Group 2

Vein Scaling in *Populous tremuloides*

Introduction:

As the primary photosynthetic centers of a tree, leaves aim to maximize the production of nutrients while minimizing energy waste. This minimization requires an efficient transport system. The vascular systems of plants are vitally important in distributing water and nutrients, as well as providing structural support (Schuetz *et al.* 2007). Through natural selection, trees and other plants optimize this system using genetic heritability and environmental factors; however, in the pursuit of optimization, certain tradeoffs occur (Gutschick, 1999). Leaf structures are known to be extremely diverse in structure with variations noted in traits ranging from linear dimensions to cell size and stomatal density. Variation among these traits emerges, but overall these same traits are optimized similarly. (Gutschick, 1999). Such closeness supports the observed variability and demonstrates how highly divergent structures can result from similar selection pressures in different plant species (Gutschick, 1999). One example of such a tradeoff was discovered by Blonder, *et al.* (2013) in a study of leaf economics in *Populus tremuloides*. Veins are required to transport nutrients, but leaf tissue cells are needed to produce those nutrients. Taking into account Dr. Blonder and Gutschick's research, we can expect a similar tradeoff between vein diameter and leaf surface area.

Each species of tree and even populations within a species respond to optimization tradeoffs differently. Since each population is exposed to different environmental factors and is subject to diverse genetic qualities, extensive variation is observed in the phenotypes expressed. To begin to understand this variation, we studied the aspen tree population in Silver Lake, Utah. Aspen trees are distinctly unique from other tree species because they reproduce sexually and asexually to form clones. These clones, which are genetically identical, often grow in clusters and are useful when observing phenotypic variation. (Callahan, *et al.*, 2013). Due to the large amount of variation in visually observable traits between clones, we expect vein widths to vary as well.

This study specifically measured the vein width versus the surface area of leaves. To lessen the impacts of negative tradeoffs, natural selection seeks to optimize scaling relationships such as the ratio between vein width and surface area. Presumably, all trees of a certain species could optimize this trait in the same way; however, because of different environmental and genetic influences, we expected this optimum trait to vary in multiple populations. Through observing the variation of this trait between trees and between clones, we aimed to observe a significant pattern in this scaling relationship in the leaves. From this information, we can conclude that such a relationship exists and that it shows significance.

Methods:

Aspen clones were identified from the Silver Lake population in Cottonwood canyon. Photographs of the aspen stand were taken at intervals throughout the summer and fall of the 2012 year. Using these photographs, clonal boundaries were estimated and eight clones were identified. Each clone had slightly different physical characteristics that allowed us to estimate their boundaries. Actual clones were identified using PCR and microsatellite analysis.

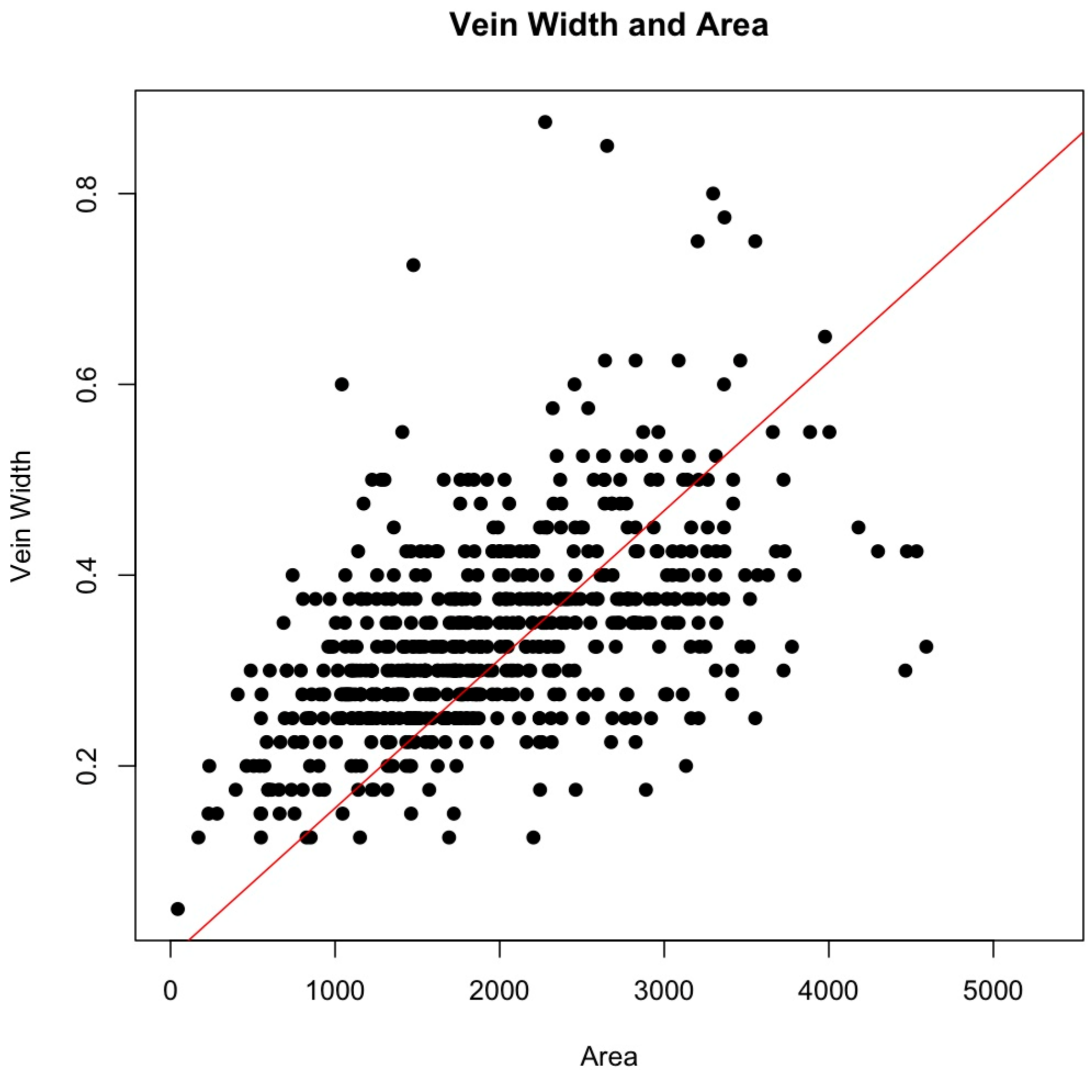
Nine to twelve trees were chosen and sampled from each clone. Branches from each of these trees were obtained and 25-40 leaves were taken from each branch using the systematic random sample procedure. These leaves were then organized and dried on leaf sheets.

To estimate the surface area of the leaves, we took width and height measurements for each of the sampled leaves. We then used these measurements to calculate the dimensions of the leaf as if it were an ellipse using the equation: $(a/2)(b/2)\pi$, where a and b are the width and height of the leaf respectively.

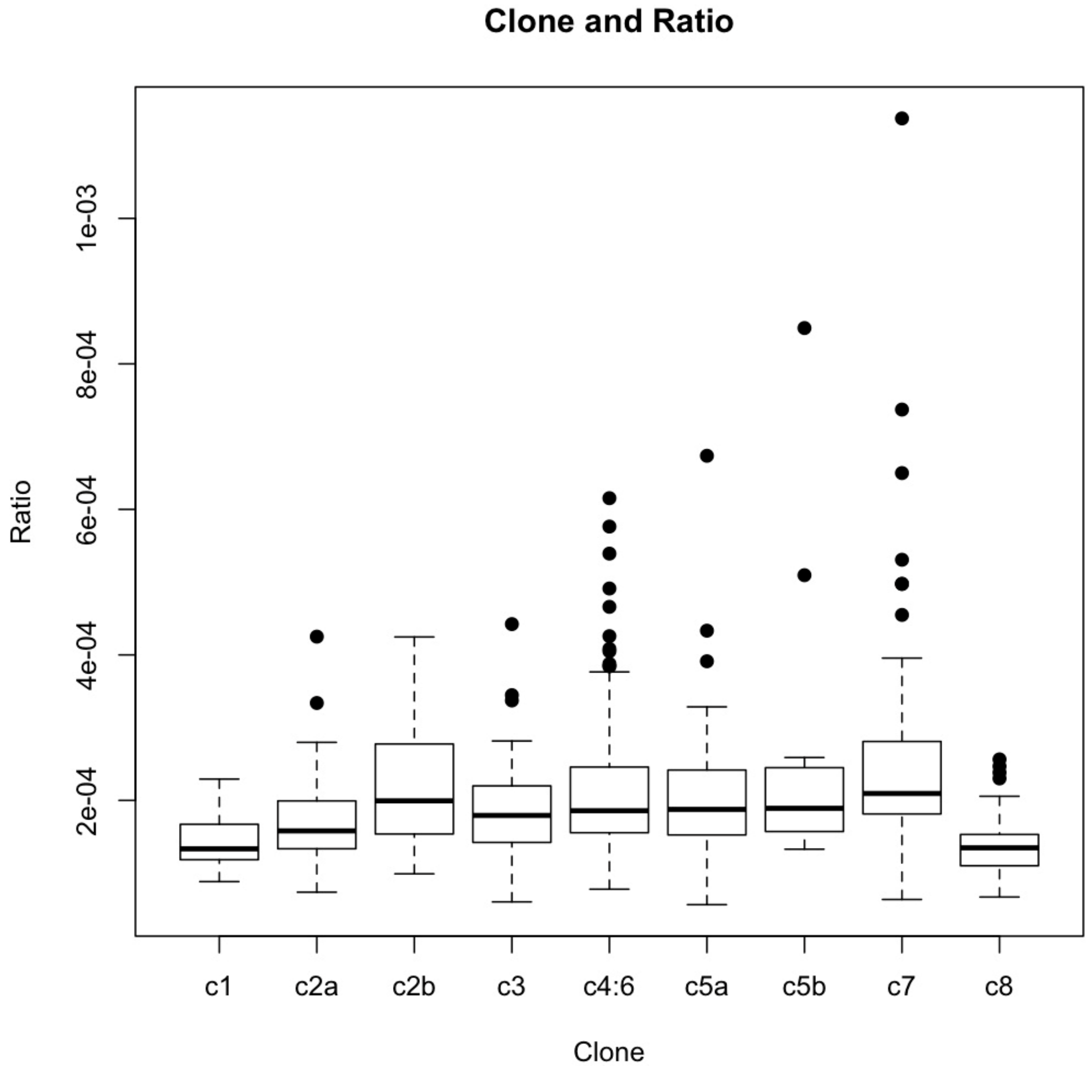
Vein widths were found by examining the leaves under a microscope. A mm standard was used and compared to the units on the microscope to measure the width of the main vein of each leaf in units, which were then converted to millimeters by dividing the value by four. Leaves were chosen by randomly selecting one leaf sheet from each tree sampled in the stand.

Once the vein widths and surface area of the leaves were determined, the ratio between these two values was calculated by dividing the vein width by the surface area of each leaf. This data was then used in a series of “analysis of variance” tests to determine the relationships between the ratio and both trees and clones (see results below). The ratio was also graphed against each clone to visually identify any relationship between the ratio and individual clones. Finally, the surface area and vein width were graphed against each other to visualize the linear correlation.

Results:



Vein Width and Area
Vein Width
0.2
0.4
0.6
0.8
0
1000
2000
3000
4000
5000
Area



Tree vs. Ratio

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
tree	69	1.845e-06	2.673e-08	3.815	<2e-16	***
Residuals	506	3.546e-06	7.007e-09			

Clone vs. Ratio

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
clone	8	9.320e-07	1.165e-07	14.81	<2e-16	***
Residuals	567	4.459e-06	7.860e-09			

Conclusion

We found that on average, leaf surface area has a positive linear correlation to vein width. Also from graph 2, we found that the mean ratio varied from clone to clone, and the analysis of variance allowed us to determine whether this variation was significant. By running this analysis, we can conclude that clonality and environment both have a significant effect on the ratio. As the leaf surface area grows, the vein width also increases to compensate for the increase in area. This demonstrates that optimization of this trait indeed occurs in this aspen population. Research informs us that both genetic and environmental factors have significant influence on the process of optimization, and our data confirms such conclusions. The ratio varies from clone to clone as well as from tree to tree. Therefore, determining clones cannot be done by simply observing this ratio. In our ANOVA, we were unable to compare which factor was more influential because both were extremely significant.

Potential environmental factors include water availability (both proximity to the water source and annual precipitation), soil quality, and terrain incline. Further study to quantify these factors could be useful in determining which of the environmental influences contribute to the leaf area/ vein width.

Works Cited:

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Callahan, Colin M., Carol A. Rowe, Ronald J. Ryel, John D. Shaw, Michael D. Madritch, and Karen E. Mock. "Continental-scale Assessment of Genetic Diversity and Population Structure in Quaking Aspen (Populus Tremuloides)." *Journal of Biogeography*(2013): n. pag. Print.

Gutschick, V. P. (1999). *Research reviews: Biotic and abiotic consequences of differences in leaf structure*. (New Mexico State University), Available from JSTOR.

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