

Conduit Diameter and Conduit Abundance Variation in Three Deciduous Tree Species in  
Northern Michigan in Relation to Species-Specific Sap Flux

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This study investigates the tradeoff between freeze-thaw cavitation resistance and rapid water transport known as the safety-efficiency tradeoff. Because large diameter vessels have higher risk of cavitation during freezing, I hypothesized that vessel size would be negatively related to vessel abundance, as trees that have small vessels will compensate with a greater abundance of vessels in order to meet its water transportation needs in areas affected by freeze-thaw cycles. I also hypothesized vessel abundance and sapwood: heartwood ratio would be negatively related, as trees with greater sapwood area will need fewer vessels to maintain their optimal water transportation rate. Because larger conduit diameters will increase the risk of embolism and death by cavitation when trees are exposed to freeze-thaw cycles, I hypothesized that high sap flux will correspond to abundant rather than large conduits. Regressions between conduit abundance and conduit diameter for each species revealed no relationship for *P. tremuloides* ( $p=0.3573$ ); however, there was a positive relationship for both *B. alleghaniensis* ( $p=0.0738$ ) and *A. saccharum* ( $p=0.02239$ ), supporting my hypothesis. ANCOVA analyses between Conduit Abundance: Conduit Diameter ratio and Sapwood: Heartwood ratio for each species indicated no significant relationship between the two factors, although *P. tremuloides* and *B. alleghaniensis* did exhibit significant intercepts. These results contrary to my hypothesis may be due to varying microclimates, water stress, or cavitation repair allowing these species to prioritize vessel diameter over vessel abundance in order to maintain high sap flux levels.

## **Introduction**

Trees are often considered major contributors to climatic conditions through their cycling of carbon through photosynthesis. However, their role in cycling water as a component of the soil-plant-atmosphere continuum is less studied. Water is taken up via the root system from the surrounding soil and travels throughout the tree through vessels and tracheids that form an expansive network in the transporting sapwood tissue from the roots to the leaves of the tree. The rate at which the water flows through the tree from root to leaf per area of sapwood is known as the sap flux. This rate can be affected by the stomatal conductance of the leaves, which the tree adjusts based on feedback response from several external factors, such as temperature and humidity.

Leaf stomata adjust by mechanically closing or opening to control how much water escapes in vapor form through the open stomata; this is called stomatal control and occurs throughout the everyday functioning of the tree. If too much water escapes through the open stomata, the tree is

at risk for cavitation. Cavitation occurs when pockets of vaporized water form spontaneously within tree vessels under excessively low pressure. These pockets of air block the flow of water through the vessel, causing cavitation, and preventing the transport of water any further. If the water pressure is extremely low, widespread cavitation can occur, causing the failure of multiple conduit vessels, and eventual tree mortality.

Alternatively, if the tree closes its stomata for too long of a period, the tree can starve from lack of carbon dioxide as cellular respiration consumes any stored sugars. Without a source of carbon dioxide to chemically transform into more carbohydrates for energy, the tree will inevitably die of carbon starvation. Thus, the tree must balance the trade-off between the risks of carbon starvation and those of cavitation. In order to avoid carbon starvation by keeping stomata open without succumbing completely to cavitation risks, there are some anatomical features that trees utilize to reduce their risk of cavitation, one of these being smaller sized conduits.

This brings about another tradeoff, as smaller conduits are not able to transport water as rapidly as larger conduits. This slowed water transport affects the transport rate of nutrients that the organism needs for daily chemical processes as well as growth and development, since these nutrients are dissolved and use the moving water as a vehicle of transportation through the sapwood tissue of the tree. This tradeoff between safety from cavitation and speed of water transport is known as the safety-efficiency tradeoff.

The safety-efficiency tradeoff is especially pertinent to trees facing droughts, which discourages stomatal opening so as to preserve any water that might escape in vapor form, lower the pressure, and ultimately cause lethal widespread cavitation. Research implies that these trees are prioritizing faster water transport by maintaining fewer but larger vessels, putting them at a higher risk for cavitation caused by freeze-thaw cycles (Zhang, et al., 2015; Petit et al., 2016).

Although other studies have suggested that in some species larger vessel diameters might not increase the cavitation risk at the tissue level for trees at risk for drought (Fichot et al. 2010), the general consensus is that 0.044 mm is the critical diameter above which complete cavitation occurs (Davis, 1999). When comparing the hydraulic efficiency of *Iriatea deltoidea* between different anatomical structures of the tree, Renninger et al found a negative relationship between conduit abundance and conduit diameters, supporting the existence of the safety-efficiency tradeoff (2013).

Another factor relevant to the safety-efficiency tradeoff is the existence of freeze-thaw cycles in some areas. Because water expands upon freezing, a tree's water transport vessels are at risk of cavitation when the expanded ice thaws. Thawing can result in residual air bubbles forming inside of the newly relaxed vessels, ultimately leading to cavitation. To reduce this risk, trees in areas that experience frequent freeze-thaw cycles could hypothetically protect themselves against cavitation by having small vessels less than 0.044 mm (Davis, 1999). These smaller vessels are associated with a lower cavitation pressure, increasing drought resistance and decreasing the temperature necessary for ice cavitation to occur (Lintunen, 2013).

This study pursued the relationship between sap flux and anatomical characteristics that trees in areas at risk for cavitation from freeze-thaw cycles might experience. I hypothesized that there would be a negative relationship between vessel size and vessel abundance, because trees that have small vessels might compensate with a greater abundance of vessels to meet their water transportation needs. I also hypothesized that there would be a negative relationship between vessel abundance and sapwood: heartwood ratio, as trees with more area of actively transporting sapwood tissue need less abundance of vessels to maintain their optimal water transportation rate. Because larger conduit diameters increase the risk of potentially lethal cavitation in these

trees when exposed to freeze-thaw cycles, I further hypothesized that high sap flux would correspond to abundant conduits rather than large conduits.

## **Methods**

### *Selection of Sites and Samples*

In order to pursue this hypothesis, I collected data from thirty codominant trees on the University of Notre Dame Environmental Research Center (UNDERC) property, which experiences frequent freeze-thaw cycles in its expanses of mixed forests. *Acer saccharum*, *Betula alleghaniensis*, and *Populus tremuloides* were selected for sampling based on patterns in sap flux data obtained through the thermal dissipation method and their abundance in the Upper Peninsula area (Bethany Blakely, personal communication). Three to five trees were sampled at each of nine different stand sites to account for local site differences (n=10 trees per species). These sites were selected based on the reference of a map representing the tree stand types on the UNDERC property. I selected visibly healthy trees that were 10-20 cm Diameter at Breast Height (DBH). From each of the selected trees, I recorded DBH and species, and then collected an increment core at chest height using an increment borer from the North-facing side of the tree. Because the *P. tremuloides* trees needed additional staining for conduit morphological measurements, I collected a second increment core from the South-facing side of all *P. tremuloides* trees.

### *Sapwood-Heartwood and Aging Measurements*

The North-side primary cores were dried in a convection oven for 43-46 hours at 45-50 °C, mounted, and stained with 0.05% Aqueous Methyl Orange from Carolina Biological Supply

Company to determine the location of the sapwood-heartwood boundary. I measured sapwood length and heartwood length on each of the dried cores from the center of the tree. I then sanded and counted the growth rings on each core in order to determine the age of each sampled tree.

#### *Conduit Abundance and Diameter Measurements*

The secondary South-side cores of the sampled *P. tremuloides* were stored in a refrigerator at 4°C to retain freshness. I measured the length of the sapwood of each of these cores and divided the sapwood length into equal thirds, with each division representing a third of the sapwood depth. I randomly selected a one-millimeter area along the length of each third of the core for data collection. To cross-section the vessels, I utilized a hand microtome on each third. This freshly exposed flat surface was stained using a black marker, and when this stain had dried, white chalk dust was pressed onto the surface. After the removal of excess chalk dust, I was able to identify the conduits which appeared white against the stained black of the rest of the core. For the *A. saccharum* and *B. alleghaniensis* trees, the conduits were easily visible in the dried cores and thus a second core was unnecessary. These cores were also divided into thirds and random one-millimeter sites in each third were selected for analysis. I photographed the cores being analyzed for conduit morphology utilizing a Leica EZ4 D Microscope Camera. I analyzed a one-millimeter squared area using Leica Application Suite EZ Microscope Camera Software to obtain counts and diameters of each of the visible conduits for each tree.

#### *Statistical Analysis*

The data was first tested for normality using a Shapiro Wilks test. Regressions with Conduit Diameter and Conduit Abundance were completed for each species sampled, and then ANCOVA analysis was performed with the dependent continuous variable of Conduit Abundance: Conduit

Diameter ratio and independent continuous variable of Sapwood: Heartwood ratio for each of the three species. All of these statistical analyses and tests were performed using the statistical program RStudio (v. 1.1.456) due to the familiarity of the researcher with the open-source program (R. Core Team, 2017).

## Results

A series of Shapiro Wilks tests revealed normal dispersion of data for Average Conduit Abundance: Average Conduit Diameter ( $p=0.1821$ ), for reciprocal-transformed Sapwood: Heartwood Ratio data ( $p=0.1382$ ), and for the Average Conduit Abundance and Average Conduit Diameter values for *P. tremuloides* ( $p=0.6022$ ,  $p=.05477$ ), for *B. alleghaniensis* ( $p=0.2116$ ,  $p=0.1265$ ), and for reciprocal-transformed *A. saccharum* ( $p=0.3027$ ,  $p=0.06356$ ).

The regressions of Average Conduit Abundance affected by Average Conduit Diameter for each species resulted in significant intercepts for each species, but a significant slope only for *A. saccharum* ( $p=0.02239$ ) and *B. alleghaniensis* ( $p=0.07380$ ; Figures 1-3). This indicates that while *A. saccharum* and *B. alleghaniensis* have a negative relationship between Average Conduit Abundance and Average Conduit Diameter, *P. tremuloides* shows no relationship, which appears counterintuitive, as *P. tremuloides* has the highest Average Conduit Abundance and smallest Average Conduit Diameter (Table 1).

ANCOVAs of Average Conduit Abundance: Average Conduit Diameter against Sapwood: Heartwood for each of the species' data revealed a significant intercept for *A. saccharum* ( $p=0.00661$ ) and for *P. tremuloides* ( $p=0.01560$ ), but not for *B. alleghaniensis*. No significant slopes were found for any of the three species (Figure 4). This indicates that although two of the

species, *A. saccharum* and *P. tremuloides*, have a significant difference in their values of Average Conduit Abundance: Average Conduit Diameter Ratio and Sapwood: Heartwood Ratio, all three species have no significant relationship between Average Conduit Abundance: Average Conduit Diameter Ratio and Sapwood: Heartwood Ratio ( $R^2 = 0.7689$ ,  $F = 15.97$ , with  $df = 5, 24$ ). Finally, the species-specific sap flux averages were obtained from Bethany Blakely at three Upper Peninsula sites and indicate that *P. tremuloides* has the highest sap flux at 35.20 g/m<sup>2</sup>s, followed by relatively lower sap fluxes for *A. saccharum* at 19.00 g/m<sup>2</sup>s and for *B. alleghaniensis* at 21.92 g/m<sup>2</sup>s (Figure 5).

## Discussion

The results of the ANCOVA analyses indicate that although there is a difference between species in their average conduit abundance and their average conduit diameter, there is no relationship between these conduit morphological characteristics for any species in relation to Sapwood: Heartwood area. This implies that different species might adapt different methodologies in conduit morphology to reduce their risk of cavitation in areas that experience freeze-thaw cycles. However, the results also imply that these species might not be utilizing increased sapwood area to increase their sap flow. This lack of relationship between conduit morphology and sapwood: heartwood area also exhibits that other factors must be responsible for differences in sap flux between the three species (Figure 5).

Studies on other species have shown variation not only in the sap flux values themselves, but also in how different species react to certain pressures. Shannon et al found that *Fraxinus nigra* responded to water stress by increasing sap flux, while no significant changes in sap flux were



found for *Acer rubrum* (2018). As both species were subjected to vapor pressure deficit, the sap flux response to water stress increased for each species at a different rate (Shannon et al, 2018). This indicates that species will react differently to abiotic stresses in terms of altering sap flux. Because differences in water use affect how trees respond to water stress, it is probable that sap flux differences are more related to these water usage differences rather than anatomical characteristics, such as conduit morphology.

The results of the regressions of conduit abundance and conduit diameter for each species suggest that there is some relationship between the two factors for *A. saccharum* and *B. alleghaniensis*, but not for *P. tremuloides*, implicating that conduit diameter has no effect on conduit abundance for *P. tremuloides*. For the other two species, there is a negative relationship between vessel diameter and vessel abundance, which indicates that as the vessel diameter increases, the vessel abundance decreases. This supports my hypothesis that as vessel diameter decreases to protect from freezing-induced cavitation vessel abundance increases to maintain the necessary sap flux.

Research has shown that the critical diameter above which complete conduit cavitation always occurs is 0.044 mm, which for this study is consistent for all three species (Davis, 1999; Table 1). The three studied species are within this critical diameter and will most likely experience partial cavitation rather than full cavitation in a freeze-thaw cycle. However, *B. alleghaniensis* is very close to this critical diameter, at a species average of 0.0437 mm (Table 1). As this species also has the highest sap flux, this may suggest that the species pushes the limits of the critical diameter in order to maintain this high sap flux. Thus in the context of the safety-efficiency tradeoff, *B. alleghaniensis* is prioritizing efficiency over safety without crossing the critical diameter.

Results imply that while sapwood area is not the major factor affecting the abundance of conduits in these species, vessel diameter is affecting abundance in *A. saccharum* and *B. alleghaniensis*. This is as I anticipated, since all these species are at risk for freeze-thaw induced cavitation, especially *B. alleghaniensis* which experiences high magnitude sap flux and would need the vessel area to maintain its high sap flux. By pushing the limits of the critical diameter, *B. alleghaniensis* can reduce its abundance of conduits. However, *P. tremuloides* does not show a relationship between the two factors, indicating that this species might prioritize efficiency over safety. This potentially may align with evidence for the healing of cavitation in the affected vessels in some species (Broderson et al, 2010). This feature may allow trees to prioritize high sap flux rather than safety from cavitation, as any vessel blockages that occur can be repaired. However, there is no current evidence that *P. tremuloides* has cavitation repair abilities. It is also possible that even though *P. tremuloides* exhibits the highest average conduit abundance (Table 1), not all of the measured conduits were conducting vessels. Research has shown that vessels that do not participate in water transportation are not always localized; rather, conducting and nonconducting vessels can intermingle in some temperate tree species, which would skew the data collected for this study for average conduit abundance and average conduit diameter (Jupa et al, 2016).

As the regression and ANCOVA results indicate, there are other factors that influence how abundant vessels are within the sapwood; some of these factors could be the surrounding microclimates and availability of necessary resources such as water for each individual tree. This study was limited in its resources and time that may have resulted in the untransformed data being abnormal at the species-level. This may be due to the more recent revelation that there is wide genetic variation in stress tolerance within some species, which may result in the abnormal

spread of data obtained in this study (Harfouche et al, 2014). A larger study could potentially account for this genetic variation through large sample size. Based on this genetic variation and the molecular complexity of stress tolerance responses, Harfouche et al recommend that a genomic approach must be taken to understand responses to abiotic factor stress, such as drought or carbon starvation (2014). This approach might provide beneficial in the pursuit of genomic engineering trees to be more drought tolerant to combat the loss of mature trees.

I propose that a more expansive study be completed in the future with more numerous replicates and species along with the measurement of potentially variable micro-climates and the use of genomic techniques to evaluate the relationship between species and their conduit morphology, such as diameter and abundance. My study provides a foundation for the potential factors that might be contributing to sap flux variations such as sapwood: heartwood ratio, conduit abundance, and conduit diameter, but age, microclimates, water stress, water use strategies, conducting vessel abundance, and natural genetic variation should be investigated as contributing factors as well. Sap flux, while simple in concept, is subject to more variables than its simplicity implies.

Understanding how trees utilize their water when subject to stress will become increasingly important as climate change continues. According to recent reports, extreme climatic events will continue to become more recurrent and droughts will increasingly affect tree water use (Brunner et al, 2015). The resulting water stress affects trees in their net primary production, seedling recruitment, vulnerability to pathogenic attacks, and susceptibility to fire devastation, leaving forests at higher risk for mass devastation (Zhao and Running, 2010; Reichstein et al., 2013). To maintain trees at the same level of contribution to the soil-plant-atmosphere continuum, it is imperative that research continues investigating how water-stress, micro-climates, increasing

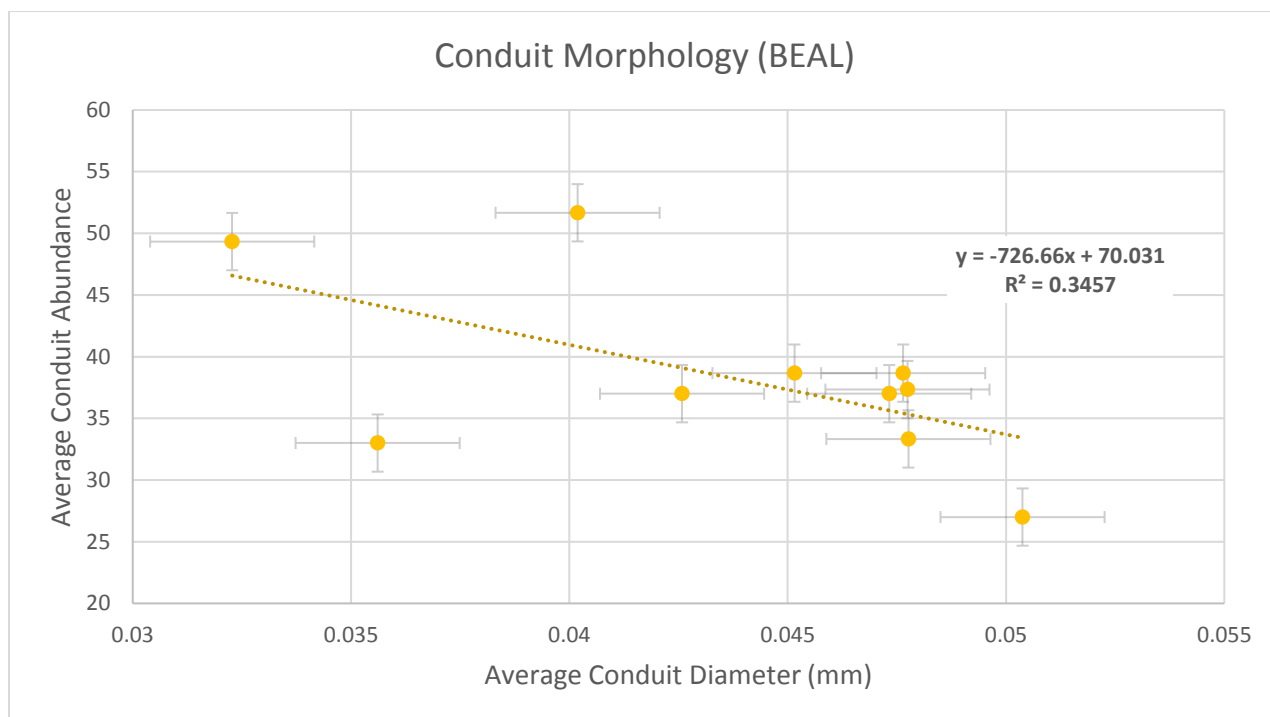
temperatures, species composition, and abiotic factors contribute to trees' stress responses to inform on how to protect forests from climate change.

## Tables

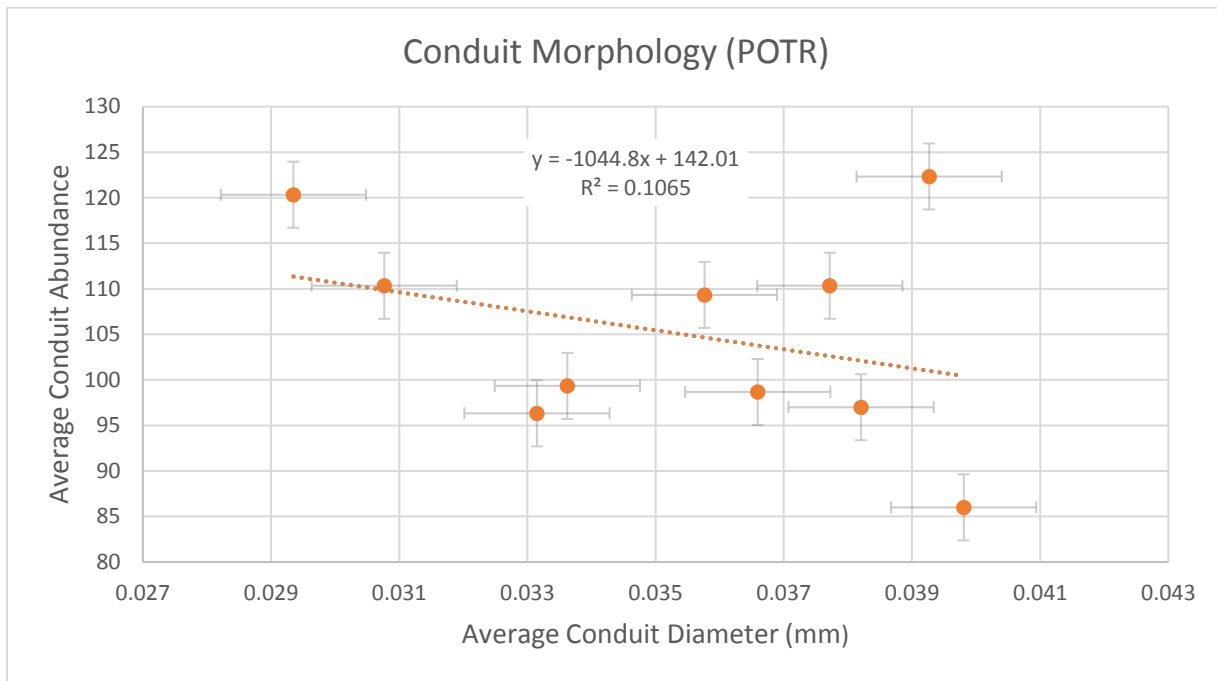
**Table 1:** The Average Sap Flux ( $\text{g/m}^2\text{s}$ ), Average Sapwood: Heartwood Ratio, Average Conduit Abundance, and Average Conduit Diameter (mm) for *A. saccharum* (ACSA), *B. alleghaniensis* (BEAL), and *P. tremuloides* (POTR)

<b>Species</b>	<b>Average Sap Flux</b>	<b>Average Sapwood: Heartwood Ratio</b>	<b>Average Conduit Abundance</b>	<b>Average Conduit Diameter</b>
<i>A. saccharum</i>	19.00	1.084	64.134	0.0401
<i>B. alleghaniensis</i>	35.20	9.556	38.300	0.0437
<i>P. tremuloides</i>	21.92	9.449	104.998	0.0354

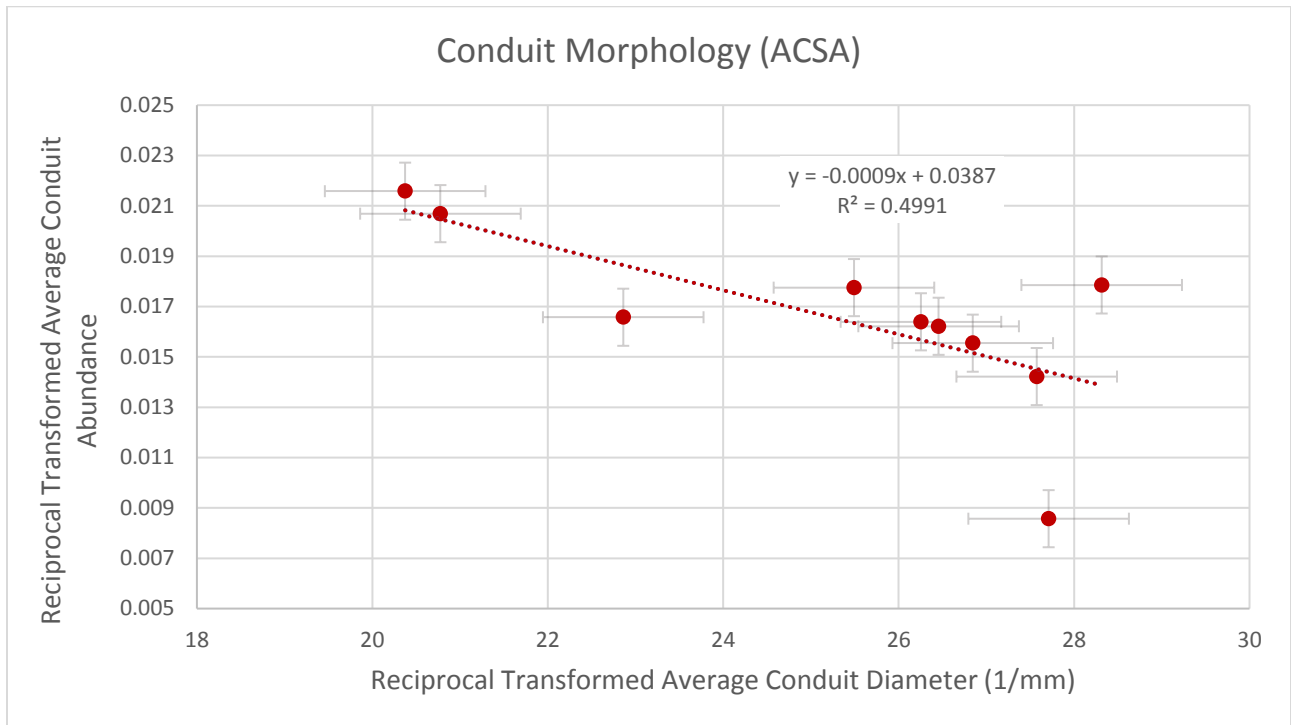
## Figures



**Figure 1: Average Conduit Abundance and Average Conduit Diameter (mm) for *B. alleghaniensis* (BEAL):** A negative relationship between Average Conduit Abundance and Average Conduit Diameter resulted in a significant intercept ( $P=0.0020$ ), a significant slope ( $P=0.0738$ ), and  $R^2 = 0.3457$  ( $df=1,8$ ).



**Figure 2: Average Conduit Abundance and Average Conduit Diameter (mm) for *P. tremuloides* (POTR):** No significant relationship was found between Average Conduit Abundance and Average Conduit Diameter and this resulted in a significant intercept ( $P=0.00578$ ), an insignificant slope ( $P=0.35733$ ), and  $R^2 = 0.1065$  ( $df=1,8$ ).

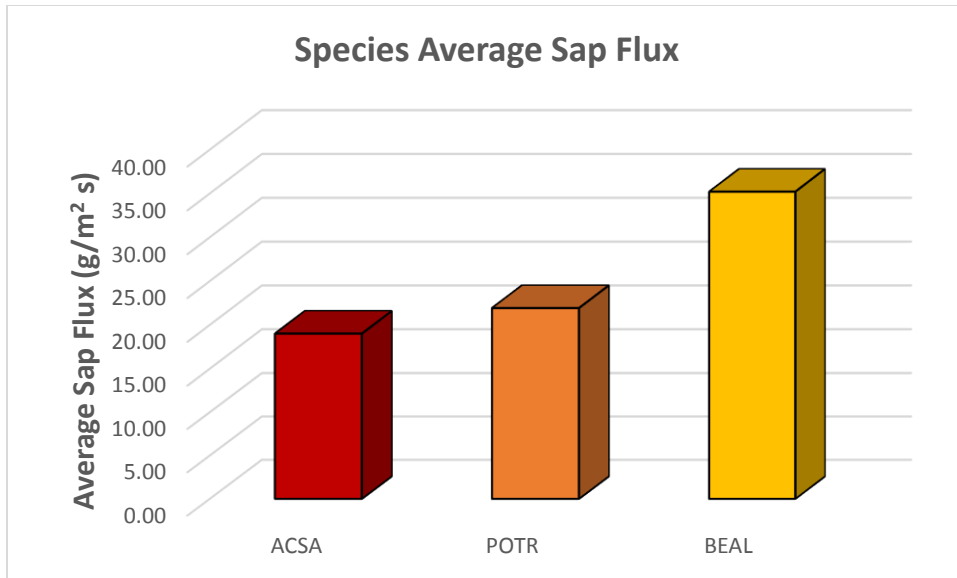


**Figure 3: Average Conduit Abundance and Average Conduit Diameter (mm) for *A. saccharum* (ACSA):** A negative relationship between the reciprocal-transformed Average Conduit Abundance and reciprocal transformed Average Conduit Diameter resulted in a significant intercept of 0.0387 ( $P=0.00119$ ), a significant slope of -0.0009 ( $P=0.02239$ ), and  $R^2 = 0.4991$  ( $df=1,8$ ).





**Figure 4: Average Conduit Abundance: Average Conduit Diameter Ratio and Reciprocal Transformed Sapwood: Heartwood Ratio for *A. saccharum* (ACSA), *B. alleghaniensis* (BEAL), and *P. tremuloides* (POTR):** No significant relationship between Average Conduit Abundance: Average Conduit Diameter Ratio and reciprocal-transformed Sapwood: Heartwood Ratio resulted in a significant intercept for ACSA ( $P=0.00661$ ) and for POTR ( $P=0.01560$ ), but an insignificant intercept for BEAL ( $P=0.40663$ ). This indicates that ACSA and POTR were significantly different from each other. No significant slopes resulted ( $P_{ACSA}= 0.77314$ ,  $P_{POTR}=0.01560$   $P_{BEAL}=0.40663$ ) and  $R^2 = 0.7689$  (df=5,24).



**Figure 5: Average Sap Flux for *A. saccharum* (ACSA), *P. tremuloides* (POTR), and *B. alleghaniensis* (BEAL):** This data was collected using the thermal dissipation probe method by Bethany Blakely at Willow Creek, UNDERC, and Sylvania sites respectively. The average sap flux for BEAL is highest of the three species at 35.20 g/m<sup>2</sup> s, while POTR and ACSA have similar lower sap fluxes at 21.92 g/m<sup>2</sup> s and 19.00 g/m<sup>2</sup> s respectively.

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