziaco et al. 2014; Castagneri et al. 2015: Sánchez-Salguero et al. 2015), and physiology (Castagneri et al. 2015; Louw et al. 2015; Sánchez-Salguero et al. 2015). The primary goal of this study was to quantify differences in water relations of Fraser fir over an elevation gradient and to correlate those data with differences in tracheid anatomy and stomatal functioning. Understanding the physiological responses to warming and water stress will enable researchers and farmers to prepare for the impacts of future warming on the Christmas tree industry in the mountains of Western North Carolina.

For this study, I tested the following hypotheses:
- H1- Warmer temperatures and less cloud immersion at lower elevations will result in a higher VPD, greater evaporative demand, and higher K_d values as well as lower stomatal conductance (g_s).

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- H2 - Higher temperatures, coupled with greater evaporative demand, will cause trees at lower elevations to have greater water stress and lower mid-day water potentials (Ψ_w); as a corollary, this would imply that if K_d does increase (H1), it does not completely compensate for the higher VPD and evaporative demand.

- H3 - To support higher sap-flow rates at the lower elevations, trees will produce tracheids with larger lumen diameters and thinner cell walls, which will increase stem K_d.

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Methods

Study Areas

Fraser fir (*Abies fraseri*) Christmas trees were sampled from farms located along an elevation gradient in the mountains of western North Carolina and eastern Tennessee. I used the elevational gradient as a surrogate for warming and selected six Christmas tree farms that ranged from 664 m to 1228 m in elevation. Using an adiabatic lapse rate of 7 ^{o}C/1000 m for the daily maximum temperature and 3 ^{o}C/1000 m for the daily minimum (Bolstad et al. 1998), this corresponds to approximately a 3.6 ^{o}C difference in daily high temperatures and a 1.6 ^{o}C difference in the daily low temperatures between the lowest and highest farms. There were two farms at each elevation, although only one per elevation was intensively sampled for physiological measurements (Table 1), and I classified them as low, middle and high elevation farms for convenience.

To minimize the presence of confounding factors, I ensured that each farm employed similar agricultural practices, including fertilization and trimming. During the growing vbcseason I sampled (2014), the farmers refrained from trimming the sample trees used in the study. Trees at each farm were most likely obtained from the Roan Mountain seed orchard (although the exact origins are unknown) and planted in comparable densities (3906 ± 152 trees/ha; mean ± se; 5 of 6 farms within the 99% confidence interval). Ten trees of similar age and size (~11-14 years old, 2.5 ± 0.5 m

tall) were selected at each site on north-facing slopes. Each tree was at least 3 m apart from other sample trees in order to maintain independence among trees.

Microclimate

Davis VantagePro2 weather stations (Davis Instruments, Hayward, California) were installed at one farm at low, middle and high elevations to record air temperature, humidity, light, wind speed and direction, and precipitation every 2 seconds, with10 minute means logged beginning in May 2014. Soil moisture measurements were recorded every week using a Hydrosense II soil moisture probe (Campbell Scientific, Logan, Utah). Three equally spaced measurements were done in the morning or early afternoon around the dripline of each tree to a depth of 20 cm.

Stomatal Conductance

Standard Conditions

Stomatal conductance was measured under standard conditions on August 7th and 13th 2014 on 5 trees from one farm at each of the three elevations, using the Li-Cor 6400xt equipped with an LED illuminated chamber (6400-02B). Approximately 48 hours before conducting the measurements on south-facing branches at chest height, needles were trimmed down to a single row that would fit in the LED chamber (the distal portions of ~ 7 needles fit in the cuvette unshaded). Standard cuvette conditions were: flow rate at 400 μmol s^{-1}, 400 μmol mol^{-1} CO_2, ~70 % RH, and temperature 23 $\pm$ 1 °C. Afterwards, sample needles were removed from their stems

and projected needle area was determined by laying needles on a flatbed scanner (CanoScan 9000F Mark II scanner, Canon USA, Melville, NY), removing image artifacts such as shadows and dust with Adobe Photoshop CC 2014 (Adobe Systems Inc., San Jose, CA), and then converting pixels from these jpg images to area in cm^2 using Black Spot, a shareware program (Varma and Osuri 2013). Accuracy of these needle area measurements was confirmed by scanning a NIST standard area disk (10 cm^2), which yielded measurement errors < 0.5%.

Diurnal and Seasonal Patterns

I measured g_s on a diurnal basis for needles on a south facing branch approximately 140 cm above the ground using the same trees measured under standard conditions. For these measurements, I used a Li-COR 6400xt portable photosynthetic gas exchange system (Li-Cor, Inc., Lincoln, NE) equipped with the conifer chamber (6400-05). Measurements were made every 3 hours from pre-dawn to dusk on each of three days during the growing season (June, July and October) at each elevation. Two days prior to making the diurnal g_s measurements, needles were clipped in order to prevent leaks around the chamber seals in the conifer chamber. Selected branches were kept in their natural orientation, and near ambient conditions were maintained in the cuvette, with flow rates ~400-500 $\mu mol\ s^{-1}$, 400 $\mu mol\ mol^{-1}$ CO_2, and cuvette relative humidity (RH) within 5% of ambient. The first set of diurnal measurements (June 2014) excluded the 2014 growth, as it had not fully developed, but the two subsequent campaigns (late July and late October, 2014) included the 2014 needles.

Xylem Water Potentials – Diurnal and Seasonal Patterns

I assessed diurnal changes in xylem water potential (Ψ_w) of an additional 5 trees per elevation using an M 1000 Scholander Pressure Chamber (PMS Instrument Company, Albany, OR). Measurements were made on the same days and times that the g_s measurements were made. For xylem water potentials, a south facing branch ~ 140 cm above the ground was wrapped in cellophane, severed from the tree and then transferred to the pressure chamber to evaluate Ψ_w.

Hydraulic Conductivity (K_h and K_d)

I calculated the hydraulic conductivity (K_h) of one-year-old twigs (formed in 2013) from 3 trees at each elevation. Deionized water was filtered through a 0.2 μm filter, degassed in a desiccator, and acidified to pH 2 using HCl. Each twig was wrapped in cellophane, clipped, and placed in an airtight plastic bag the day prior to measurements. Within 12 hours, the bark and attached needles were peeled off, the ends recut underwater with a sharp razor, and the length and diameter of each twig measured. Then they were submerged for 12 in acidified water in the desiccator at 575 Torr to remove any native embolisms. Hydraulic conductivity measurements were carried out according to Sperry et al. (1988), with some modifications to ensure there was no water loss through the needle traces. Branches were enclosed in a separate, sealed tube at equivalent pressure to that entering the basal end of the twig in order to prevent water loss from exposed needle strands. A pressure head of 1,091 mm, corresponding to a pressure of 10.2 kPa was applied to each twig. The flow of

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water through each branch was measured 3 times for 3 minutes each by collecting sap in micro-centrifuge tubes, and then the mean was calculated to get the estimate of K_h. Conductivity was expressed as the flow rate (Kg/s) divided by the pressure gradient (MPa/m) according to (Sperry et al. 1988):

$$K_h = Kg\ m\ MPa^{-1}\ s^{-1}$$

after correcting for temperature ($20^{o}C$). Rates were then normalized according to twig cross sectional area (d, m^2):

$$K_d = K_h/d = Kg\ MPa^{-1}\ s^{-1}\ m^{-1}$$

Xylem Anatomy

The lumen size, wall thickness, and density of tracheids were examined using scanning electron microscopy (FEI Quanta 200 Scanning Electron Microscope; Hillsboro, OR). Three trees from each of the primary farms were sampled for xylem anatomy, using 3 south-facing branches, at approximately 140 cm above ground, from each tree. I wrapped each sample in cellophane, placed it into a labeled bag with a damp paper towel, and sealed it. Using a Leica vibratome (Leica VT1000 S, Buffalo Grove, IL), I then isolated the 2013 growth and sliced 3-4 cross sections. The vibratome was set to make 200 μm thick slivers at a frequency of 60 Hz and a speed of 1.5 mm/s. The sections were submerged in 1 M glutaraldehyde for at least 6 hours and subsequently underwent dehydration using an increasing concentration of ethanol. After dehydration, the samples were critical point dried using the stasis setting in the Tousimis Autosamdri-931 (Tousimis; Rockville, Maryland) and were gold plated using a Polaron SEM coating system (Polaron E5400; Hatfield, PA). For

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imaging, I photographed the cross and tangential sections at 2000x magnification

using a camera mounted on the scanning electron microscope. Using ImageJ (NIH;

Bethesda, MA), I measured the lumen areas of 30 cells/tree along a line of cells. For

density, each image threshold was adjusted to isolate individual cells, making the

image black and white instead of gray scale, circularity was limited to 0.15 to 1.00 to

prevent the measurement of non-cell entities in the image, and size was limited to 15

μm^2 and larger.

Statistical Methods

      Statistical tests were performed using SigmaPlot 13.0 (Systat Software Inc.;

San Jose, CA). Repeated measures ANOVAs and post-hoc tests (Tukey's) were used

to analyze differences among elevations for diurnal g_s and diurnal water potential

Hydraulic conductivity, soil moisture, and various aspects of tracheid anatomy were

analyzed using one-way ANOVAs. Average daily evaporative demand was calculated

using the daily average temperature and average relative humidity (RH) using the

formula: kPa*h/day. A non-linear regression was used for the soil moisture data

across the elevation categories. All graphs were constructed using Microsoft Excel

2011 (Microsoft, Redmond, WA). If assumptions for parametric tests were not met,

non-parametric alternatives were used. Significance for all analyses was assumed if p

< 0.05.

Results

Microclimate

Observed temperature differences were close to the expected temperature changes based on the adiabatic lapse rate provided in Bolstad et al. (1998) for the southern Appalachians. Mean maximum air temperatures were 3.8°C warmer at the low elevation than high elevation, which is close to the expected increase in temperature of 3.6°C. The middle elevation maximum temperature was 1.3°C warmer which is approximate to the expected increase of 1.4°C (Table 2). Daily minimum temperatures were expected to increase 3°C per every 1000 m decrease in elevation, but exhibited the opposite trend. In relation to the high elevation minimum temperature, I measured a 1.0°C decrease in daily minimum temperature at middle elevation and 0.5°C decrease at low elevation. This is likely due to cold air drainage from the surrounding topography of the low elevation site and more intensive cold air drainage at the middle elevation site.

Daily maximum VPD and evaporative demand were both highest at low elevation (1.53 kPa and 11.21 kPa*h day^{-1} respectively). The mean and minimum VPD were lowest at the middle elevation (0.29 ± 0.01 and 0.01 ± 0.00).

Mean maximum daily solar radiation was lowest at the high elevation site (186.7 W/m^2), intermediate at the middle elevation site (191.1 W/m^2) and highest at the low elevation site (202.4 W/m^2). Mean solar insolation, or the cumulative

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radiation over a day, displayed a similar trend (4858, 4586, and 4465 W*h m^{-2} day^{-1} at low, middle, and high elevations, respectively), due to more frequent cloud cover and/or cloud immersion at the high and middle elevations.

Maximum and mean wind velocities were highest at the high elevation site (2.4 m/s and 0.98 m/s respectively). The middle elevation site had the lowest maximum and mean wind velocities, at 1.2 m/s and 0.40 m/s, while at low elevation these values were 1.8 m/s and 0.43 m/s.

Precipitation amounts over the sample season were similar for the low and high elevation sites (405 and 403 mm), but approximately 50 mm higher at middle elevation (456 mm). Soil moisture showed no statistically significant differences among the elevations ($p=0.1505$) and averaged between 21 and 31 for all sites.

Stomatal Conductance

Standard Conditions

Stomatal conductance (g_s) under standard conditions at 1500 μmol m^{-2} s^{-1} PAR, high elevation trees had the lowest g_s (0.041 ± 0.02 mol m^{-2} s^{-1}, $p=0.011$) (Figure 1). Trees at middle elevations had greater g_s than high elevations and low elevation did not differ from either high or middle elevations (0.132 ± 0.01and 0.101 ± 0.01 mol m^{-2} s^{-1} respectively, $p = 0.011$).

Diurnal Patterns

Over the course of a day, g_s exhibited a more drastic afternoon drop at low elevations than at middle and high elevations (Figure 2). At high elevation g_s peaked in the afternoon during the spring, while at the middle and low elevations g_s showed a

steady decline over the course of the day after peaking in the morning. In late July,

the mid-elevation site showed a peak in the afternoon, though the data set is

incomplete because high RH and condensation on the needles precluded making

accurate g_s readings earlier in the day.

Across the elevations, high elevation g_s tended to be higher than low and

middle elevation g_s but did not show a significant difference (Figure 3). As the

growing season progressed, the g_s across all three elevations decreased, driven

entirely by the middle and high elevations (p=0.001, Figure 3).

Water Potentials – Diurnal and Seasonal Patterns

    Predawn Water Potentials

Predawn water potentials (Ψ_w) in June did not differ among the sites

(p=0.1492) (Figure 5). In July predawn Ψ_w measurements were lowest at high

elevation and highest at the middle elevation (-0.096 ± 0.01and -0.396 ± 0.01 MPa

respectively, p<0.0001) (Figure 5). In the October campaigns, the predawn Ψ_w did not

differ between the low and middle elevations, while the high elevation trees exhibited

the most water stress (p<0.0001) (Figure 5).

    Mid-day Water Potentials

Early in the growing season, in June, the midday Ψ_w was the lowest (most

negative) at the low elevation site while the high elevation trees had the highest

potentials (-1.318 ± 0.04 and -0.79 ± 0.02 MPa respectively, p = 0.004 Figure 6). In

July and October, Ψ_w at the low and middle elevation sites did not differ (Figure 6),

but in July, the high elevation trees had the lowest midday Ψ_w, possibly due to higher

VPD on that day ($p>0.0001$, Figure 6). In contrast, trees at the low elevation site exhibited the lowest Ψ_w in October ($p=0.0043$, Figure 6).

Hydraulic Conductivity (K_d)

Hydraulic conductivity was not statistically different for trees across the different elevations ($p = 0.191$) (Figure 7).

Wood Anatomy

Tracheid lumen areas (μm^2) did not differ significantly among the sites (Figure 8), but tracheid density count did differ between the middle and high elevations (4888 ± 318.8 and 3468 ± 331.4 tracheids/mm^2 respectively, Figure 9). High elevation wood growth showed a significantly lower density of tracheids than the middle and low elevation wood ($p=0.033$). Cell wall thickness also differed among sites ($p = 0.033$) (Figure 9). Cells at middle elevation had thicker cell walls than those from either high or low elevations ($p=0.008$) (Figure 10).

Discussion

Microclimate

The maximum lapse rate was close to the predicted temperature trends, showing that daytime temperatures did indeed exhibit a warming gradient. This warming gradient is within the range of temperatures predicted by the IPCC (2013) for a projected doubling of atmospheric CO_2, and thus is of a realistic magnitude to investigate potential impacts of future climate change on this species. Topographical differences among sites may have accounted for some of the variation in temperature trends, especially the anomalous trend for daily minimum temperature. The high elevation site was located at the peak of the surrounding land and began to warm shortly after sunrise, while the middle and low elevations were bordered by higher topography, causing cold air drainage, and thus decreases in temperature, as well as in VPD for the first few hours of the day. The middle elevation site in particular experienced cold-air drainage to a more drastic degree, perhaps because the site was located at the bottom of a valley surrounded by hills that were approximately 20-30 m above the site. The low elevation sites were located on hillsides near the bottom of hills and cold air could also have been a factor in affecting these minimum temperatures.

The maximum temperatures and VPDs followed the expected adiabatic trend, namely they were higher at the lowest elevation site and decreased with increasing

elevation. However, on some days, the VPD could be lower at the higher elevation site, which shows that local weather conditions can vary substantially over this elevation range. Topography also influenced wind speeds, with the less-sheltered high elevation site experiencing higher maximum and mean wind speeds than the middle and low elevations, which were more sheltered. Thus, while a warming effect was achieved across the range of elevations, there were additional factors that may have influenced tree responses, such as wind speed and early morning minimum temperatures. For example, higher wind speeds can alter diameter and height growth in trees, while variation in wind speed can affect the deposition velocity of gases to tree canopies, with potential impacts on photosynthesis and transpiration.

Cloud cover and immersion, inferred from solar radiation data, was lower at the middle and lower elevations as compared to that at high elevation. Although clouds reduce irradiance of all wavelengths, they increase the relative proportion of diffuse to direct sunlight (Reinhardt et al. 2010). Since Fraser fir has 3-dimensional needles in a radial arrangement on the branches, they can effectively utilize this diffuse light (Berry and Smith 2013), which can penetrate further into the canopy because it reaches the needles from all directions, as compared to conditions where there are no clouds and radiation impinges on the tree from only one direction. Clouds also lower temperatures, VPD, and evaporative demand compared to those days without cloud events (Berry and Smith 2012, Reinhardt and Smith 2008b). The altered temperature and humidity ranges could result in trees at lower elevations experiencing a greater evaporative demand, which could lower soil moisture, increase

water stress in the trees, and alter the penetration of radiation into the dense crowns, which would subsequently affect carbon gain through photosynthesis.

Although the middle elevation sites showed a trend for higher soil moisture, perhaps the result of the slightly higher precipitation received during the 2014 growing season, the small sample size and large intra-site variation precluded this from being statistically significant. Because soil moisture contents were essentially equivalent across all elevations, there was little evidence of greater drought at the lower elevation, contrary to expectations prior to this study. However, values obtained in just one year may not be typical of those over the lifespan of a typical commercially grown tree, which can range from 10 – 15 years. Further long-term examination of soil conditions could address whether soils tend to be drier at lower elevations.

Stomatal Conductance

Standard Conditions

High light conditions, at 1500 μmol m^{-2} s^{-1}, the middle elevation had a higher g_s than the high elevation but neither middle or high differed from the low elevation. High elevation Fraser firs may have adapted to increased water acquisition with more frequent cloud events and lower VPD (Berry et al. 2014a) and could be more sensitive to decreased VPD. but the reason for the lower g_s in trees at high elevations is not easily explained, especially in view of the fact that photosynthesis, chlorophyll content, N amounts, and specific leaf weights did not differ among trees from the three elevations (Cory 2015).

Diurnal Patterns in g_s

Stomatal conductance showed different diurnal trends across elevations and throughout the growing season. For low elevation trees, after an early morning peak, g_s decreased throughout the rest of the day over the growing season. Middle elevation trees also followed this same pattern for the June and October diurnal campaigns, but in the July diurnal they showed a mid-day increase in g_s that can be explained by a midday precipitation event which decreased the VPD. As is common for many conifers, including Fraser fir and ponderosa pine, trees exhibit lower g_s in response to a drier and warmer environment, primarily because g_s decreases with increases in VPD (Reinhardt and Smith 2008a; Reinhardt and Smith 2008b; Stout and Sala 2003). The lack of any consistent pattern in diurnal measurements of g_s across elevations strongly suggests that this parameter is governed more by local weather conditions and that there were no developmental changes resulting from warming that caused any consistent changes in the magnitude of g_s. In other words, warming, per se, has not affected this parameter, at least over the elevational range observed in this study. Since g_s is particularly responsive to local and immediate weather conditions, it can be higher or lower at any of our elevations, depending on the weather conditions at each site. Over the course of an entire growing season, and knowing that on average the VPD is greater at low elevations than high elevations, the expectation would be that g_s on a cumulative basis should be lower at the low elevation site. One way to assess this is to analyze the ratio of $^{12}C/^{13}C$ in the needles and/or wood of these trees. Under those conditions where water stress results in lower g_s (i.e., more closed stomata), discrimination against ^{13}C is reduced, and this can be detected by stable

carbon isotope analysis. These analyses are currently being done, although they will not be available in time for this thesis.

Xylem Water Potentials – Diurnal and Seasonal Patterns

Water potential is affected by the elevation and the time during the growing season, explained by precipitation and temperature. At the highest elevation, a high VPD resulted in a more negative Ψ_w at mid-season, during a diurnal that was notably without the morning cloud-immersion experienced in the other two diurnals, which implies that fewer instances of cloud events will lead to greater water stress and less net photosynthesis due to warmer temperatures and lower stomatal conductance, as demonstrated by previous research (Berry and Smith 2013; Bruijnzell 2001; Johnson and Smith 2006; Oliveira et al. 2014).

Are trees stressed enough to exhibit detrimental effects, growing outside their native range, because of the lack of water and higher evaporative demand? The lowest water potentials observed ranged from -1.2 MPa to a minimum of -1.8 MPa. These values are far above (less negative) then those required to induce embolisms in this species (Berry et al. 2015), which suggests that these trees, while perhaps under more water stress at these lower elevation farms, are not suffering from enough water stress (at least during the year of my study) to cause cavitation leading to xylem embolisms, which in turn, would reduce *Kd*. The data suggest that at least when these trees are small (less than 4 m height), they are able to supply enough water to the needles to avoid catastrophic failure of the xylem. Recent analyses of the vulnerability of the xylem to embolisms in these farm trees suggest that cavitation

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does not begin until minimum Ψ_w reaches at least -3.0 MPa (Stevens, unpublished data), allowing for an operating range with a fairly large safety margin (~1.0 MPa).

Therefore, Christmas tree growers in the southern Appalachians can be assured that trees will not suffer catastrophic xylem failure as long as precipitation amounts continue as observed during the year of this study, and there are no major changes in either pattern of rainfall (no extended droughts) or RH that could cause severe soil water shortages. Localized predictions of climate change in this region show only modest increases in air temperature for the near future, and little or no change in precipitation, which would be good news for Christmas tree farmers (citation supplied by me).

Hydraulic Conductivity (K_d)

Hydraulic conductivity of branches did not differ among elevations, similar to what Knutzen et al. (2015) found in European beech trees along a moisture gradient. Conductivity of ponderosa pine and Douglas fir also did not change over a ~60 m elevation gradient (and associated moisture gradient), though ponderosa pine displayed a higher vulnerability to embolism. Instead, Ponderosa pine compensated using lower stomatal conductance and greater carbon allocation to sapwood (Stout and Sala 2003). These data suggest that Fraser fir has little capacity to modify its xylem anatomy in response to warming. Given the modest temperature increases predicted for the southern Appalachians, the K_d suggests that this species may be able to persist even if it cannot alter its ability to deliver water through the xylem to the

needles, and that the Christmas tree industry will be able to continue to raise trees in most locations.

Wood Anatomy

The fact that lumen area did not differ among sites along the elevational range may partially explain the lack of difference in the K_d that was observed. Although the degree of warming was not enough to affect xylem lumen development, early summer precipitation is known to result in tracheids with larger lumen areas (Olano et al. 2012). Should the pattern of precipitation change, especially if early season precipitation amounts decline, then differences could arise between the elevations in terms of lumen diameters, especially if it dries out more at lower elevations than higher elevations. However, there is some evidence that higher elevations could be subject to greater warming than lower elevations, which could partially compensate for any such trends.

The density of tracheids did exhibit a significant difference along the elevational gradient. High elevation trees had a lower cell density than did middle or low elevation trees. The cause of this variation in density is not clear at this point. However, a reduction in density suggests a lower K_d, but *since K_d did not differ* among trees at different elevations, it is quite possible that the density differences are too small to be detected by such a method.

The cell walls of the middle elevation were significantly thicker than those of the low and high elevation. Wood formation is the result of a complex interaction between previous-year starch reserves, water availability, and temperature

constraints. The low elevation is likely limited by precipitation and water availability, while the high elevation is limited by low temperatures and a shorter growing season, as shown in Sidor et al. (2015) with Norway spruce.

It is apparent that the xylem structure and function of Fraser fir trees is relatively unresponsive to conditions associated with warming along an elevational gradient. Lack of anatomical adaptation suggests that should significant warming occur, that this species would have to adapt to changes in water demand by other means, such as changes in allometry (perhaps by reducing needle area relative to xylem conducting area) or by increasing fine root density, or by changes in stomatal behavior over the course of a day. Experiments under controlled conditions, where seedlings or saplings are grown at different temperatures should be conducted to verify such speculation. Scots pine revealed a marked decrease in lumen areas, particularly in xeric sites, as well as a decrease in number of tracheids and resin ducts in dead trees relative to living trees (Hereş et al. 2014). A lower cell count in low elevation trees could be a marker for reduced physiological functioning.

Conclusions

Climate warming involves a suite of changes that can include daily and seasonal temperatures, in addition to changes in the amount and distribution of precipitation, as well as a rise in the cloud ceiling. All of these changes can have consequences for the water relations of trees growing along an elevational gradient. The elevation gradient used in this project gave expected values, showing that it is appropriate for the research questions at hand. Under standard conditions there were few differences in physiological responses except for lower g_s at high elevations for reasons unknown. Diurnal patterns of g_s suggest conductances peak earlier at low elevations due to warmer afternoon temperatures. Anatomy showed little change over elevation suggesting that compensation for water stress is mostly controlled by stomatal closure. Pre-dawn water potentials indicated little or no soil water stress over the season. Mid-day water potentials were highly variable and dependent on current weather conditions but did not go below -1.8 MPa, indicating that these trees probably did not suffer embolisms under field conditions. While the anatomy of Fraser firs did not reveal extreme differences along an elevation gradient, the physiology suggests that, with increased environmental stress, Fraser fir Christmas trees will begin to alter water and carbon balances. Without an increase of water input, an increase in temperature can result in low water availability and more negative water potentials. As these changes become more extreme, the trees will have to compensate by either closing stomata due to low VPD and water availability, as

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shown in the diurnal campaigns, or increasing the solute potential and inducing a greater water potential gradient across the soil-root pathway. These compensatory strategies decrease gas exchange or introduce higher risk to drought-induced embolism. The low and high elevations are more negatively affected by climate warming; high elevations, which supplement the water supply with cloud water, are experiencing fewer cloud immersion events while low elevations are undergoing notably warmer temperatures with out any additional precipitation input. With the warming-induced changes, farmers will need to alter agricultural practices by planting trees farther apart or investing in an irrigation regime.

References

Anderegg WRL, Anderegg LDL (2013) Hydraulic and carbohydrate changes in experimental drought-induced mortality of saplings in two conifer species. Tree Physiology 3:252-260.

Anderegg WRL, Anderegg LDL, Berry JA, Field CB (2014) Loss of whole-tree hydraulic conductance during severe drought and multi-year forest die off. Oecol 175:11-23.

Arnold RJ, Jett JB, McKeand SE (1994) Natural variation and genetic parameters in Fraser fir for growth and Christmas tree traits. Can J For Res 24:1480-1486

Berry CZ, Hughes NM, Smith WK (2014a) Cloud immersion: an important water source for spruce and fir saplings in the southern Appalachians. Oecol 174:319-326.

Berry CZ, Smith WK (2012) Cloud pattern and water relations of *Picea rubens* and *Abies fraseri*, southern Appalachian mountains, USA. Agr For Met 162:27-34.

Berry CZ, Smith WK (2013) Ecophysiological importance of cloud immersion in a relic spruce-fir forest at elevational limits, southern Appalachian Mountains, USA. Oecol 173:637-648.

Berry CZ, White JC, Smith WK (2014b) Foliar uptake, carbon fluxes and water status are affected by the timing of daily fog in saplings from a threatened cloud forest. Tree Phys 34:459-470.

Berry CZ, Johnson DM, Reinhardt K (2015) Vegetation-zonation patterns across a
temperate mountain cloud forest ecotone are not explained by variation in
hydraulic functioning on water relations. Tree Phys 00:1-11.

Bolstad PV, Swift L, Collins F, Régnière J (1998) Measured and predicted air
temperatures at basin to regional scales in the southern Appalachian
Mountains. Agr For Met 91:161-176

Bowling DR, McDowell NG, Bond BJ, Law BE, Ehleringer JR (2002) ^{13}C content of
ecosystem respiration is linked to precipitation and vapor pressure deficit.
Oecol 131:113-124.

Brodribb TJ, Cochard H (2009) Hydraulic failure defines the recovery and point of
death in water-stressed conifers. Plant Phys 149:575-584.

Bruijnzeel LA (2001) Hydrology of tropical montane cloud forests: a reassessment.
Land Use Water Resour Res 1:1–18.

Castagneri D, Petit G, Carrer M (2015) Divergent climate response on hydraulic-
related on xylem anatomical traits of *Picea abies* along a 900-m altitudinal
gradient. Tree Phys 00:1-10.

Chevin L-M, Collins S, Lefèvre F (2013) Phenotypic plasticity and evolutionary
demographic responses to climate change: taking theory out to the field. Funct
Ecol 27:966-979

Cogbill CV, White PS (1991) The latitude-elevation relationship for spruce-fir forest
and treeline along the Appalachian mountain chain. Veget 94:153–175

Cory ST (2015) Growth and ecophysiological response of Fraser fir (*Abies fraseri*)
Christmas trees along an elevational gradient (Master's thesis). Retrieved
from author.

Crimmins TM, Crimmins MA, Bertelsen CD (2010) Complex responses to climate
drivers in onset of spring flowering across a semi-arid elevation gradient. J of
Ecol 58:1042-1051.

Eldhuset TD, Nagy NE, Volařík D, Børja I, Gebauer R, Yakovlev IA, Krokene P
(2012) Drought affects tracheid structure, dehydrin expression, and above-
and belowground growth in 5-year-old Norway spruce. Plant Soil 366:305-
320.

Emerson JL, Frampton J, McKeand SE (2008) Genetic variation in early growth and
bud production among natural populations of Fraser fir. Hort Science 43:661-
666.

Gebauer R, Volarik D, Urban J, Borja I, Nagy NE, Eldhuset TD, Krokene P (2013)
Altered light conditions following thinning affect xylem structure and
potential hydraulic conductivity of Norway spruce shoots. Eur J For Res
133:111-120.

Gleason SM, Westoby M, Jansen S, Choat B, Hacke UG, Pratt RB, Bhaskar R,
Brodribb TJ, Bucci SJ, Cao K-F, Chochard H, Delzon S, Domec J-C, Fan Z-
X, Feild TS, Jacobsen AL, Johnson DM, Lens F, Maherali H, Martínez-
Vilalta J, Mayr S, McCulloh KA, Mencuccini M, Mitchell PJ, Morris H,
Nardini A, Pittermann J, Plavcová L, Schreiber SG, Sperry JS, Wright IJ,
Zanne AE (2015) Weak tradeoff between xylem safety and xylem-specific

hydraulic efficiency across the world's woody plant species. New Phyto doi:10.1111/nph.12646.

Hacke UG, Janesen S (2009) Embolism resistance of three boreal conifer species varies with pit structure. New Phyto182:675-686.

Hajek P, Leuschener C, Hertel D, Delzon S, Schuldt B (2014) Tradeoffs between xylem hydraulic properties, wood anatomy and yield in *Populus*. Tree Phys34:744-756.

Hereş A-M, Camarero JJ, López BC, Martínez-Vilalta J (2014) Declining hydraulic performances and low carbon investments in tree rings predate Scots pine draught-induced mortality. Trees 28:1737-1750.

Hinesley LE, Derby SA (2004) Growth of Fraser fir Christmas trees in response to annual shearing. Hort Science 39:1644-1646.

Hinesley LE, Derby SA (2004) Growth of Fraser Fir Christmas Trees In Response to Annual Shearing. Hort Science 39:1644-1646.

Hinesley LE, Snelling LK (1997) Basal pruning Fraser fir Christmas trees. Hort Science 32:324-326.

Hinesley LE, Wright RD (1989) Biomass and nutrient accumulation in Fraser-fir Christmas trees. Hort Science 24:280-282.

Huntington TG (2006) Evidence for intensification of the global water cycle: review and synthesis. J of Hydro 319:83-95.

IPCC (2013) Climate change 2013: The Physical Science Basis. Contribution of Working Group 1 to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor,

S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds).
Cambridge University Press, Cambridge, United Kingdom and New York,
NY, USA.

Johnson DM, Smith WK (2006) Low clouds and cloud immersion enhance
photosynthesis in an understory species of a southern Appalachian spruce-fir
forest (USA). Am J Bot 93:1625-1632.

Knutzen F, Meier C, Leuschner C (2015) Does reduced precipitation trigger
physiological and morphological drought adaptations in European beech
(*Fagus sylvatica* L.)? Comparing provenances across a precipitation gradient.
Tree Phys 35:949-963.

Kubiske ME, Abrams MD (1990) Pressure-volume relationships in non-rehydrated
tissue at various water deficits. Plant Cell Environ 13:995-1000.

Kulaç S, Nzokou P, Guney D, Cregg BM, Turna I (2012) Growth and physiological
response to Fraser fir [*Abies fraseri* (Pursh) Poir.] seedlings to water stress:
seasonal and diurnal variations in photosynthetic pigments and carbohydrate
concentration. Hort Science 47:1512-1519.

Louw EL, Hoffman EW, Theron KI, Midgley SJE (2015) Physiological and
phonological response of *Protea* 'pink ice' to elevated temperatures. SA J Bot
99:93-102.

Maherali H, Williams BL, Paige KN, Delucia EH (2002) Hydraulic differentiation of
Ponderosa pine populations along a climate gradient is not associated with
ecotypic divergence. Funct Ecol 16:510-521.

Matyssec R, Wieser G, Patzner K, Blaschke H, Häberle KD (2009) Transpiration of

forest trees and stands at different altitude: consistencies rather than contrasts? Eur J For Res 178:579-596.

McDowell N, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J, Sperry J, West A, Williams DG, Yepez EA (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while other succumb to drought? New Phyto 178:719-739.

Meinzer FC (1982) The effect of vapor pressure on stomatal control of gas exchange in Douglas fir (*Pseudotsuga menziesii*) saplings. Oecol 54:236-242.

Meinzer F, Goldstein G, James M (1984) The effect of atmospheric humidity on stomatal control of gas exchange in two tropical coniferous species. Can J Bot 62:591-595.

Negret BS, Pérez F Markesteijn L, Castillo MJ, Arnesto JJ (2013) Diverging drought-tolerance strategies explain tree species distribution along a fog-dependent moisture gradient in a temperate rain forest. Oecol 173:625-635.

Oberhuber W, Kofler W, Schuster R, Wieser G (2015) Environmental effects on stem water deficit in co-occurring conifers exposed to soil dryness. Int J Biomet 59:417-426.

Olano JM, Eugenio M, García-Cervigón AI, Folch M, Rosas V (2012) Quantitative tracheid anatomy reveals a complex environmental control of wood structure in continental Mediterranean climate. Int J Plant Sci 173:137-149.

Oliveira RS, Eller CB, Bittencourt PRL, Mulligan M (2014) The hydroclimatic and ecophysiological basis of cloud forest distributions under current and projected climates. A of Bot 113:909-920.

Oosting HJ, Billings WD (1951) Comparison of virgin spruce-fir forest in the

northern and southern Appalachian system. Ecol 32:84-103.

Parker WC, Colombo SJ (1995) A critical re-examination of pressure-volume

analysis of conifer shoots: comparison of three procedures for generating *PV*

curves on shoots of *Pinus resinosa* Ait. Seedlings. J of Exp Bot 46:1701-1709.

Potter KM, Frampton J, Josserand SA, Nelson CD (2008) Genetic variation and

population structure in Fraser fir (*Abies fraseri*): a microsatellite assessment of

young trees. Can J For Res 38:2128-2137.

Ranjitkar S, Luedleing E, Shrestha KK, Guan K, Xu J (2013) Flowering phenology of

tree rhododendron along an elevation gradient in two sites in the Eastern

Himalayas. Int J Biomet 57:225-240.

Reinhardt K, Smith WK (2008a) Impacts of cloud immersion on microclimate,

photosynthesis and water relations of Abies fraseri (Pursh.) Poiret in a

temperate mountain cloud forest. Oecol 158:229-238.

Reinhardt K, Smith WK (2008b) Leaf gas exchange of understory spruce-fir saplings

in relict cloud forests, southern Appalachian Mountains, USA. Tree Phys

28:113-122.

Reinhardt K, Smith WK, Carter GA (2010) Clouds and cloud immersion alter

photosynthetic light quality in a temperate mountain cloud forest. Botany

88:462-470.

Richter BS, Benson DM, Ivors KL (2011) Microbial Profiling of Cultural Systems for

Suppression of Phytophthora Root Rot in Fraser Fir. Plant Dis 95:537-546.

Sánchez-Salguero R, Camarero JJ, Hevia A, Madrigal-González J, Linares JC,

Ballesteros-Canovas JA, Sánchez-Miranda A, Alfero-Sánchez R, Sangüesa-Barreda G, Galván JD, Gutiérrez E, Génova M, Rigling A (2015) What drives the growth of Scots pine in continental Mediterranean climates: drought, low temperatures, or both?. Ag For Met 206:151-162.

Sidor CG, Popa I, Vlad R, Cherubini P (2015) Different tree-ring responses of Norway Spruce to air temperature across an altitudinal gradient in the Eastern Carpathians (Romania). Trees 29:985-997.

Sperry JS, Donnelly JR, Tyree MT (1988) A method for measuring hydraulic conductivity and embolism in xylem. Plant Cell Environ 11:35-40.

Stout DL, Sala A (2003) Xylem vulnerability to cavitation in *Pseudotsuga menziesii* and *Pinus ponderosa* from contrasting habitats. Tree Phys 23:43-50.

Tyree MT, Hammel HT (1972) The measurement of the turgor pressure and the water relations of plants by the pressure-bomb technique. J Exp Bot 74:267-282.

Tyree MT, Richter H (1981) Alternative methods of measuring water potential isotherms: some cautions and clarifications. J Exp Bot 128:643-653.

Vitasse Y, Hoch G, Randen CF, Lenz A, Kollas C, Scheepens JF, Körner C (2013) Elevational adaptation and plasticity in seedling phenology of temperate deciduous tree species. Oecol 171:663-678.

Watt MS, Whitehead D, Mason EG, Richardson B, Kimberly MO (2003) The influence of weed competition for light and water on growth and dry matter partitioning of young *Pinus radiata* at a dryland site. For Ecol and Man 183:363-376.

Ziaco E, Biondi F, Rossi S, Deslauriers A (2014) Climatic influences on wood

anatomy and tree-ring features of Great Basin conifers at a new mountain

observatory. App Plant Sci 2:1400054.

Tables and Figures

Site Name	Elevation (m)	Moist Adiabatic Lapse of Daily Max Temps (°C)	Moist Adiabatic Lapse of Daily Min Temps (°C)	Elevation Category	Geographical Coordinates	County, State	Tree Height (m)	Planting Density (# trees per ha)
Carroll1	664	3.9	1.7	Low	36°22'0.95"N 82° 0'54.47"W	Johnson, TN	2.38 ± 0.07	4499
Carroll2	710	3.6	1.6	Low	36°23'3.37"N 81°51'19.69"W	Johnson, TN	2.62 ± 0.06	3660
Thad	1021	1.4	0.6	Middle	36°10'42.10"N 81°45'37.76"W	Watauga, NC	2.28 ± 0.04	4026
Ronnie	1048	1.3	0.5	Middle	36°1047.10"N 81°45'44.17"W	Watauga, NC	2.49 ± 0.06	3681
Jim	1224	-	-	High	36°17'23.86"N 81°40'55.92"W	Watauga, NC	2.36 ± 0.04	4090
Greene	1228	-	-	High	36°16'13.51"N 81°44'3.41"W	Watauga, NC	2.59 ± 0.07	3477

Table 1. Summary information on sample sites. Bold represents a site at which

weather stations were installed, gas exchange and water potentials were measured,

and microscopy was done. Lapse rates at the low and middle elevation sites are the

expected increase in temperature compared to high elevation sites, based on 7 °C/km

for daily maximum and 3 °C/km for daily minimum temperatures reported by Bolstad

et al. (1998) for the southern Appalachian Mountains. Tree heights are mean ± s.e

(Cory 2015).

Parameters	Low Elevation (710m)	Middle Elevation (1021m)	High Elevation (1224m)
Mean Daily air temperature (°C)	18.2 ± 0.4	16.9 ± 0.3	16.6 ± 0.3
Daily minimum air temperature (°C)	12.5 ± 0.4	12.0 ± 0.3	13.0 ± 0.3
Daily maximum air temperature (°C)	25.6 ± 0.4	23.1 ± 0.4	21.8 ± 0.4
Mean Daily VPD (kPa)	0.47 ± 0.02	0.29 ± 0.01	0.40 ± 0.01
Daily minimum VPD (kPa)	0.03 ± 0.00	0.01 ± 0.00	0.17 ± 0.01
Daily maximum VPD (kPa)	1.53 ± 0.06	1.02 ± 0.04	0.97 ± 0.04
Daily evaporative demand (kPa*h/d)	11.21 ± 0.48	7.02 ± 0.33	9.54 ± 0.37
Mean Daily solar radiation (W/m^2)	202.4 ± 6.7	191.1 ± 6.3	186.7 ± 6.4
Daily integrated insolation (W*h m^{-2} d^{-1})	4858 ± 160	4586 ± 152	4465 ± 156
Mean Daily wind speed (m/s)	0.43 ± 0.02	0.40 ± 0.02	0.98 ± 0.06
Daily maximum wind speed (m/s)	4.89 ± 0.12	4.39 ± 0.11	6.21 ± 0.22
Cumulative Precipitation (mm)	404.8	456.2 ±	403.4

Table 2. Weather data measured from May 25th – October 31st 2014. Each measurement was taken every 2 seconds and then averaged over 10 minutes intervals (Cory 2015).
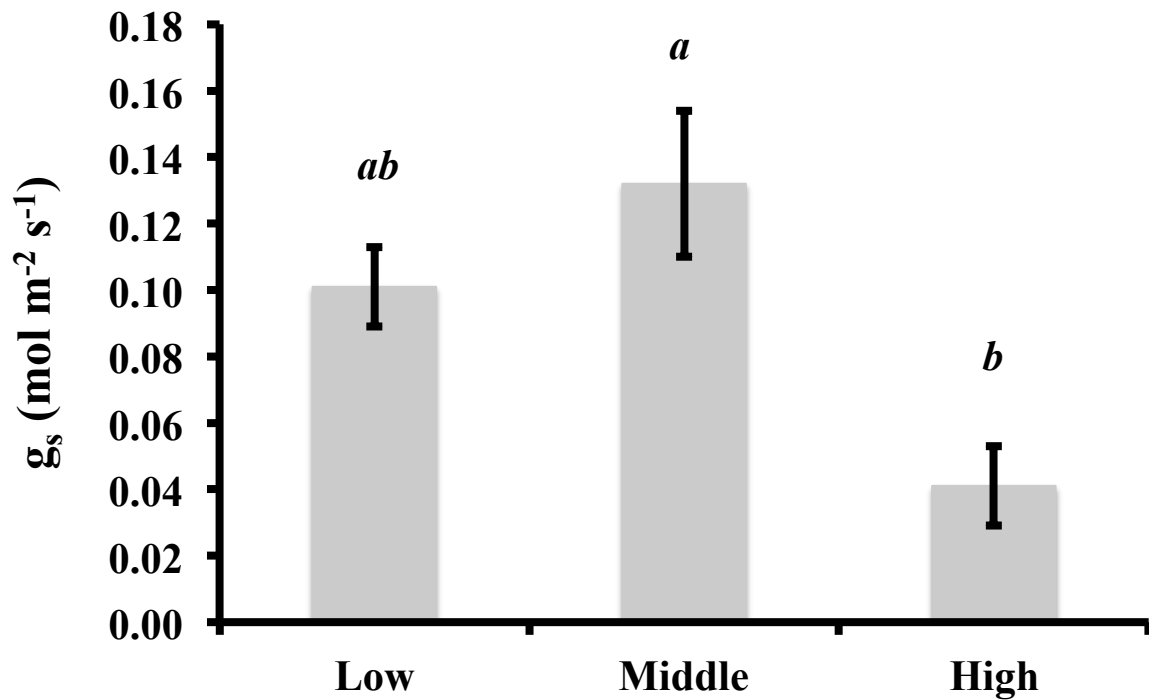
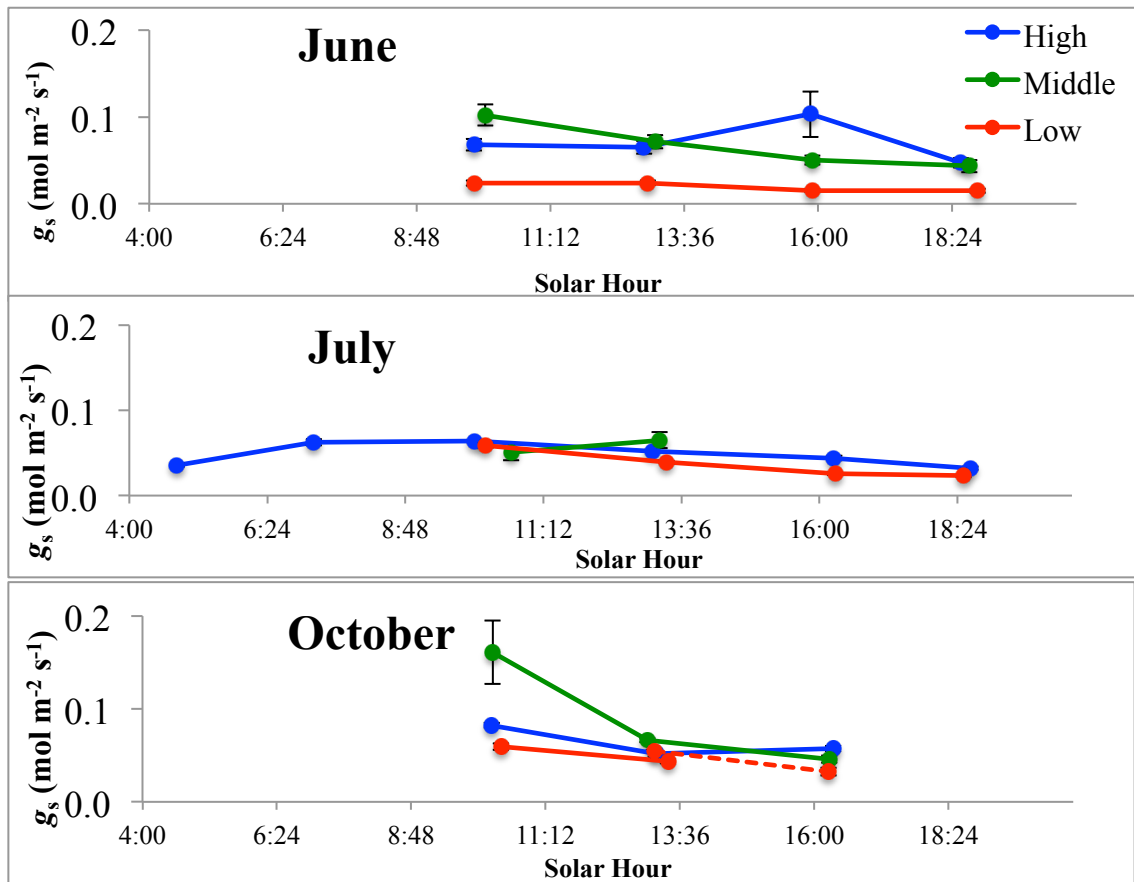Figure 1. ANOVA results on g_s under standard conditions at 1500 PAR ($n=5$, $p=0.0011$).

Figure 2. Stomatal conductance (g_s) of 2013 shoots across the growing season, measured during the summer of 2014. The first diurnal was on June 2-18 (a), second diurnal on July 23-29 (b) and third diurnal on October 2-20(c). Missing points are due to condensation on needles or precipitation events.
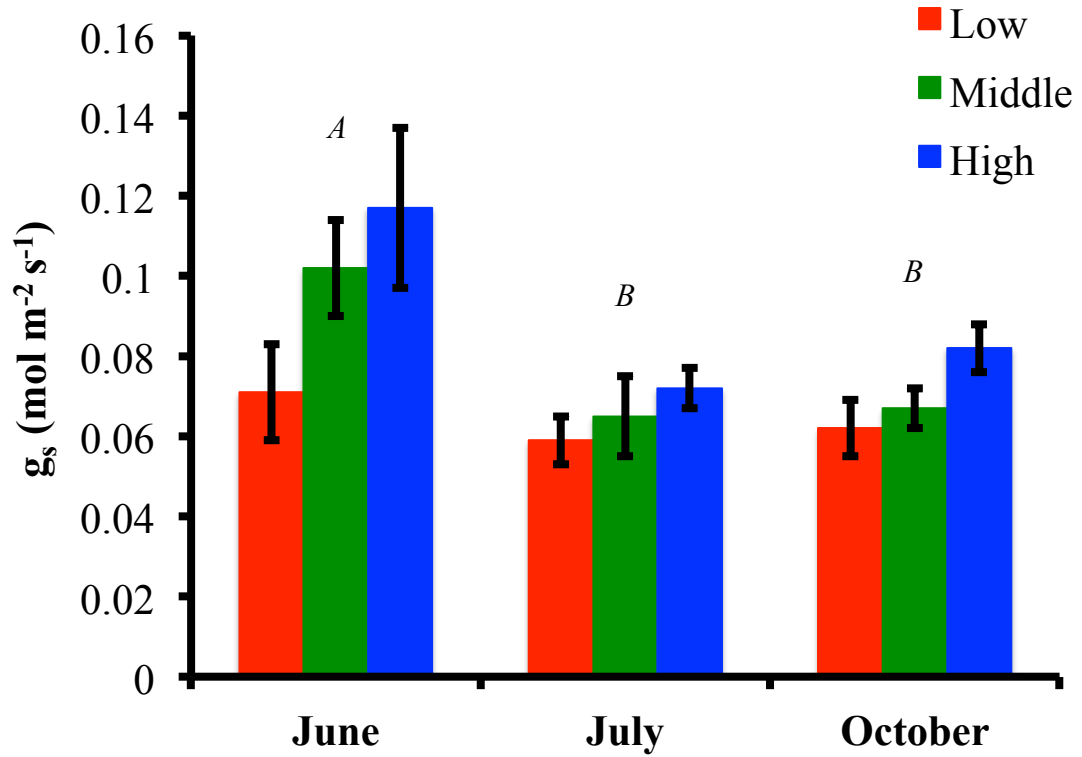
Figure 3. Above is a repeated measures ANOVA performed on maximum daily g_s over the growing season showing the first diurnal on June 2-18, the second diurnal on July 23-29, and the third diurnal on October 2-20. The sites showed no difference but the June diurnals showed significantly higher g_s than the July and October ($p = 0.0085$).
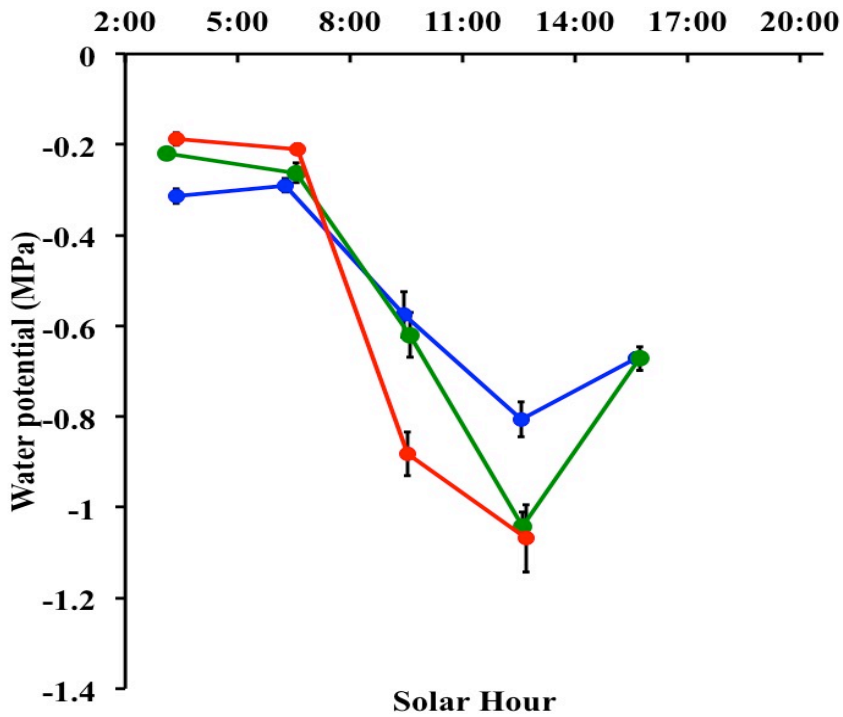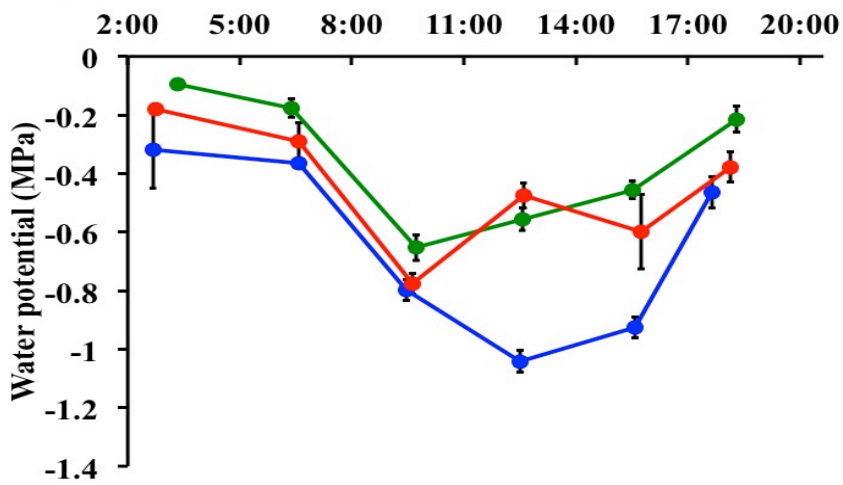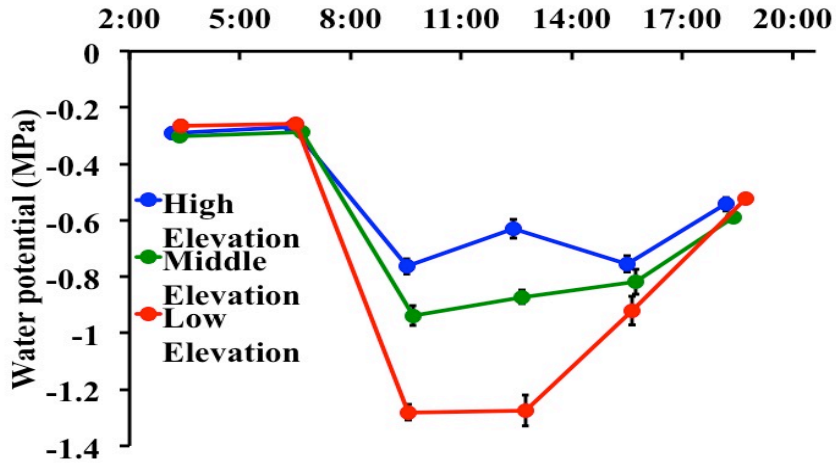
Figure 4. Water potentials (ψ) of the first (a), second (b), and third (c) diurnal days, on June 2-18, July 23-29, and October 2-20 2014 respectively.
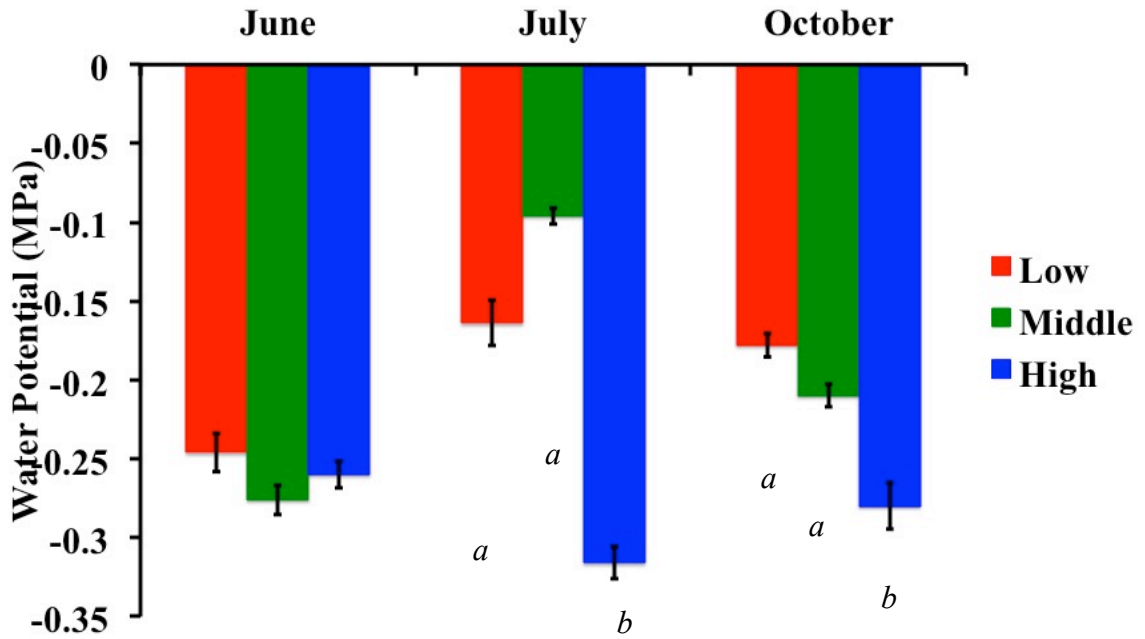
Figure 5. Repeated measures ANOVA performed on maximum (predawn) daily water potential over the growing season (*n*=5).
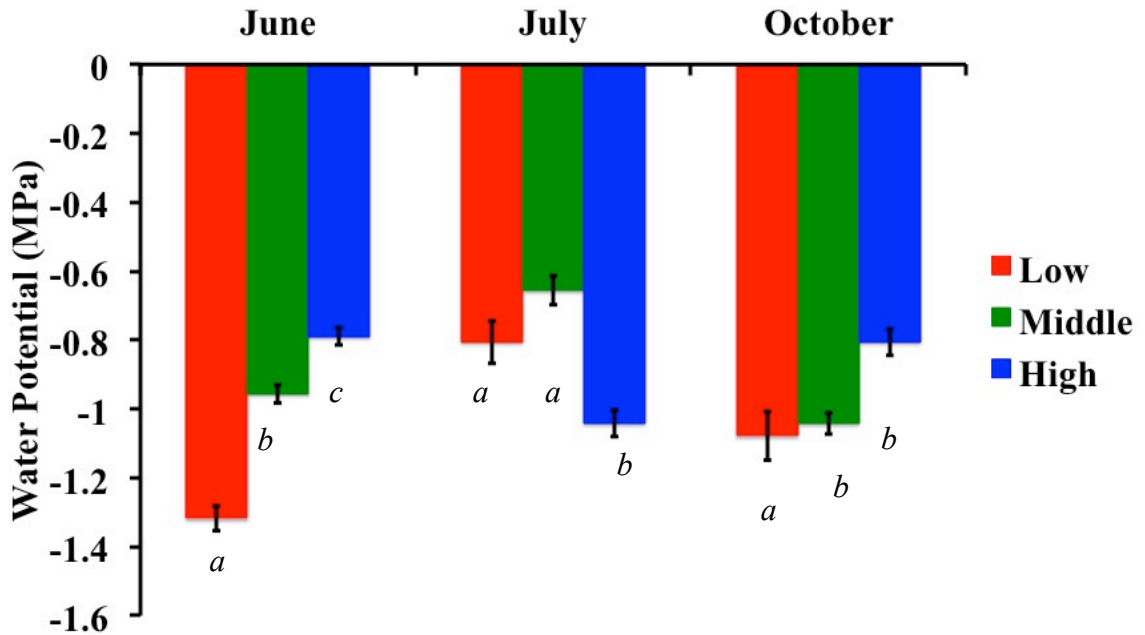
Figure 6. Repeated measures ANOVA performed on minimum daily water potential over the growing season (*n*=5).
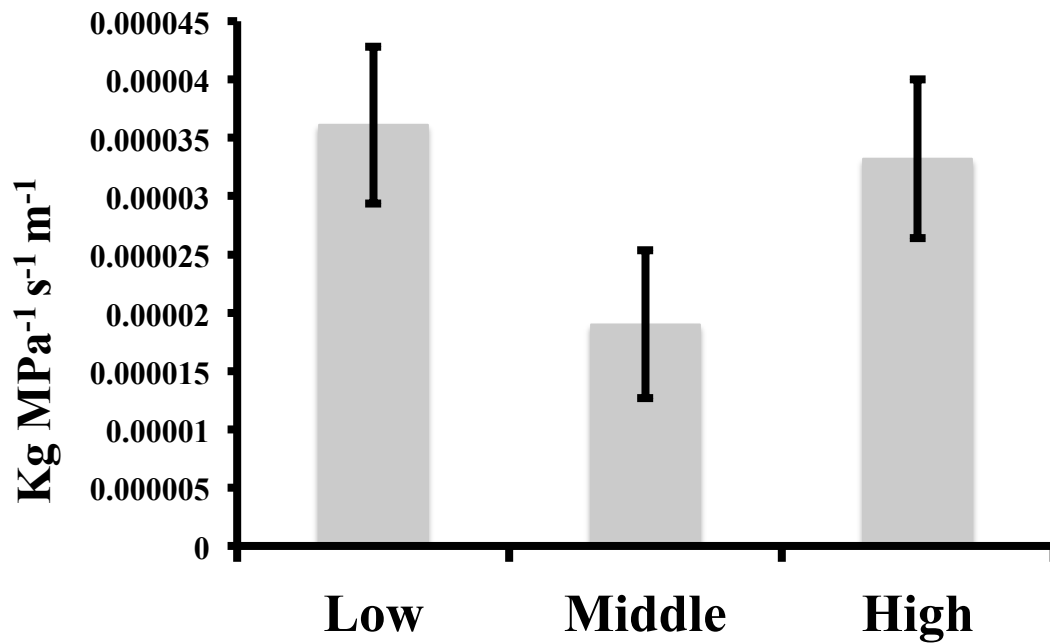
Figure 7. Conductivity among sites does not exhibit a significant difference ($p = 0.191$)
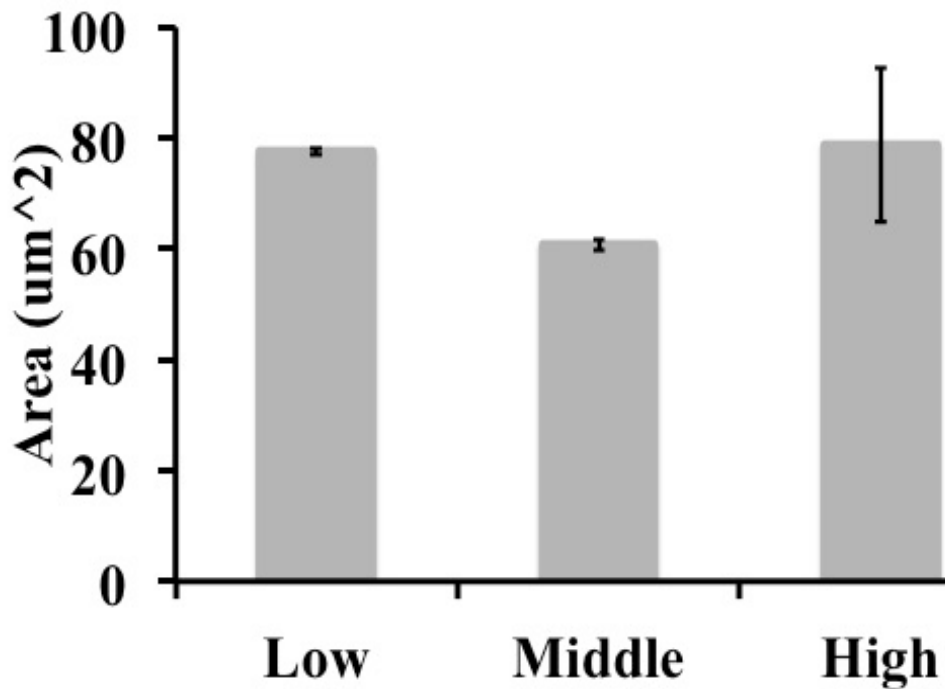
Figure 8. The tracheid lumen areas do not differ among the three elevations ($p =$ 0.254)
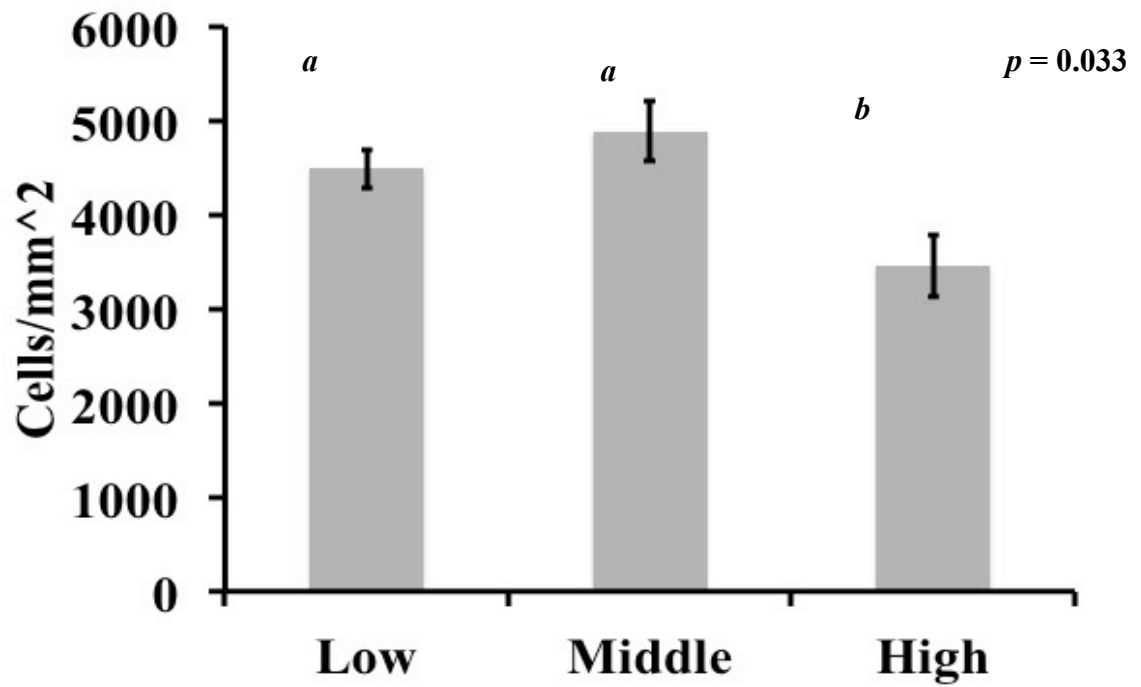

Figure 9. Cell density count of the microscopy images across elevations show a

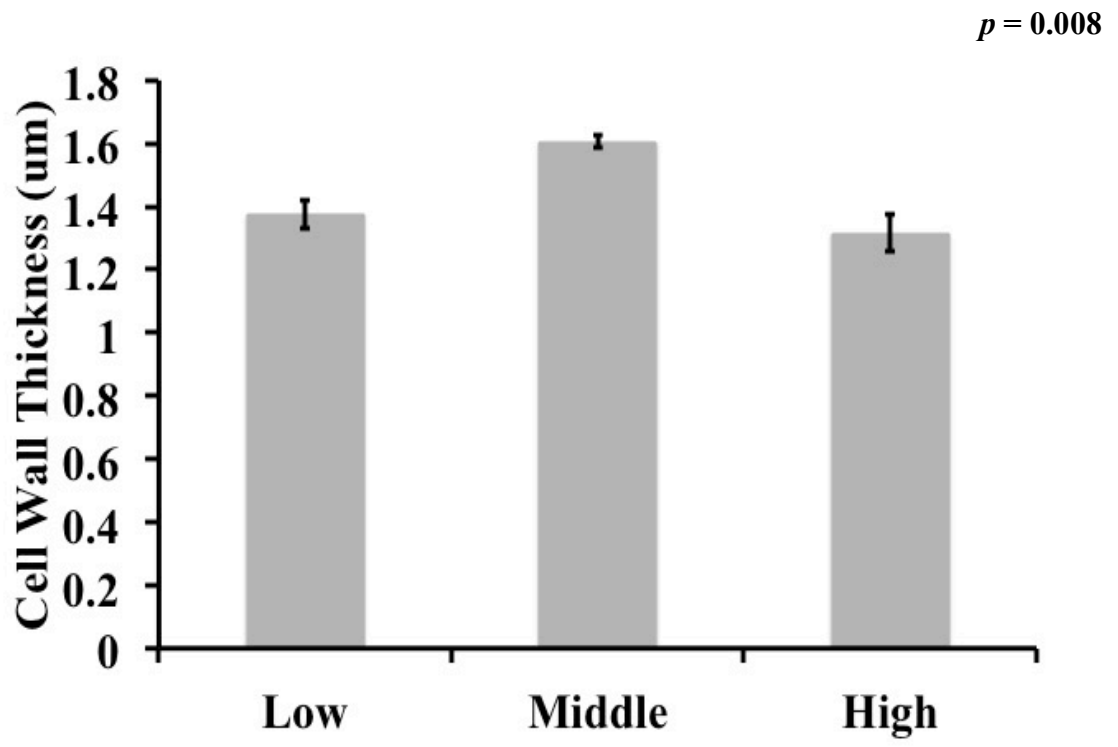significantly lower cell count in the high elevation site (n=3, p=0.033).

p = 0.008

Figure 10. Cell wall thickness of tracheids among sites (*n*=3, *p* = 0.008).

Vita

Lauren Kathleen Wood was born in 1988 in Mount Airy, North Carolina. She attended North Carolina School of Science and Mathematics from 2004 to 2006. She then toured the UNC school system and the east coast pursuing agricultural, viticultural, and educational experiences. She was awarded a Bachelor of Science degree in Biology in May 2013 from Appalachian State University. She then became a graduate student in the Department of Biology at Appalachian State University and was awarded her Masters degree in 2016. After being awarded a Master of Science in Biology, she will begin doctoral work with Dr. Donald R Young and Dr. Julie Zinnert at Virginia Commonwealth University.