Relationships of hydraulic traits and wood morphology in Douglas-fir branches from wet vs. dry regions and sites

by
Herman Noe Flamenco, Jr.

A PROJECT

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in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Forest Management
(Honors Associate)

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AN ABSTRACT OF THE THESIS OF

Herman Noe Flamenco, Jr. for the degree of Honors Baccalaureate of Science in Forest Management presented on May 11, 2015. Title: Relationships of hydraulic traits and wood morphology in Douglas-fir branches from wet vs. dry regions and sites.

Abstract approved:

Barbara Lachenbruch

It is well known that water transport in xylem depends on its anatomy, but recent research shows that water transport through different parts of the growth ring are affected by the water potential in different ways. The goal of the project was to learn the extent to which wood morphology is related to several hydraulic properties, making use of samples and an existing dataset on hydraulic properties of 2-year old branches from 11-year old trees of Douglas-fir (*Pseudotsuga menziesii* subsp. *menziessi* Mirb. (Franco)) from several provenances that were grown in a site with low or high annual precipitation. The dataset provided the specific conductivity (K_s) of samples that were well hydrated, the percent loss of conductivity of those samples after being subjected to -4 MPa (PLC@4), the percent of water mass that was lost between hydration and -4 MPa, the density of the sample, and the sample itself that had been stained after the -4 MPa treatment. The pattern of stain showed the flow paths that were still operative at -4 MPa. There were 36 samples each from an

Oregon Coast Range provenance and a low elevation Siskiyou provenance. Half of the samples from each provenance were grown in a Coast Range site, and half near Medford (for 18 trees in each of four treatment combinations). The specific hypotheses were the following.

1) PLC@4 is positively correlated with K_s and with the percentage of water lost between 0 MPa and -4 MPa, and is negatively correlated with wood density.
2) PLC@4 is positively correlated with the percentage of the growth ring that is from certain locations (the first-formed earlywood of the inner growth ring, etc.).
3) K_s is positively correlated with the percentage of the growth ring that is from certain locations (the earlywood of both rings, and the outer ring).

Using image analysis, I determined the percentage of the growth ring in each of four locations, and then with the help of a statistician, analyzed the data to test the hypotheses. The data supported hypothesis 1 and 3 but not 2. The most interesting result was that the percentage of the cross section in a particular growth ring (e.g. earlywood, latewood), was not significantly correlated to drought vulnerability. This result suggests that the anatomy of the individual samples may be better predictors of drought vulnerability than the proportion of cross-sectional area alone.

Key Words: Douglas-fir, hydraulic architecture, wood morphology, embolism

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I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

Herman Noe Flamenco, Jr., Author

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Table of Contents

List of Figures

Introduction

The way a tree branch performs hydraulically in any given environment depends on its wood anatomy and its stomatal behavior. Plants from dry provenances may be anatomically different and have different stomatal behavior than plants from wet provenances. The same can be said of plants growing in dry versus wet sites. This study focuses on one aspect of the wood's adaptations and plasticity as part of a larger study on the plasticity of hydraulic architecture in young Douglas-fir (*Pseudotsuga menziessii*) trees.

It is expected that a tree will have adaptations that are suitable to the environment in which it is found (Bansal et al. 2015). We presume that these adaptations are genotypic at a regional level. Adaptations can also be plastic responses when different phenotypes within a provenance are observed. Below I will detail some genotypic and phenotypic responses to moisture in conifers, focusing on the wood hydraulic traits.

Many studies have shown a trade-off between being able to transport water quickly (specific conductivity, Ks) and resisting the formation of embolisms as the tension in the water increases. This relationships is called the safety vs. efficiency tradeoff (Domec et al. 2008). The current dataset that I started with in this project showed such a tradeoff (Figure 1), meaning that a sample that had high permeability (Ks) tended to be more vulnerable to embolism (have lower PLC@4) than one with lower conductivity. In other words, morphological features that conferred high conductivity appeared to also confer high risk of embolism.

Species with higher wood densities tend to have lower vulnerability to embolism (Hacke et al. 2001), lower specific conductivity, K_s, and a tradeoff between vulnerability to embolism and K_s (Kavanagh et al. 1999, Domec and Gartner 2002, Domec et al. 2008, Rosner et al. 2008). Genotypes with a higher wood density, tend to be more drought resistant, than those with a low wood density (Dalla-Salda et al. 2008). There is also some research that is looking into the hypothesis that trees with a lower transition-wood density are more resistant to embolism (Dalla-Salda et al. 2014). The transition wood is the portion of the ring that is between the earlywood and the latewood. The 2-year old segments that were used broke up into (starting from the pith and moving out): earlywood (A) and latewood (B) of the inner growth ring, initial earlywood of the second growth ring (C), and remainder of the ring (D) (Figure 2).. Latewood had not quite began to form, yet the tissue was still different enough from earlywood that it was considered to be separate.

Douglas-fir has a wide growing range, from very wet to very dry provenances. This gives way to two variations of Douglas-fir, var. *menziesii* and var. *glauca*. The coastal variety (*menziesii*) has green needles, while the interior variety (*glauca*) needles are more bluish, though either can exhibit both colors. The cones between the two also differ. Coastal Douglas-fir has straight, flat bracts, as opposed to the protruding and reflected bracts of the interior variety. It is also typical for the coastal variety to be greater in height, diameter and volume, and age (Lavender and Hermann 2014).

My research was primarily concerned with relating several aspects of wood structure to hydraulic characteristics already studied on these samples. Image analysis and correlation analysis were used to learn which morphological traits are most strongly correlated with K_s and PLC@4, to better understand how plant morphology contributes to tree growth and survival.

K_s is a measure of the stems porosity (Tyree and Ewers 1991). Essentially it is water (m3) crossing a unit stem cross-sectional area (m2) per time (s) with a given pressure gradient across it (MPa/m). Based on that one can see that K_s increases if the number of tracheid increases, which means it increases with an increase in diameter. PLC represents the loss of water conductivity of the stem due to embolism. As the segment is submitted to an increasingly negative xylem pressure, K_s is measured, which shows loss in conductivity (Hacke et al 2001). It is a useful tool for simulating drought stress.

For this research, wood anatomy was of importance. For Douglas-fir, the distinction between earlywood and latewood is quite visible (Figure 2). Earlywood forms earlier in the year and is followed by the latewood. Earlywood is produced in more abundance, with wider lumen diameters, and is less dense than latewood. Because of those characteristics, earlywood is expected to be more conductive. Wood density plays a role in the hardy structure of a tree, but is also of importance to tracheid structure (Domec and Gartner 2002). Tracheid with thickened and lignified cell walls are able to resist embolism brought on by embolized neighbors. The thicker walls are able to support more stress. Staining samples is a useful tool for visualizing embolism. These samples were stained after they were subjected to 4 MPa, resulting in an array of staining patterns (Figure 3). The stain indicates the parts of the wood that were still conductive after the simulated drought. 4 MPa was the pressure used because based on random samples, it was the point in which samples lost 50% of their conductivity.

The project aimed to relate wood morphology, specifically the proportion of each of the growth rings that were a specific type of wood, to K_s, wood density, and PLC@4 (a measure of drought susceptibility). The specific hypotheses that were tested are the following:

1. PLC@4 is positively correlated with the percentage of water lost at -4 MPa.
2. PLC@4 is negatively correlated with wood density.
3. PLC@4 is positively correlated with percentage of earlywood, particularly in the portions that are C + D and is negatively correlated with the portion that is D.
4. K_s is positively correlated with the earlywood portions of C and C+D.

Materials and Methods

Plant Material:

Plant materials came from the Douglas-fir Seed Source Movement Trial, a set of trials in nine locations, each of which has replicated plant material from Douglas-fir seed sources from 12 regions. The trial was developed and is maintained by USDA FS

PNW research scientists (Gould et al. 2010). The current study made use of two of their provenances, Oregon Coastal North (ORCSTN) and Oregon Siskiyou Lower Elevation (ORSISL) that were grown in common gardens at two of their sites, Nortons near Eddyville, Oregon (in the Oregon Coast Range) and Stone near Medford, Oregon (on the western edge of a valley in southern Oregon). All plant material came from six half-sib families of Douglas-fir (meaning their seeds came from six mother trees) at each of two sites. The particular half-sib families were selected to have climates most similar to the climate of one of the two common garden sites. The estimates of mean climatic conditions at the two test sites and of the regions from which the seeds came are given in Appendix 1.

Study from which samples derive:

The samples are from a larger project (Lachenbruch, unpublished) that is asking the degree to which the morphology of a stem in one year affects biomass traits of the wood produced the second year in coastal Douglas-fir. One of the foci of that larger study is the genotype x environment interactions for hydraulic performance of the xylem of the two-year old branch segments. It was expected that plants from the dry site would have lower K_s and a lower vulnerability to embolism, and that the samples from the wetter provenance would show the largest effects.

Hydraulics Measurements (collected before my involvement):

The original study included collecting data on the hydraulic properties of the samples. One 5-year old vigorous branch was selected from each of eighteen trees of the two provenances at each of the two sites. From those branches, the last two years growth was harvested and placed immediately in a bucket of water. The 2-year old stems were cut to length (avoiding the bend if any at the very base of the growth, to get a relatively straight 4-cm long segment), the bark was removed, and the samples were rehydrated from field conditions to have the maximum possible moisture content. That rehydration was accomplished by putting them in a beaker of water that was placed inside a desiccator, and then a vacuum was drawn. Samples were left there until their mass leveled off (4-7 days). In that state, the samples were weighed and then specific conductivity (K_s) was measured on the two-year old wood. The same samples were then subjected to a treatment that simulates a drought of -4 MPa (by applying a positive pressure of 4 MPa to part of the sample for 30 seconds). They were weighed a second time to learn the mass loss between 0 and -4 MPa, and then K_s was measured a second time to allow calculation of the percentage loss of hydraulic conductivity that occurred at -4 MPa relative to 0 MPa (PLC@4MPa). At that point, 0.5% aqueous acid fuchsin stain was pushed through the samples, coloring only the part of the xylem that still conducted water at -4MPa. Samples were then cut at their midpoint, and photographed with a Keyence VHX-1000 Digital Microscope. Xylem density was estimated as dry mass/green volume, where green volume was estimated from geometry using segment length (using calipers), and the cross-sectional area (calculated from the outer xylem and pith diameters, taken at two perpendicular bearings at the segment's midpoint, under the microscope).

Cross Sectional Area:

In order to correlate the hydraulic properties in the database with the wood properties, I needed to determine the proportion of a sample's cross-section that belonged to each of four categories, which I called A, B, C, and D (Figure 2). A was the area of the inner growth ring's earlywood, and B was its latewood. C was the area of the first portion of the outer (second) growth ring's earlywood and D was the remainder, which could include earlywood and some latewood. The growth ring boundary was simple to determine visually, as was the A/B boundary, by looking at cell wall thicknesses and the radial lumen diameters. The C/D boundary was mostly determined on the basis of lumen diameter, subjectively (by eye), with the D area appearing denser.

I delineated the zones of A, B, C, and D directly on the images that had already been made. Note that that image was of the cut surface of the stem (it was not a thin section). I was aided by consulting thin sections I had made of each sample, which I could view in the microscope or on-screen as the same time as I was looking at the other image (Figure 4). The area of the cross sections and the proportion of A, B, C, D, and pith of each sample were determined using images of the cross sections and a combination of Microsoft Paint and ImageJ. The images of the cross sections were obtained from pictures taken with a Keyence VHX-1000 Digital Microscope. The pith, EW, LW, and inner and outer EW2 were delineated in Paint with the help of stained images of the samples. Once cross sections were delineated, ImageJ was used to get the area of each section. The scale was set in ImageJ using the scale included in the picture. A line was drawn the length of the scale and in the “Set Scale” command the length and units were input. The polygon tool was used to trace the delineations. First the background was deleted, then everything else other than the outer EW2. The image “Threshold” was then adjusted so that the ring was red and the rest of the image black. Then the command “Analyze Particles” was used to calculate the area. This process was repeated until an area for each section was known. The values were input into Excel and then totaled to know the total area of the cross section.

Staining/Permanent Slides:

Microtome sections of the samples were taken and mounted on a slide by wrapping the sections and slide with string. The sections were then stained and permanently mounted following advised instructions from Peter Kitin. The sections were first bathed in a Safranin/Astrablue stain for 3 minutes. It was prepared by mixing 0.8 grams of Safranin powder in 100 ml of distilled water and 0.5 grams of Astrablue powder in 100 ml of distilled water plus 2 ml of acetic acid (Gartner and Schweingruber, 56). The Safranin stains lignified cell structures red and the Astrablue stains unlignified cell structures blue, allowing one to better analyze cell structures. They were then rinsed in 25% ethanol (EtOH) for ten minutes, two times. They were then bathed in 75% EtOH for 10 minutes and then in 96% EtOH for ten minutes. The sections were then transferred into absolute EtOH for ten minutes twice in different baths before finally being immersed in histoclear for a few minutes. Permount was then used to place a permanent glass cover on the sections. The slides were then left on a hotplate to dry. Once finished, pictures were taken (Figure 4).

Data analysis:

Data analyses were done at the USDA PNW Research Station in Olympia, Washington, by Kevin R. Ford, PhD.

Before testing the data for the hypotheses I was testing, we first needed to learn information from the original data (Figure 5 and 6). Dr. Ford analyzed for correlations between provenances and hydraulic traits, and between sites and hydraulic traits. The correlation is of importance to see how provenance and site affect these traits. Although there were strong trends that trees from the ORCSTN genetic source had higher loss of conductivity than did trees from the ORSISL source at both growing sites, the means were not significantly different at $p>0.05$ (Figure 11). The growth environment (Nortons, vs. Stone), however, had a significant effect (Figure 11). The ORCSTN genetic source had significantly higher K_s than the ORSISL genetic source at Nortons, which is the more mesic of the sites, but not at Stone (Figure 12). The results of these analysis directed whether further analyses included all samples pooled, compared the response of the genetic sources, and/or compared the response of the plants at the two sites.

A linear mixed model (LMM) was used to test the hypotheses. This model was necessary because some of the data being analyzed were categorical (provenance and sites). PLC@4 was logit transformed to meet the assumption of a normal error distribution. Likelihood ratio tests were used to test the significance of the correlation between the two hydraulic traits, Ks and PLC@4. The correlation coefficient R^2 for general linear mixed models (GLMM) was calculated to assess the goodness-of-fit of the models for all of the models in which the traits were significantly correlated.

Results

Proportion of water lost at -4 MPa

The proportion of water lost after the PLC@4 treatment was positively correlated (Figure 7). This tells us that water lost in response to embolism increases with the loss of conductivity. For a given amount of water lost, on average samples from Stone had lower PLC@4, meaning they were less drought susceptible, than samples from Nortons. Provenances were not included in the analysis since there was no effect of provenance on this relationship (Figure 5).

Wood density

Wood density and PLC@4 are negatively correlated (Figure 8). For a given wood density, on average a sample from Stone had a lower PLC@4, meaning they were less drought susceptible, than a sample from Nortons. Provenances were not included in the analysis since there was no effect of provenance on this relationship (Figure 5).

Proportion loss of conductivity at 4 MPa and growth ring compositions

There was no significant correlation between PLC@4 and the proportion of the growth ring’s cross section that was earlywood (Figure 9), C +D (Figure 10), or D (Figure 11). Samples had very little variation in the proportion of earlywood that they were composed of, but there was much variation in PLC@4. For example, a sample with 95-99% of earlywood could have PLC@4 ranging from just over 0 to almost 80% (Figure 9). The portion that was C+D ranged within samples, sites, and PLC@4. The portion of the xylem in D was on the other spectrum of the portion that was earlywood, but the results are scattered like that of C+D. Provenances were not included in the analysis since there was no effect of provenance on this relationship (Figure 5).

Specific conductivity vs. growth ring proportions

The specific conductivity, K_s, was positively correlated with the proportion of the cross section that is the outer ring (Figure 12). The correlation co-efficient, however, was rather low, at 0.28, meaning that only 28% of the variation in K_s could be accounted for by the proportion of the cross section in the outer ring. Provenances were not included in the analysis since there was no effect of provenance on this relationship (Figure 5).

Discussion

While some of the hypotheses were supported by this study, others were not. The first hypothesis was that PLC@4 is positively correlated with the percentage of water lost at -4 MPa. This hypothesis was supported by the data. This result can be useful for breeding programs in that it may be possible to assess drought vulnerability without having to perform the time-consuming and technically difficult measurements of PLC (percent loss of conductivity). The data suggest that one could vacuum infiltrate samples, weigh them, pressurize them as one would for a PLC measurement, and then re-weigh to learn the mass of water lost, and then use that value as a proxy of the actual PLC. This method would avoid having to make any of the conductivity measurements.

Secondly, PLC@4 was negatively correlated with wood density. This result was expected because at high water tension, tracheids are at risk of implosion. The mechanical design that gives tracheids their resistance to this mechanical implosion causes cells to have thicker cell walls (t) relative to the diameter of their lumen (d), as $(t/b)^2$. Hacke et al. (2001) and then many more researchers (Domec et al. 2009) have shown empirically that the water tension at which samples have lost half their conductivity is positively correlated with this mechanical value. The interpretation is that a plant will be designed to have no more mechanical strength than it needs because of the hydraulic stresses. If a plant had a very high $(t/b)^2$, but didn't have much tension in the xylem, it would have been wasting its biomass. Having a high t/b^2 means that cell walls are relatively thick compared to the lumen diameter, so that will cause a high wood density.

The third and fourth hypothesis related to PLC@4 and K_s. Contrary to expectation that the proportion of A and C+D would be positively correlated with PLC@4 and negatively correlated with D, results showed that neither were correlated. C and K_s also resulted in not being positively correlated. Results supported the hypothesis that C+D and K_s are positively correlated, though it is possible that it may not be as significant as it was expected to be. This suggests that the proportion of growth rings in different partitions cannot be used in this system to estimate water transport, nor vulnerability to drought. This negative result, however, leads to the hypothesis that if anatomy and proportions of the partitions of the growth ring were examined together, then it could be possible for this hypothesis to be supported.

It is likely that K_s and PLC@4 are determined by pit geometry (Lachenbruch et al. 2011, Lachenbruch and McCulloh 2014), but the exact components needed to be measured are cumbersome. Instead, tracheid diameter (Domec et al. 2002) and wood density (Rozenberg et al. 1999, Dalla-Salda et al. 2014, Martinez-Meier et al. 2015) are often used as proxies. Tracheid diameter is justified because a small diameter tracheid has small diameter pits with small mesh holes in the membrane so that the membranes do not have to move far to close off a pit, all of which are important geometric traits. As for density, it is correlated with having thicker cell walls and narrower lumens, which as discussed above, appears to have co-evolved with hydraulics so that both hydraulics and the cell wall integrity limit water transport at the same level of xylem tension (Hacke et al. 2001, Domec et al. 2009).

In future work, I intend to look at the correlation of the samples' PLC@4 with their micro-density profiles. I sawed precision-length segments from each sample, which I sent to the Rozenberg lab in Orleans, France. Drs. Dalla-Salda and Martinez-Meier were spending one year there doing research. They x-rayed each sample. When I arrive in Bariloche, Argentina into Drs. Dalla-Salda and Martinez-Meier's lab in late June, I will begin looking at the x-ray data for these samples, in relation to the staining patterns (which indicated embolism) and the values of PLC@4. These data will allow us to learn if these samples follow the same patterns as reported by Dalla Salda et al. (2014). Meanwhile, data will be collected from the thin sections that I made. Claudia Andersen and Shannon Dunfee, undergraduates in the College of Forestry, are using those sections to find the representative value of tracheid wall thicknesses (t) and lumen diameters (b) for each of the four partitions of these growth rings. We will then be able to calculate the $(t/b)^2$ of those partitions.

References

Bansal, S, CA Harrington, PJ Gould and JB St. Clair. 2015. *Climate-related genetic variation in drought-resistance of Douglas-fir* (*Pseudotsuga menziesii*). Global Change Biology. 21, 947-958.

Dalla-Salda, G, A Martinez-Meier, H Cochard and P Rozenberg. 2008. *Variation of wood density and hydraulic properties of Douglas-fir* (*Pseudotsuga menziesii* (Mirb.) Franco) *clones related to a heat and drought wave in France*. Elsevier. 257, 182-189.

Dalla-Salda, G, A Martinez- Meier, ME Fernandez, AS Sergent, P Rozenberg and E Badel. 2014. *Dynamics of cavitation in a Douglas-fir tree-ring: transition-wood, the lord of the ring?*. The Journal of Plant Hydraulics. e-0005, 1-11.

Domec, JC and BL Gartner. 2002. *Age- and position-related changes in hydraulic versus mechanical dysfunction of xylem: inferring the design criteria for Douglas-fir wood structure*. Tree Physiology. 22, 91-104.

Domec, JC, B Lachenbruch, FC Meinzer, DR Woodruff, JM Warren and KA McCulloh. 2008. *Maximum height in a conifer is associated with conflicting requirements for xylem design*. PNAS. 105(33), 12069-12074.

Domec, JC, JM Warren, FC Meinzer and B Lachenbruch. 2009. Safety factors for xylem failure by implosion and air-seeding within roots, trunks and branches of young and old conifer trees. IAWA Journal. 30, 100-120.

Gartner, H and FH Schweingruber. *Microscopic Preparation Techniques for Plant Stem Analysis*. Kessel Publishing House. 2013.

Gould, J, CA Harrington and B St. Clair. 2010. Incorporating genetic variation into a model of budburst phenology of coast Douglas-fir (Pseudotsuga menziesii var. menziesii). NRC Research Press. 41, 139-150.

Hacke, UG, JS Sperry, WT Pockman, SD Davis and KA McCulloh. 2001. *Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure*. Oecologia. 126, 457-461.

Kavanagh, KL, BJ Bond, SN Aitken, BL Gartner and S Knowe. 1999. *Shoot and root vulnerability to xylem cavitation in four populations of Douglas-fir seedlings*. Tree Physiology. 19, 31-37.

Lachenbruch, B, JR Moore and R Evans. 2011. *Chapter 5: Radial Variation in Wood Structure and Function in Woody Plants, and Hypotheses for Its Occurrence*.

Lachenbruch, B and KA McCulloh. 2014. *Traits, properties, and performance: how woody plants combine hydraulic and mechanical functions in a cell, tissue, or whole plant*. New Phytologist. 204, 747-764.

Lachenbruch, B. 2015. *Plastic Hydraulic Architecture*. Unpublished.

Lavender, DP and RK Hermann. 2014. *Douglas-fir the Genus Pseudotsuga*. 15-17.

Martinez-Meier, A, ME Fernandez, G Dalla-Salda, J Gyenge, J Licata, P Rozenberg. 2015. *Ecophysiological basis of wood formation in ponderosa pine; Linking water flux patterns with wood microdensity variables*. Forest Ecology and Management. 346, 31-40.

Rosner, S, A Klein, U Muller and B Karlsson. 2008. *Tradeoffs between hydraulic and mechanical stress responses of mature Norway spruce trunk wood*. Tree Physiol. 28(8), 1179-1188.

Rozenberg, P, A Franc, C Mamdy, J Launay, N Schermann, JC Bastein. 1999. *Genetic control of stiffness of standing Douglas-fir; from the standing stem to the stadardised wood sample, relationships between modulus of elasticity and wood density parameters. Part II*. Annals of Forest Science. 56, 145-154.

Tyree, MT and FW Ewers. 1991. *The Hydraulic Architecture of Trees and Other Woody Plants*. New Phytologist. Vol. 119, No. 3, 345-360.

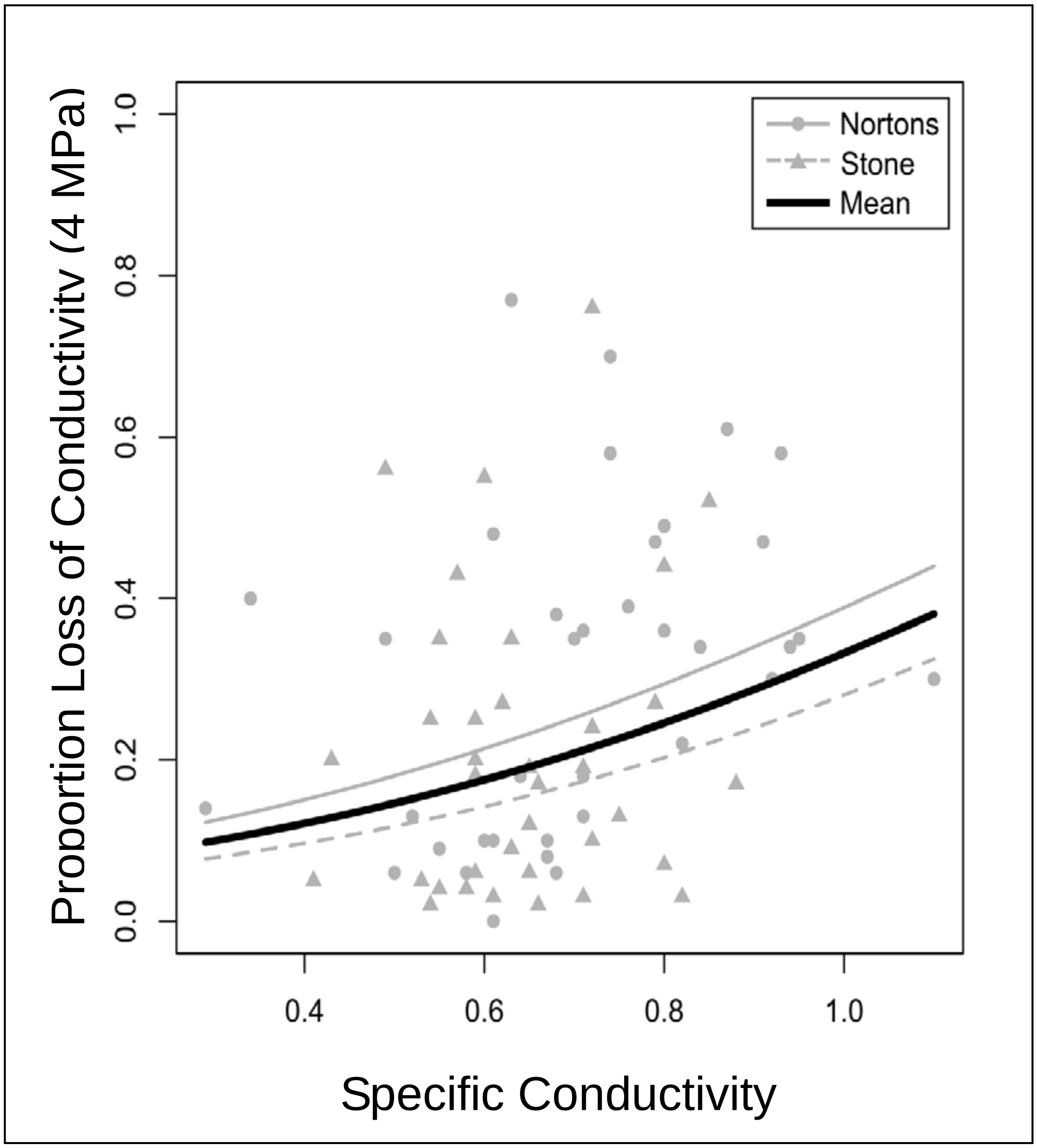

Figure 1.PLC@4 vs K_s:

Proportion loss of conductivity after samples were subjected to 4MPa was positively correlated with specific conductivity, controlling for the effect of site on proportion loss of conductivity ($p = 0.035$, $R^2_{GLMM(c)} = 0.14$).

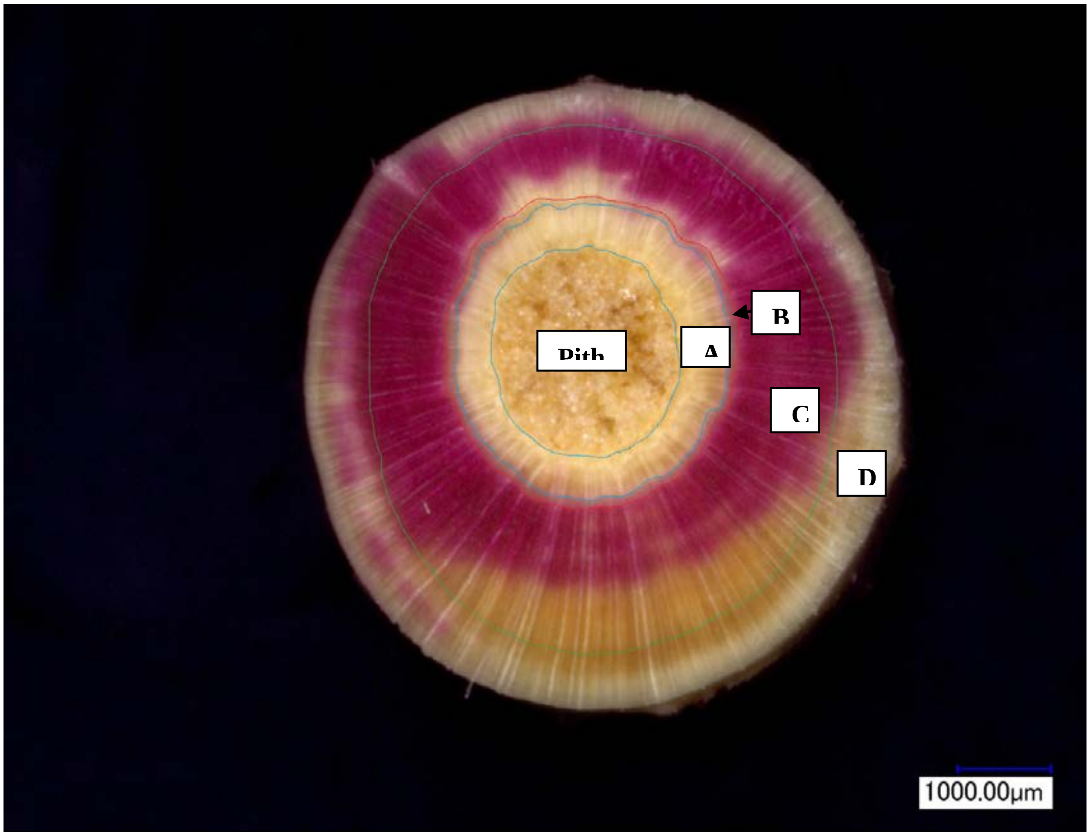

Figure 2. Growth Rings:

Tree rings have been delineated in the following order beginning from the center: pith, the earlywood (A) or latewood (B) of the inner growth ring, and the initial earlywood (C) and the remainder of the ring (D), which was earlywood transitioning toward latewood (Dalla-Salda et al. 2014).

Figure 3. Staining Pattern:

The image above is of a cross-section that is fully conductive. The image below is of a cross section after having been subjected to a pressure of 4 MPa. The loss in conductance can be seen due to the difference in staining where water movement occurred.

Figure 4.Stained and Mounted Cross-Sections:

These images were taken after the microtome sections had been mounted on slides. These were helpful in delineating the different growth rings. The top image is a close up of one of the samples. The bottom image shows the entire cross-section. These images will also be used to measure lumen diameter for future research.

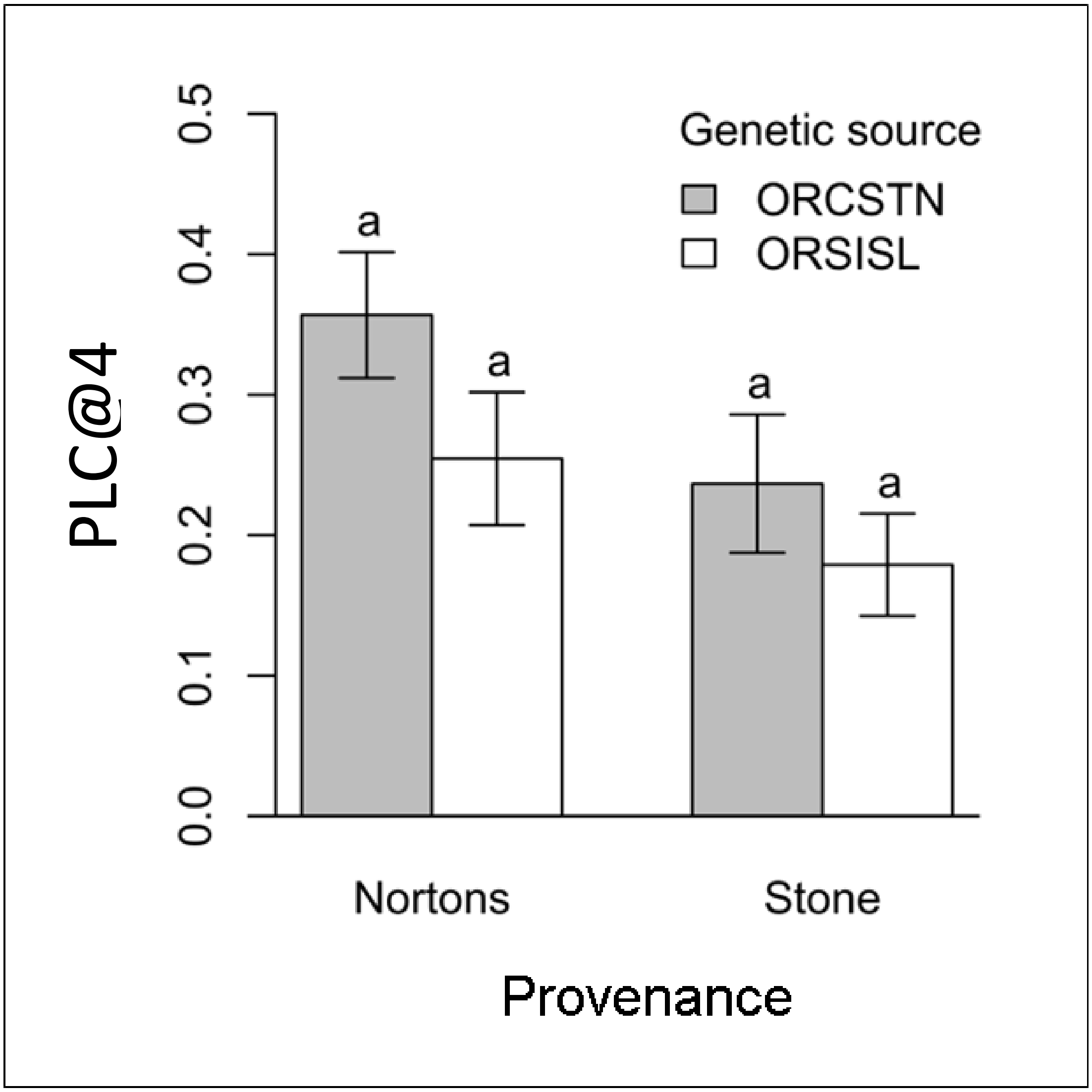

Figure 5. PLC@4 vs Provenances:

Genetic and environmental effects on proportion loss of conductivity (PLC). Bars show means with standard errors. No significant interaction between genetics and environment (p=0.48). Main effect of genetic source was not significant (p=0.12), but the main effect of growth environment was significant (p=0.029). Groups with the same letter were not significantly different (multiple comparison test, α=0.05). Though none of the groups were significantly different from any of the others at the α=0.05 level, the difference between ORCSTN at Nortons and ORSISL at Stone was marginally significant (adjusted p = 0.063).

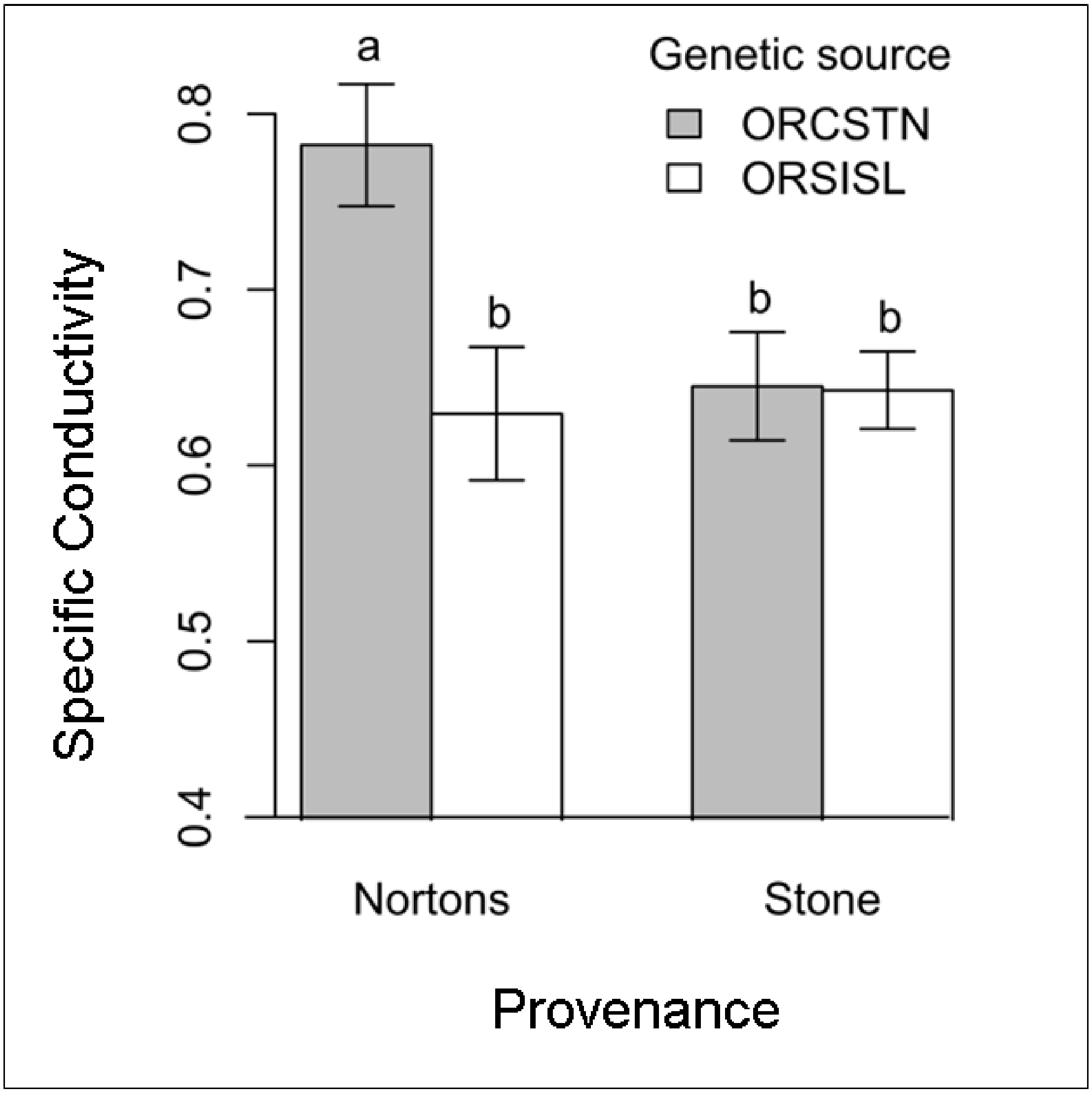

Figure 6. K_s vs. Provenance:

Genetic and environmental effects on specific conductivity. Bars show means with standard errors. There was a significant interaction effect of growth environment and genetic source on specific conductivity ($p = 0.017$). Groups with the same letter were not significantly different (multiple comparison test, $\alpha=0.05$). Trees from ORCSTN grown at Nortons had greater specific conductivity than each of the other three groups, and there were no significant differences amongst the other three groups.

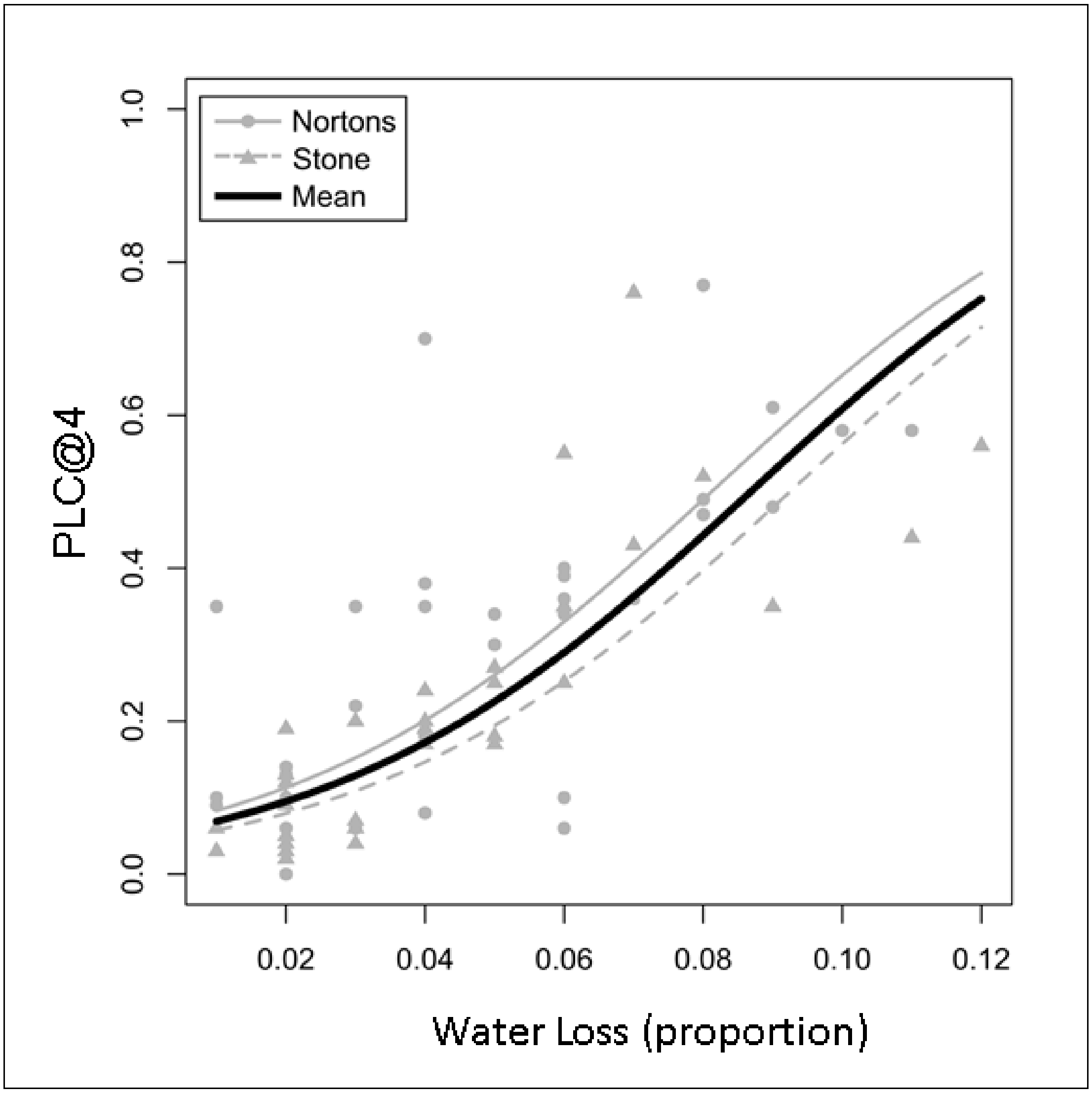

Figure 7. PLC@4 vs Proportion of Water Removed:

Proportion loss of conductivity after being subjected to 4 MPa was positively correlated with proportion of water removed, controlling for the effect of site percent loss of conductivity ($p < 0.0001$, R2GLMM(c) = 0.56).

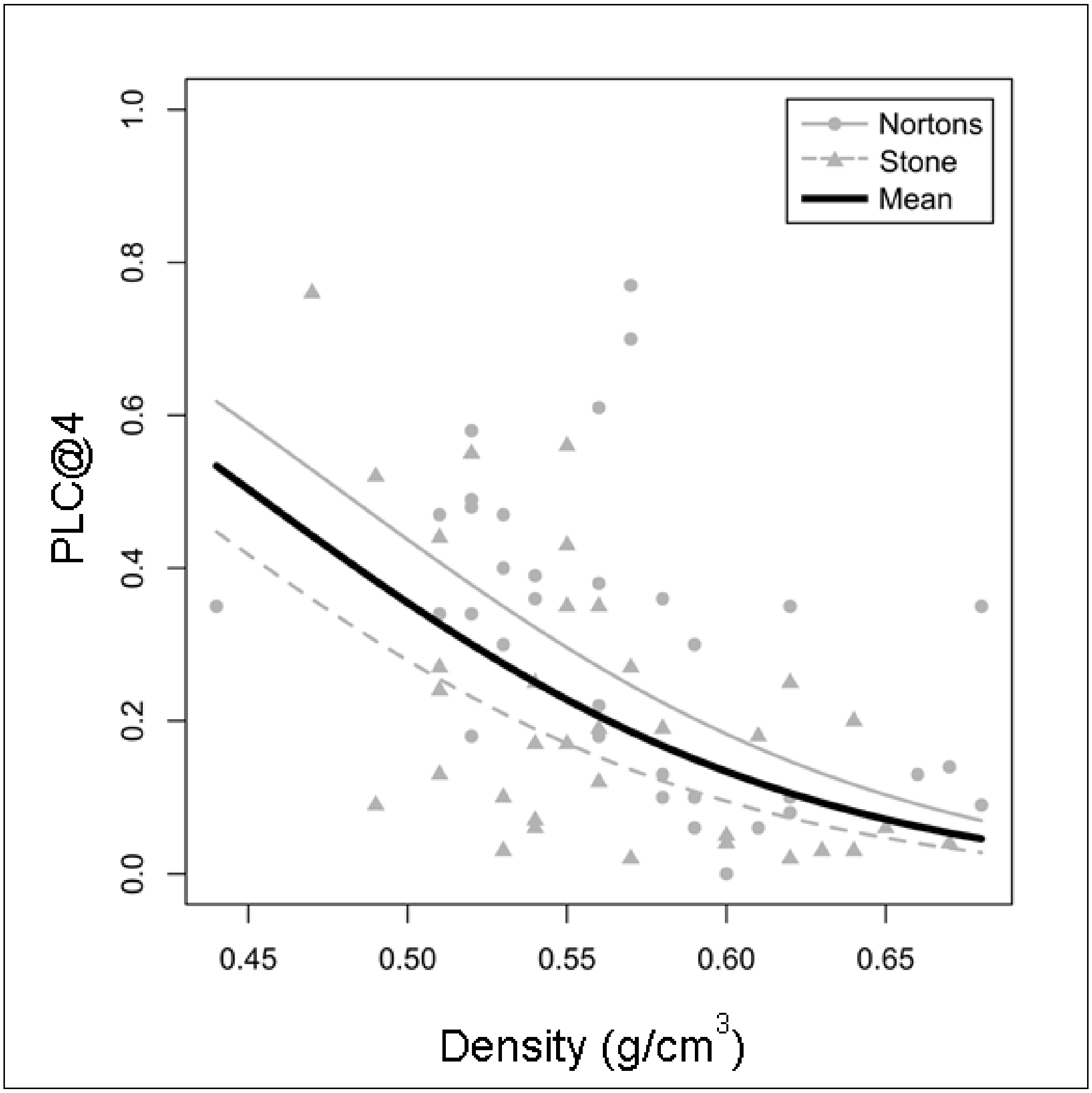

Figure 8. PLC@4 vs Density

Proportion loss of conductivity after samples were subjected to 4 MPa was negatively correlated with density, controlling for the effect of site on proportion loss of conductivity ($p < 0.0001$, $R^2_{GLMM(c)} = 0.30$).

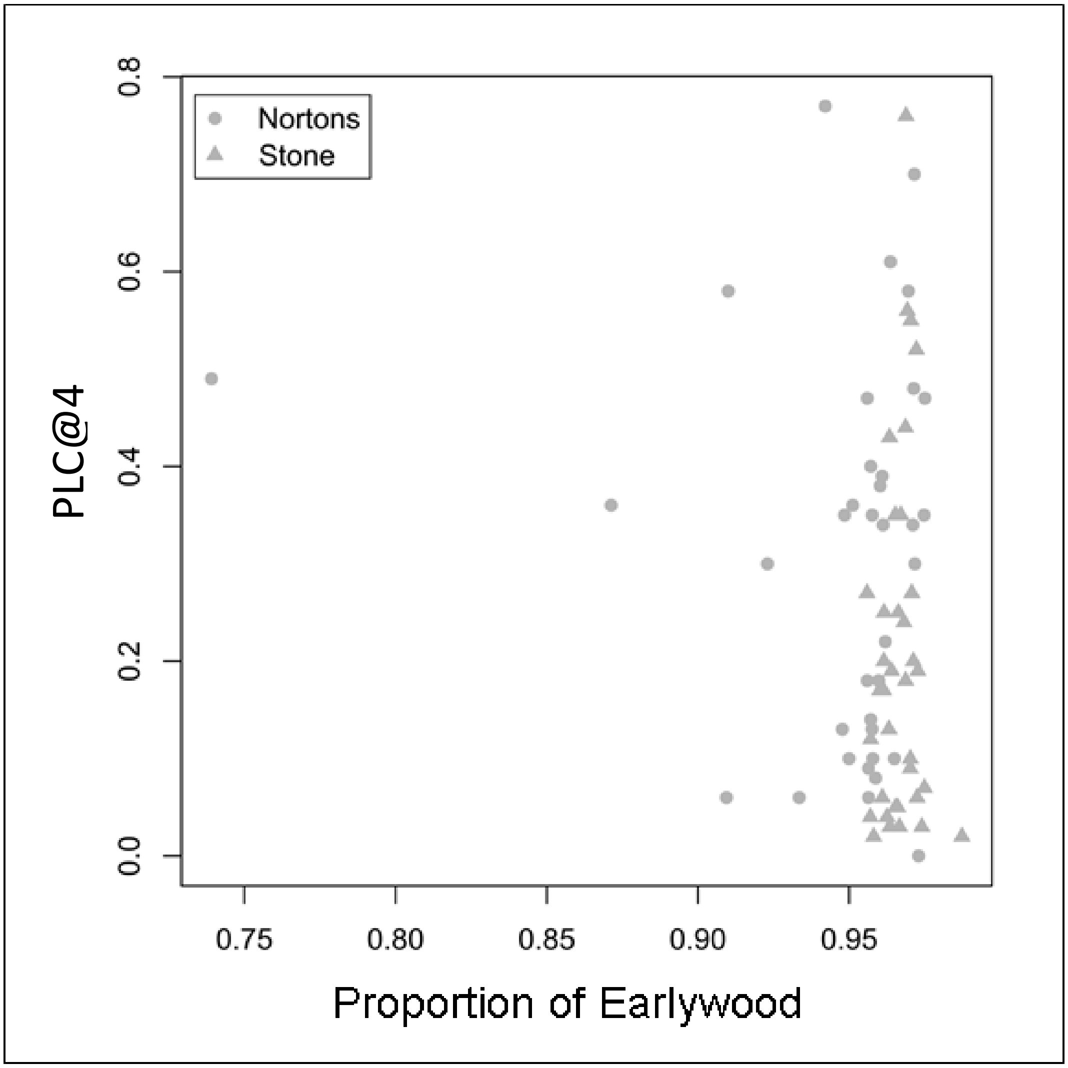

Figure 9. PLC @4 vs Proportion of Earlywood

Proportion loss of conductivity after samples were subjected to 4 MPa was not correlated with proportion of xylem tissue that was earlywood, controlling for the effect of site on proportion loss of connectivity ($p = 0.50$).

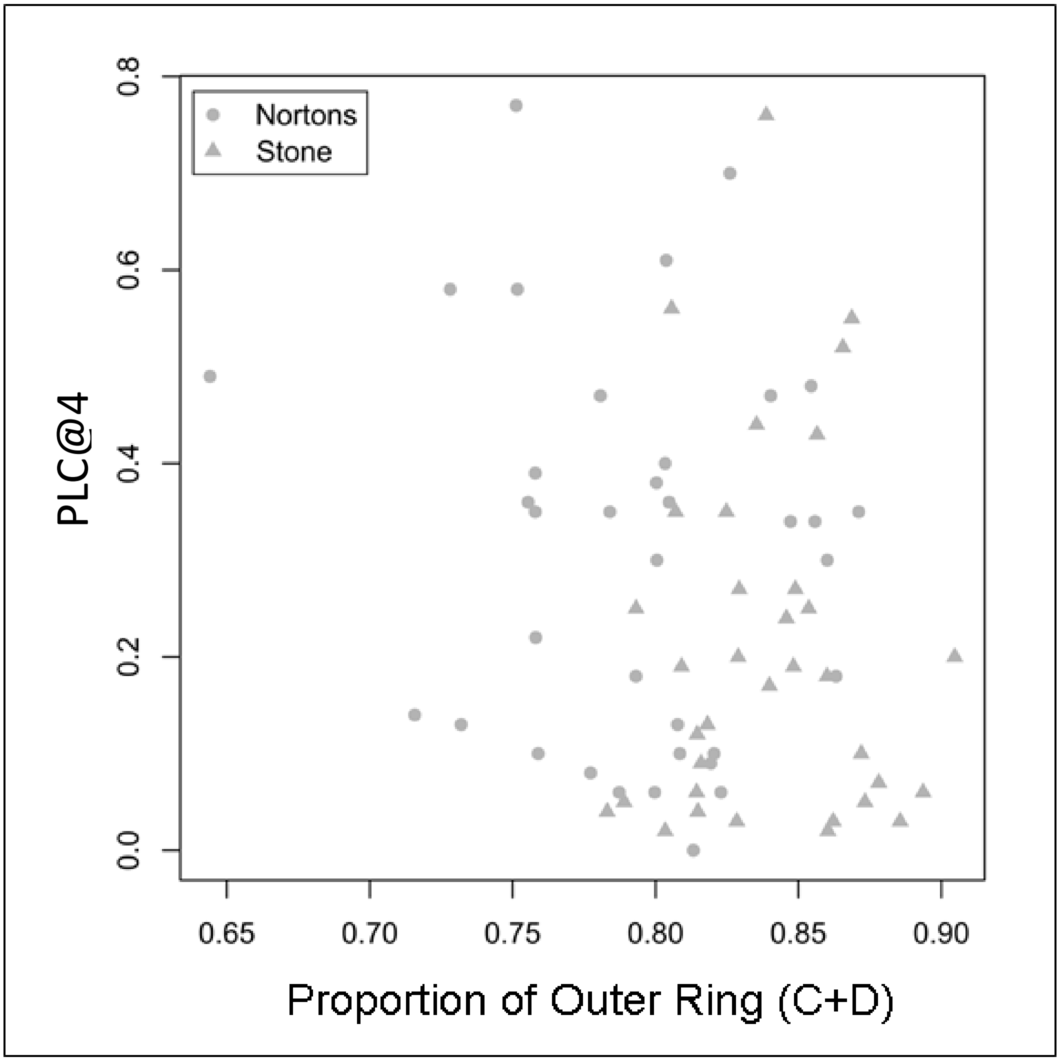

Figure 10. PLC@4 vs Proportion C+D

Proportion loss of conductivity after samples were subjected to 4 MPa was not correlated with proportion of xylem tissue in the outer ring, controlling for the effect of site on proportion loss of connectivity (p = 0.36).

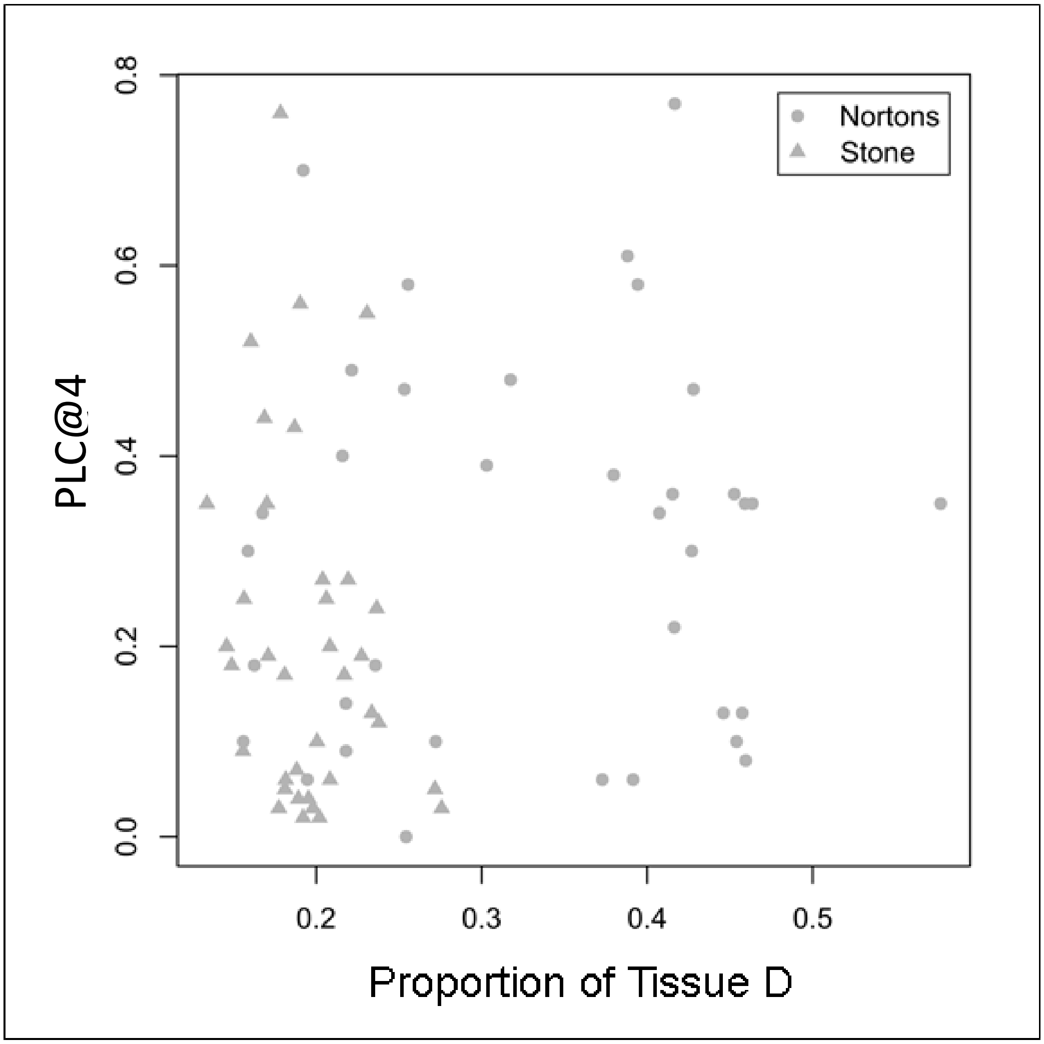

Figure 11. PLC@4 vs Proportion D

Proportion loss of conductivity after samples were subjected to 4 MPa was not correlated with proportion of xylem tissue that was in tissue type “D,” controlling for the effect of site on PLC@4 (p=0.95).

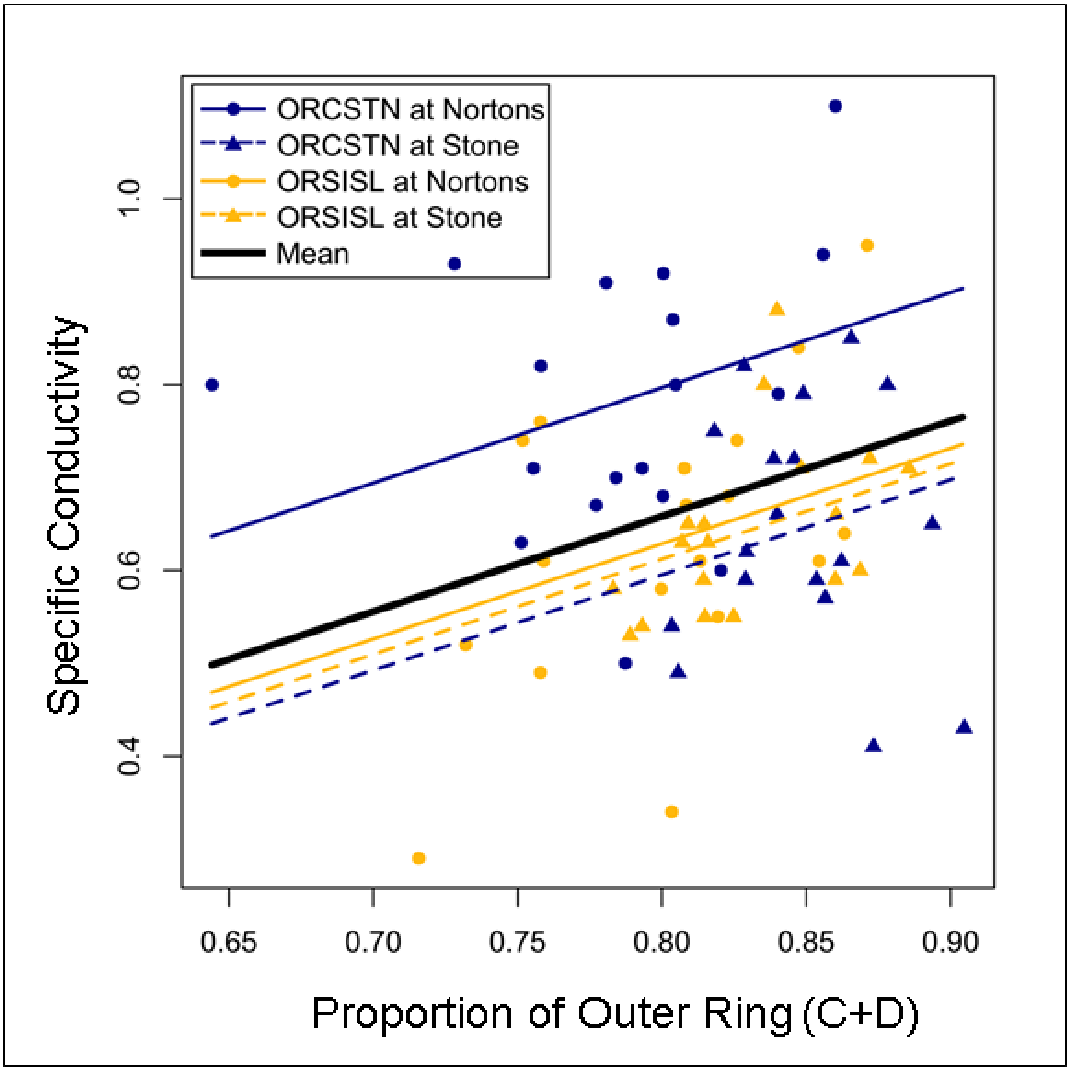

Figure 12. K_s vs. Proportion C+D

Specific conductivity after samples were subjected to 4 MPa was positively correlated with proportion of xylem tissue in the outer ring, controlling for the interactive effects of site and region on specific conductivity ($p = 0.0091$, $R^2_{GLMM(c)} = 0.28$).

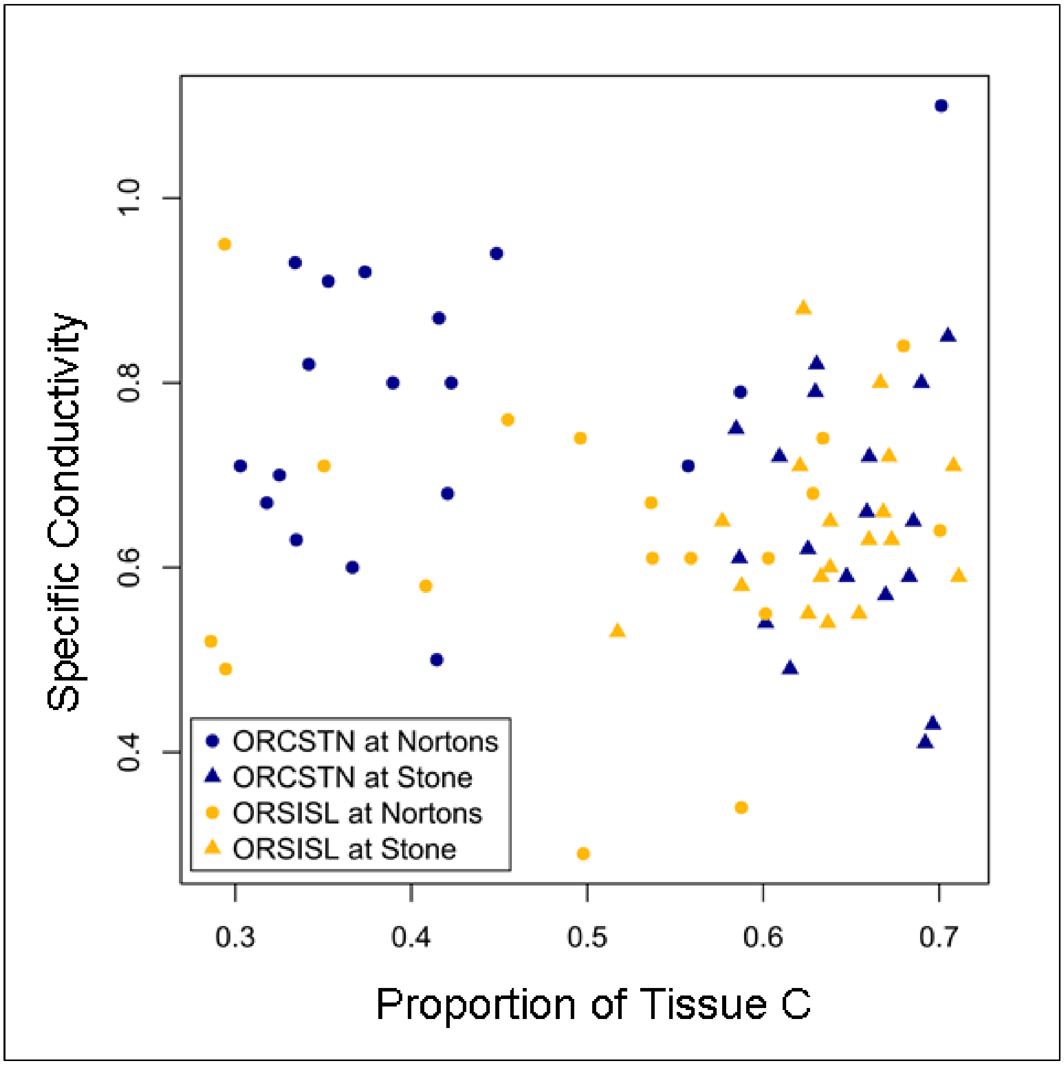

Figure 13. K_s vs Proportion C

Specific conductivity after samples were subjected to 4 MPa was not correlated with proportion of xylem tissue that was in tissue type "C", controlling for the interactive effects of site and region on specific conductivity (p = 0.24).

Chapter 2: International Interest in Wood Density to Predict Tree Physiological Success

Conifers are economically important worldwide. With the predicted rise in temperatures, it is of question whether conifers can continue to be grown successfully in plantations, and perhaps even naturally. It is predicted that conifers will begin to experience high rates of mortality as temperatures continue to rise (McDowell and Allen 2015). Researchers are interested to learn which traits are of most importance for tree survival, and whether those traits can be used as predictors of which trees are more prone to drought induced mortality. Wood density is one such trait that is of international importance for its potential use as a predictor for tree dieback and mortality (Rosner et al. 2013, Dalla-Salda et al. 2014), and wood quality (Lachenbruch et al. 2010).

Europe has recently had two severe heat waves, in 2003 and 2006, which led to much decline (dieback or slowed growth) and mortality of Norway spruce (*Picea abies*) in Norway (Rosner et al. 2013) and Douglas-fir (*Pseudotsuga menziesii*) in France (Martinez-Meier et al. 2008, a, b). The incident lasted for over six months in some areas and was the most severe drought recorded there during the last 50 years (Breda et al. 2006). Douglas-fir in France experienced partial and complete foliage necrosis as well as differences in the 2003 growth ring (Martinez-Meier et al. 2008, a). The formation of the 2003 growth ring ended sooner that the years prior and post heat wave.

Dalla-Salda et al. (2009) found that the surviving Douglas-fir clones were those that had a higher wood density and expects that this genetic response to drought will help with the survival of trees as temperatures increase (Martinez-Meier et al. 2008, a, Ruiz Diaz et al. 2014).

The relationship between wood density and drought vulnerability has been studied in many species. In general, high wood density is associated with better tree performance during drought. For example, trees that survived drought tended to have higher wood density in a large number of species and regions: Norway spruce in France and Norway (Bouriard et al. 2005, Rosner et al. 2013); ponderosa pine (*Pinus ponderosa*) in Argentina (Martinez-Meier et al. 2015)), Douglas-fir in France and Argentina (Dalla-Salda et al. 2009), and tropical savannah trees (six dominant tree species) in Brasilia, Brazil (Bucci et al. 2004).

Where it has been looked at, the exact anatomy associated with the higher wood density in more drought-hardy individuals is variable in different species and studies. The work in *Schefflera macrocarpa*, *Styrax ferrugineus*, *Ouratea hexasperma*, *Caryocar brasiliense*, *Erythroxylum suberosum*, and *Kielmeyera coriaceae* and Norway spruce showed an increase in the amount of latewood, with the same earlywood amount, giving an increase in wood density (Bucci et al. 2004, Bouriard et al. 2005). Reasoning for that was that latewood was more affected by changes in temperature. In Norway spruce, Bouriard et al. (2005) showed a tradeoff between wood density (and improved drought-hardiness) and radial growth rate of the trees. Other work in Norway spruce showed that individual trees that survived the heat wave had higher wood density than those that die or had significant dieback, and that the divergence in wood density started many years before the drought came (Rosener et al. 2007). The survivors had a narrower zone of wood that was transitional between earlywood and latewood than those that died (Rosner et al. 2007). Martinez-Meier et al. (2008, a, b) had similar findings in surviving Douglas-fir, comparing growth rings from the drought year (2003) to the 2002 and 2004. Ruiz Diaz et al. (2014) looked further at the Douglas-fir trees that survived vs. died in the 2003 height wave in France. Comparing pairs of surviving and dead trees, they showed that surviving trees had denser wood in all three regions studied, but that the densest part of the ring varied depending on the region.

The reasons that wood density confer drought resistance are also being studied internationally. Work in the lab in which I did my senior thesis is investigating the role of wood density in Douglas-fir trees in the Pacific Northwest. That work is characterizing the anatomy of the locations within the wood that are no longer conducting water during drought (see Chapter 1, above). Water moves through wood according to the local pressure gradients. The water is in tension (e.g., it is being stretched), and drought can cause air bubbles to be pulled into the water-conducting cells (tracheids or vessels). When that happens, the cell becomes embolized (air-filled). Being air-filled means the cell cannot transport water. What is more damaging though is if the air in one cell then spreads to the next. The spread of embolism is controlled by the pits, which are the tiny holes between tracheids or vessels that allow for lateral water movement. The pits essentially have valves on them, and the characteristics of those valves determines whether the air bubble can spread. Therefore, there seems to be a relationship between wood density and these pits' functioning, and that can occur in two ways in a conifer. Latewood is much denser than earlywood, and it is known that latewood is not drought resistant (the pit membranes cannot stretch enough to close off the pits; Domec and Gartner 2002). If a tree had a higher proportion of latewood, the wood will have a higher density, but it should also be more susceptible to drought, not less. That means that the positive density/drought-hardiness relationship should be driven by wood other than the latewood, which includes the early earlywood (the largest cells with thin cell walls), the earlywood (large cells with thin cell walls) and the transition wood (intermediate-sized cells with intermediate-thickness of cell walls).

DallaSalda et al. (2014), working on conifer trees in both France and Argentina, has published data showing that as the drought (water tension) increases, the first embolism (air-filling) occurs in the latewood. That result is consistent with Domec and Gartner's 2002 result that latewood embolizes first because the pit membranes are too stiff to stretch and block a pit. The next wood to embolize is the early earlywood, most likely because the pit membranes have expanded to a large size (given the large size of cells) leaving large pores that air bubbles can pass through with less pressure gradient (Lachenbruch, personal communication). Those embolisms spread with increasing drought into the rest of the earlywood (DallaSalla et al. 2014), leaving the transition wood as the last zone to embolize. Other work, on 12 conifer species and 36 hardwood species (Hacke et al. 2001) mostly from Utah, California, the Great Basin and Sonoran deserts, and North Carolina, showed a strong relationship of wood density to resistance to embolism in wood (Hacke et al. 2001). The explanation was that there was no point, evolutionarily, in plants making wood that is stronger than necessary for avoiding 'implosion' if there is a drought. That the tension that would cause implosion of tracheids and vessels was related to $(t/b)^2$ (t is cell wall thickness, b is the diameter of the inside of the cell), and that t/b is directly related to wood density, explaining the general relationship (all other things being equal) (Hacke et al. 2001).

The 'all other things being equal' statement is important. High wood density does not necessarily mean that a tree will be drought resistant. For example, Johnson et al. (2005) reported on trees in coastal Oregon that had a leaf pathogen that caused the trees to have high latewood proportion and high wood density. The trees had such little earlywood that they would not be able to transport much water. Other studies show that if the wood is made dense by other types of traits (like compression wood, made on the underside of a leaning tree), the wood will be less drought hardy (Mayr and Cochard, 2003, working in the Alps). The cell walls have different properties in different situations, and that is not taken into account in Hacke et al's calculations (2001), and so positive relationship between wood density and drought resistance does not always hold (reviewed in Lachenbruch et al. 2011).

Wood density is also of international interest for estimating wood quality (Zobel and van Buijenen 1983), for example in New Zealand (Acuna and Murphy 2006), Chile (Lasserre et al. 2009), Europe (Moore 2011), Argentina (Martinez-Meier et al. 2008, a,b, Dalla-Salda et al. 2014), and the United States (Lachenbruch et al. 2010). Lachenbruch et al. (2010) found that density was a good predictor of wood stiffness and strength in Douglas-fir as long as only the mature wood was considered, because in juvenile wood, which has different cell wall properties, the relationship does not hold (Domec and Gartner 2002). Radiata pine or Monterey pine (*Pinus radiata*), a species of great economic importance (grown commercially in New Zealand and Chile), typically has a large loss in value due to intra-ring checking (the breaking of the wood in the radial direction, that occurs in live trees) (Putoczki et al. 2007), which occurs in many cases in locations that have had severe summer drought (Grabner et al. 2006, Putoczki et al. 2007). There are probably various factors that lead to intra-ring checking occurrences including cell wall structure (Putoczki et al. 2007) and changes in water pressure ((Almeras and Gril 2007), but it seems likely that where wood is very dense, it will be less flexible, and so the tension in the water column may be more likely to make the wood separate (Grabner et al. 2006).

Researchers are also interested in management practices that can improve wood quality, such as fertilization and thinning regimes (Josza and Brix 1989, Kang et al. 2004, Lasserre et al. 2009). Close spacing tends to result in higher wood density, although the research of Lasserre et al. (2009) with radiata pine grown in a plantation in New Zealand did not support that trend, meaning the effects that silvicultural treatments have on wood density vary across species and site. Another application of wood density comes from its relationship with pulp, which is used for paper. Barnett and Bonham (2004) report that lower wood density, which is related to high proportion of earlywood, makes better pulp because earlywood fibers have high microfibril angle, drape better and make for softer, more flexible, and more opaque paper. On the other hand, higher wood density gives higher ‘yield’, which is the amount of pulp that can be made from a volume of wood.

It may also be possible to have breeding programs to control wood density. The findings of Martinez-Meier et al. (2008, b) suggest that the relationship between mean ring density and heat could be of importance for tree breeding programs, since mean ring density often can be genetically controlled. Rozenberg et al. (2002) results show that is possible for density peaks to be used to breed drought resistant Norway spruce at no cost of lowering wood density and decreasing wood quality, although more research is needed because the time at which density peaks is not consistent, so the physiological mechanism is unclear. Dalla-Salda et al. (2011) reported significant differences among clones in wood density and hydraulic characteristics, showing promise for use in improving drought resistance in Douglas-fir.

This international research concerning wood density is of utmost economic importance and for the continuing survival of forests worldwide. With expected warming temperatures, breeding for drought resistant trees becomes more of a priority. Wood density is one possible trait that can be of use. It is also beneficial that it can be used as a proxy for wood quality, which is important for high quality wood products as well as for pulp.

References

Acuna, M and G Murphy. 2006. *Estimating internal wood properties of logs based on real-time, NIR measurements of chainsaw wood chips from a harvester/processor*. Council on Forest Engineering (COFE) Conference Proceedings. 463-472.

Almeras, T and J Gril. 2007. *Mechanical analysis of the strains generated by water tension in plant stems. Part I: stress transmission from the water to the cell walls*. Tree Physiology. 27, 1505-1516.

Barnett, JR and VA Bonham. 2004. *Cellulose microfirbril angle in the cell wall of wood fibres*. Biological Reviews of the Cambridge Philosophical Society. 79 (2), 461-472.

Bouriaud, O, JM Leban, D Bert and C Deleuze. 2005. *Intra-annual variations in climate influence growth and wood density of Norway spruce*. Tree Physiology. 25, 651-660.

Breda, N, R Huc, A Granier and E Dreyer. 2006. *Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences*. Annals of Forest Science, 63, 625–644.

Bucci, S.J, G Goldstein, FC Meinzer, FG Scholz, AC Franco and M Bustamante. 2004. *Functional convergence in hydraulic architecture and water relations of tropical savanna trees: from leaf to whole plant*. Tree Physiology. 24, 891-900.

Dalla-Salda, G, A Martinez-Meier, H Cochard and P Rozenberg. 2009. *Variation of wood density and hydraulic properties of Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) clones related to a heat and drought wave in France*. Forest Ecology and Management. 257, 182-189.

Dalla-Salda, G, A Martinez-Meier, H Cochard and P Rozenberg. 2011. *Genetic variation of xylem hydraulic properties shows that wood density is involved in adaptation to drought in Douglas-fir (Pseudotsuga menziesii (Mirb.)).* Annals of Forest Science. 68, 747-757.

Dalla-Salda, G, A Martinez-Meier, ME Fernandez, AS Sergent, P Rozenberg and E Badel. 2014. *Dynamics of cavitation in a Douglas-fir tree-ring: transition-wood, the lord of the ring?.* The Journal of Plant Hydraulics. e-0005, 1-11.

Domec, JC and BL Gartner. 2002. *Age- and position-related changes in hydraulic versus mechanical dysfunction of xylem: inferring the design criteria for Douglas-fir wood structure*. Tree Physiology. 22, 91-104.

Grabner, M, P Cherubini, P Rozenberg and B Hannrup. 2006. *Summer drought and low earlywood density induce intra-annual radial cracks in conifers*. Scandinavian Journal of Forest Research. 21, 151-157.

Hacke, UG, JS Sperry, WT Pockman, SD Davis and KA McCulloh. 2001. *Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure*. Oecologia. 126, 457-461.

Johnson, GR, AT Grotta, BL Gartner and G Downes. 2005. *Impact of the foliar pathogen Swiss needle cast on wood quality of Douglas-fir*. Canadian Journal of Forest Research. 35, 331-339.

Jozsa LA and H Brix. 1989. *The effects of fertilization and thinning on wood quality of a 24-yearl old Douglas-fir stand*. Canadian Journal of Forest Research. 19, 1137-1145.

Kang, KY, SY Zhang and SD Mansfield. *The effects of initial spacing on wood density, fibre and pulp properties in jack pine (Pinus banksiana Lamb.)*. Hotzforachung. 58, 455-463.

Lachenbruch, B, GR Johnson, GM Downes and R Evans. *Relationships of density, microfibril angle, and sound velocity with stiffness and strength in mature wood of Douglas-fir*. Canadian Journal of Forest Research. 40, 55-64.

Lasserre, Jp, EG Mason, MS Watt and JR Moore. 2009. *Influence of initial planting spacing and genotype of microfibril angle, wood density, fibre properties and modulus of elasticity in Pinus radiata D. Don corewood*. Forest Ecology and Management. 258, 1924-1931.

Martinez-Meier, A, L Sanchez, G Dalla-Salda, MJM Pasotino, JY Gautry, LA Gallo and P Rozenberg. 2008. *Genetic control of the tree-ring response of Douglas-fir (Psedotsuga menziesii (Mirb.) Franco) to the 2003 drought and heat-wave in France*. Annals of Forest Science. 65 (102), 1-8. a.

Martinez-Meier, A, L Sanchez, MJM Pastorino, LA Gallo and P Rozenberg. 2008. *What is hot in tree rings? The wood density of surviving Douglas-firs to the 2003 drought and heat wave.* Forest Ecology and Management. 256, 837-843. b.

Martinez-Meier, A, ME Fernandez, G Dalla-Salda, J Gyenge, J Licata and P Rozenberg. 2015. *Ecophysiological basis of wood formation in ponderosa* *pine: Linking water flux patterns with wood microdensity variables*. Forest Ecology and Management. 346, 31-40.

Mayr S and H Cochard. 2003. *A new method for vulnerability analysis of small xylem areas reveals that compression wood of Norway spruce has lower hydraulic safety that opposite wood*. Plant, Cell and Environment. 26, 1365-1371.

McDowell, NG and CD Allen. 2015. *Darcy's law predicts widespread forest mortality under climate warming*. Nature Climate Change. 1-4.

Moore, J. 2011. *Wood properties and uses of Sitka spruce in Britain*. Forestry Commission. 1-48.

Putoczki, TL, H Nair, B Butterfield and SL Jackson. 2007. *Intra-ring checking in Pinus radiata D. Don: the occurrence of cell wall fracture, cell collapse, and lignin distribution*. Trees. 21, 221-229.

Rosner, S, A Klein, U Muller and B Karlsson. 2007. *Hydraulic and mechanical properties of young Norway spruce clones related to growth and wood structure*. Tree Physiology. 27, 1165-1178.

Rosner, S., S. Andreassen, I. Børja, L. Dalsgaard, R. Evans, R. Hentschel, Z. Kayler, J. Svetlik and S. Solberg. 2013. *Is recent occurrence of top dieback in Norway spruce triggered by drought stress?* NRC Research Press. 154-161.

Rozenberg, P., J. van Loo, B. Hanrrup and M. Grabner. 2002. *Clonal variation of wood density record of cambial reaction to water deficit in Picea abies (L.) Karst*. Annals of Forest Science 59: 533-540.

Ruiz Diaz Britez, M., A.-S. Sergent, A. Martinez Meier, N. Breda and P. Rozenberg. 2014. *Wood density proxies of adaptive traits linked with resistance to drought in Douglas- fir (Pseudotsuga menziesii (Mirb.) Franco)*. Trees. 28, 1289-1304.

Appendix 1

This appendix contains climate data that was used to choose appropriate study sites and trees for a larger project (Lachenbruch unpublished) that is asking the degree to which the morphology of a stem in one year affects biomass traits of the wood produced the second year in coastal Douglas-fir. All climate data were estimated by the PRISM Oregon Climate Model using inputs of latitude, longitude, and elevation from the 30 year period of 1961-1990 (Daly et al. 2002). The data were extracted and downscaled using the tool ClimateWNA (Wang et al. 2013). Nortons was chosen to represent the relatively wet site and Stone as the relatively dry site. Nortons is at about 485 m elevation in the central Oregon Coast Range west of Corvallis, OR, USA. Stone is about 300 km south of Nortons, at lower elevation (185 m), and in the broad inland valley near Medford, OR, USA between the Siskiyou Mountains and the Cascades Range. Populations for the study were then selected by geographic proximity to each of the sites: Oregon Coastal North (ORCSTN) near Nortons and Oregon Siskiyou Low Elevation (ORSISL) near Stone. Then three families per population that had climate (focusing mainly on summer precipitation and annual heat: moisture index) that was most similar to the nearby site were selected for the study. Thus, in total, at each of the two sites (Nortons and Stone) we studied six trees from three families that originated near Nortons and three families that originated near Stone.

Family	Population	Latitude	Longitude	Elevation	MAT	MWMT	MAP	MSP	AHM	SHM	NFFD	CMD
	ORCSTN	44.642	-123.752	228	10.9	17.2	2653	346	8.6	52.9	308	243
		0.159	0.059	113	0.9	0.6	673	78	2.1	11.1	18	42
	ORSISL	42.623	-123.088	471	12.1	21.0	826	120	27.6	183.0	297	640
		0.184	0.036	26	0.3	0.7	132	22	4.2	33.4	1	59
	Nortons	44.664	-123.687	415	9.7	15.9	1711	236	11.5	67.3	282	303
	Stone	42.349	-122.939	185	13.0	22.4	535	87	43.0	258.8	298	809
6016	ORCSTN	44.403	-123.837	241	11.8	18.2	2297	299	9.5	60.7	324	299
8045	ORCSTN	44.672	-123.749	61	11.3	16.8	1931	266	11	63.2	321	249
8524	ORCSTN	44.85	-123.67	381	9.5	16.5	3732	472	5.2	34.9	278	181
3094	ORSISL	42.923	-123.03	429	11.6	19.9	1041	155	20.7	128.4	296	543
3285	ORSISL	42.472	-123.116	491	12.3	21.5	713	101	31.2	212.1	297	690
3286	ORSISL	42.475	-123.118	493	12.3	21.5	725	103	30.8	208.4	299	686

MAT=Mean Annual Temperature
MWMT=Warmest Month Temperature
MAP=Precipitation
MSP=Summer Precipitation (May to September)
AHM=Annual Heat:Moisture Index (high is more arid)
SHM=Summer Heat:Moisture Index (high is more arid)
NFFD=Frost Free Days
CMD=Hargreaves Climatic Moisture Deficit

References:

Daly, C, M Halbleib, JI Smith, WP Gibson, MK Doggett, GH Taylor, J Curtis and PP Pasteris. 2008. *Physiographically sensitive mapping of climatological temperature and precipitation across the conterminous United States*. International Journal of Climatology. DOI: 10.1002/joc.1688.

Wang T, A Haman and DL Spittlehouse. 2013. Climate WNA v4.7s: *A program to generate climate normal, annual, seasonal and monthly data for genecology and climate change studies in Western North America (WNA) region*. Centre for Forest Conservation Genetics. Web. <http://cfcg.forestry.ubc.ca/projects/climate-data/climatebcwna/>

Appendix 2

This appendix contains original data from a larger project (Lachenbruch unpublished) that is asking the degree to which the morphology of a stem in one year affects biomass traits of the wood produced the second year in coastal Douglas-fir, as well as data collected during this study. Nortons was chosen to represent the relatively wet site and Stone as the relatively dry site. Nortons is at about 485 m elevation in the central Oregon Coast Range west of Corvallis, OR, USA. Stone is about 300 km south of Nortons, at lower elevation (185 m), and in the broad inland valley near Medford, OR, USA between the Siskiyou Mountains and the Cascades Range. Populations for the study were then selected by geographic proximity to each of the sites: Oregon Coastal North (ORCSTN) near Nortons and Oregon Siskiyou Low Elevation (ORSISL) near Stone. Then three families per population that had climate (focusing mainly on summer precipitation and annual heat: moisture index) that was most similar to the nearby site were selected for the study. Thus, in total, at each of the two sites (Nortons and Stone) we studied six trees from three families that originated near Nortons and three families that originated near Stone.

Individual Tree ID	**Site grown**	**Sample**	**Population**	**Family**	**K$_s$**	**Loss of conductivity at 4 MPa (%)**	**Water removed (% of original)**	**Xylem area (m2)**	**Subsample wood density (g/cm3)**	**A**	**B**	**C**	**D**
4371	Nortons	PSME 0150	ORCSTN	8524	0.00080	0.49	0.08	3.19E-05	0.52	10%	9%	42%	22%
2458	Nortons	PSME 0151	ORCSTN	6016	0.00071	0.36	0.07	4.05E-05	0.54	12%	3%	30%	45%
1824	Nortons	PSME 0156	ORSISL	3285	0.00061	0.48	0.09	4.09E-05	0.52	12%	3%	54%	32%
8984	Nortons	PSME 0159	ORCSTN	8524	0.00093	0.58	0.11	2.18E-05	0.52	18%	9%	33%	39%
8896	Nortons	PSME 0160	ORCSTN	8045	0.00094	0.34	0.05	4.27E-05	0.51	11%	4%	45%	41%
6660	Nortons	PSME 0163	ORSISL	3306	0.00058	0.06	0.03	2.71E-05	0.61	13%	7%	41%	39%
6288	Nortons	PSME 0164	ORSISL	3094	0.00064	0.18	0.04	1.90E-05	0.52	9%	4%	70%	16%
8979	Nortons	PSME 0165	ORCSTN	8524	0.00092	0.30	0.05	2.64E-05	0.53	12%	8%	37%	43%
7211	Nortons	PSME 0166	ORCSTN	6016	0.00080	0.36	0.06	2.88E-05	0.58	15%	5%	39%	42%
4297	Nortons	PSME 0167	ORCSTN	8045	0.00087	0.61	0.09	3.21E-05	0.56	16%	4%	42%	39%

8981	Nortons	PSME 0168	ORCSTN	8524	0.00050	0.06	0.06	1.81E-05	0.61	12%	9%	41%	37%
4300	Nortons	PSME 0170	ORCSTN	8045	0.00070	0.35	0.04	4.69E-05	0.68	17%	4%	32%	46%
1823	Nortons	PSME 0171	ORSISL	3285	0.00076	0.39	0.06	2.24E-05	0.54	20%	4%	45%	30%
1873	Nortons	PSME 0173	ORSISL	3306	0.00061	0.10	0.02	9.71E-06	0.58	19%	5%	60%	16%
6608	Nortons	PSME 0174	ORSISL	3285	0.00029	0.14	0.02	1.80E-05	0.67	24%	4%	50%	22%
2452	Nortons	PSME 0176	ORCSTN	6016	0.00060	0.10	0.06	1.96E-05	0.59	14%	4%	37%	45%
4395	Nortons	PSME 0177	ORCSTN	8524	0.00067	0.08	0.04	2.75E-05	0.62	18%	4%	32%	46%
4286	Nortons	PSME 0178	ORCSTN	8045	0.00082	0.22	0.03	3.51E-05	0.56	20%	4%	34%	42%
6675	Nortons	PSME 0179	ORSISL	3306	0.00049	0.35	0.01	1.96E-05	0.62	19%	5%	29%	46%
6591	Nortons	PSME 0180	ORSISL	3285	0.00071	0.13	0.02	2.30E-05	0.58	15%	4%	35%	46%
1885	Nortons	PSME 0181	ORSISL	3306	0.00055	0.09	0.01	6.50E-06	0.68	14%	4%	60%	22%
1460	Nortons	PSME 0182	ORSISL	3094	0.00084	0.34	0.06	3.81E-05	0.52	12%	3%	68%	17%

4385	Nortons	PSME 0183	ORCSTN	8524	0.00068	0.38	0.04	3.76E-05	0.56	16%	4%	42%	38%
2453	Nortons	PSME 0184	ORCSTN	6016	0.00091	0.47	0.08	2.17E-05	0.51	18%	4%	35%	43%
8893	Nortons	PSME 0187	ORCSTN	8045	0.00071	0.18	0.04	3.38E-05	0.56	17%	4%	56%	24%
6310	Nortons	PSME 0188	ORSISL	3094	0.00074	0.58	0.10	3.26E-05	0.52	22%	3%	50%	26%
6587	Nortons	PSME 0189	ORSISL	3285	0.00052	0.13	0.02	2.11E-05	0.66	22%	5%	29%	45%
6670	Nortons	PSME 0190	ORSISL	3306	0.00074	0.70	0.04	4.67E-05	0.57	15%	3%	63%	19%
1462	Nortons	PSME 0191	ORSISL	3094	0.00095	0.35	0.03	5.80E-05	0.44	10%	3%	29%	58%
8895	Nortons	PSME 0192	ORCSTN	8045	0.00063	0.77	0.08	1.81E-05	0.57	19%	6%	33%	42%
1820	Nortons	PSME 0193	ORSISL	3285	0.00067	0.10	0.01	2.45E-05	0.62	16%	4%	54%	27%
6275	Nortons	PSME 0194	ORSISL	3094	0.00068	0.06	0.02	9.74E-06	0.59	13%	4%	63%	19%
7204	Nortons	PSME 0195	ORCSTN	6016	0.00110	0.30	0.05	5.24E-05	0.59	11%	3%	70%	16%
1895	Nortons	PSME 0196	ORSISL	3306	0.00061	0.00	0.02	4.06E-05	0.60	16%	3%	56%	25%

1501	Nortons	PSME 0198	ORSISL	3094	0.00034	0.40	0.06	2.69E-05	0.53	15%	4%	59%	22%
7193	Nortons	PSME 0199	ORCSTN	6016	0.00079	0.47	0.08	3.01E-05	0.53	13%	3%	59%	25%
8994	Stone	PSME 0200	ORCSTN	8524	0.00072	0.24	0.04	2.08E-05	0.51	12%	3%	61%	24%
1893	Stone	PSME 0201	ORSISL	3306	0.00053	0.05	0.02	3.22E-05	0.60	18%	3%	52%	27%
4384	Stone	PSME 0202	ORCSTN	8524	0.00075	0.13	0.02	2.06E-05	0.51	14%	4%	58%	23%
6671	Stone	PSME 0203	ORSISL	3306	0.00059	0.06	0.01	1.45E-05	0.65	15%	4%	63%	18%
1458	Stone	PSME 0205	ORSISL	3094	0.00054	0.25	0.05	1.54E-05	0.54	17%	4%	64%	16%
7217	Stone	PSME 0206	ORCSTN	6016	0.00065	0.06	0.03	1.33E-05	0.54	8%	3%	69%	21%
6279	Stone	PSME 0207	ORSISL	3094	0.00072	0.10	0.02	3.02E-05	0.53	10%	3%	67%	20%
8915	Stone	PSME 0208	ORCSTN	8045	0.00062	0.27	0.05	1.29E-05	0.51	13%	4%	63%	20%
6593	Stone	PSME 0209	ORSISL	3285	0.00088	0.17	0.04	2.21E-05	0.54	12%	4%	62%	22%
2474	Stone	PSME 0210	ORCSTN	6016	0.00059	0.20	0.03	9.06E-06	0.64	13%	4%	68%	15%

1814	Stone	PSME 0211	ORSISL	3285	0.00071	0.19	0.02	2.90E-05	0.58	12%	3%	62%	23%
1825	Stone	PSME 0224	ORSISL	3285	0.00071	0.03	0.01	1.59E-05	0.64	9%	3%	71%	18%
2475	Stone	PSME 0225	ORCSTN	6016	0.00057	0.43	0.07	1.04E-05	0.55	11%	4%	67%	19%
1866	Stone	PSME 0226	ORSISL	3306	0.00065	0.12	0.02	2.22E-05	0.56	14%	4%	58%	24%
7190	Stone	PSME 0227	ORCSTN	6016	0.00085	0.52	0.08	1.83E-05	0.49	11%	3%	70%	16%
1473	Stone	PSME 0228	ORSISL	3094	0.00055	0.35	0.06	2.35E-05	0.56	14%	3%	65%	17%
4373	Stone	PSME 0229	ORCSTN	8524	0.00072	0.76	0.07	1.71E-05	0.47	13%	3%	66%	18%
6596	Stone	PSME 0230	ORSISL	3285	0.00063	0.35	0.09	1.56E-05	0.55	16%	3%	67%	13%
6655	Stone	PSME 0232	ORSISL	3306	0.00063	0.09	0.02	1.79E-05	0.49	15%	3%	66%	16%
4283	Stone	PSME 0233	ORCSTN	8045	0.00043	0.20	0.04	7.75E-06	0.64	7%	3%	70%	21%
6285	Stone	PSME 0234	ORSISL	3094	0.00060	0.55	0.06	3.02E-05	0.52	10%	3%	64%	23%
8964	Stone	PSME 0235	ORCSTN	8524	0.00080	0.07	0.03	3.97E-05	0.54	10%	3%	69%	19%

6303	Stone	PSME 0236	ORSISL	3094	0.00080	0.44	0.11	2.42E-05	0.51	13%	3%	67%	17%
4315	Stone	PSME 0237	ORCSTN	8045	0.00049	0.56	0.12	1.47E-05	0.55	16%	3%	62%	19%
1819	Stone	PSME 0238	ORSISL	3285	0.00059	0.18	0.05	2.09E-05	0.61	11%	3%	71%	15%
4374	Stone	PSME 0239	ORCSTN	8524	0.00079	0.27	0.05	2.35E-05	0.57	12%	3%	63%	22%
6606	Stone	PSME 0240	ORSISL	3285	0.00065	0.19	0.04	2.63E-05	0.56	15%	4%	64%	17%
2472	Stone	PSME 0241	ORCSTN	6016	0.00059	0.25	0.06	2.26E-05	0.62	11%	3%	65%	21%
1480	Stone	PSME 0242	ORSISL	3094	0.00066	0.02	0.02	2.29E-05	0.57	13%	1%	67%	19%
7214	Stone	PSME 0243	ORCSTN	6016	0.00082	0.03	0.02	3.65E-05	0.53	14%	3%	63%	20%
6673	Stone	PSME 0244	ORSISL	3306	0.00058	0.04	0.02	9.64E-06	0.67	17%	4%	59%	20%
8910	Stone	PSME 0245	ORCSTN	8045	0.00054	0.02	0.02	1.82E-05	0.62	15%	4%	60%	20%
1886	Stone	PSME 0246	ORSISL	3306	0.00055	0.04	0.03	1.03E-05	0.60	15%	4%	63%	19%
8980	Stone	PSME 0247	ORCSTN	8524	0.00066	0.17	0.05	2.93E-05	0.55	12%	4%	66%	18%

8919	Stone	PSME 0248	ORCSTN	8045	0.00041	0.05	0.02	1.05E-05	0.60	9%	3%	69%	18%
4301	Stone	PSME 0249	ORCSTN	8045	0.00061	0.03	0.02	2.33E-05	0.63	10%	4%	59%	28%

K_s=specific conductivity
A=earlywood inner growth ring
B=latewood inner growth ring
C=earlywood outer growth ring
D=rest of outer growth ring