Examining the Ratio of the Circumference to Average Leaf Size of Quaking Aspen (*Populus tremuloides*)

Species of aspen such as *Populus tremuloides* (Quaking Aspen) can form stands of genetically identical clones with unique characteristics that can be used to distinguish between clonal groups. Quaking Aspens have one of the largest distributions of any tree that is native to North America (Little, 1971). They can be found as far north as Alaska to as far south as Mexico and in varying ecosystems and elevations, which makes it an excellent tree to study (Little, 1971). Aspen can reproduce both sexually via seed and pollen and asexually via tree sprouts that form clone colonies (Barnes, 1966). The leaves on quaking aspen seedlings are a narrow oval shape, tapering to a point at each end. During the first growing season there is no flattening of the leaf stem or any lateral branching, the second growing season the leaves are more egg shaped and there is vertical branching (Howard, 1996). This means that over the course of the aspens life the shape of its leaves vary. The leaves of the tree are largely responsible for photosynthesis and other related processes, including transpiration. Both of those processes are dependent on the leaf’s size and surface area.

Colonies of aspen clones are formed when trees reproduce using tree sprouts or “suckers” (Barnes, 1966). The suckers should be genetically identical to the original tree (Salmon, 2006). Clones are often considered to be one large organism, for example, the Pando clone located in Fishlake National Forest, is considered the largest living organism ever discovered at an astonishing 6,600 tons. Large clones such as Pando are thought to exist only in an environment that has the right balance of disturbances and stability, meaning that asexual reproduction is predominant, but genetic variation still occurs due to accumulated mutations and sexual reproduction (Grant, 2010).
Each tree will have its own unique circumference due to different combinations of environmental and genetic factors along with age. Contained within the trunk of the tree is the tree’s vascular system, which consists of the xylem and phloem. The xylem and phloem are responsible for water and nutrient transport. On the perimeter of the trunk is the bark of the tree. The bark is the tough outer shell of the tree, which protects the delicate vascular system from the outside environment, but the bark of aspens is unique in that it also carries out photosynthesis (Pearson and Lawrence, 1958). This makes it easier to survive the winter months without leaves, but it also makes it more vulnerable to the environment. The ratio of leaf size to trunk circumference is a characteristic that could be used to distinguish between clones.

**Hypothesis**

We hypothesize that the ratio of the circumference of the tree to its average leaf size is a constant; a larger circumference tree will have larger leaves and a smaller circumference tree will have smaller leaves. It is expected that as the circumference of the tree increases the average leaf size of the tree will increase at the same rate, creating a positive linear correlation.

Additionally if the entire sample was plotted we would expect an overall positive linear trend with high variance due to the many different clones but different clones are expected to cluster together on the scatterplot allowing for the clones to be identified. Different clones are expected to maintain their correlations but possibly have different slopes. Lastly we expect the variance of the ratio of circumference to leaf size within independent aspen clones to be less than the variance of this ratio when applied to all aspen trees sampled.

**Methods**

The aspen trees that were studied are located within a stand at Silver Lake in Brighton, Utah (Upper Big Cottonwood Canyon). Before sampling the trees, boundaries of an area that appeared to contain clones were drawn on a map from Google maps. Next ten different trees were chosen within the predetermined boundaries. Each tree was marked with twine and a marker with group number and tree number. Pictures and a description of each tree were taken. The circumferences of each of the aspen trees were measured at chest height using a tape measure. Next, approximately 20-30 leaves were collected from each tree. Leaves from each tree were collected in a variety of ways, including a large slingshot and tree clippers. The height of each tree was found with the iPhone app iHypsometer. The rough bark was measured starting from the ground to the end of the rough bark, using a tape measure. Each tree was labeled with
permanent aluminum tags. The leaves were then labeled and fastened to printer paper and scanned, making it easier to measure their dimensions. Since each leaf varies in shape as explained in the introduction, the “area” of each leaf was determined by multiplying the length and width of each individual leaf. A program in R was used to find the length times width values for each leaf. Once the areas of each leaf were determined, the average leaf size was calculated using the mean function in R. Lastly, the ratio of the circumference of a tree to its average leaf size was found also using R. The process was repeated for each tree. By identifying different microsatellites, the trees were placed into clonal groups. A graph of average leaf size vs. circumference was created using R to determine if the ratio is some constant. A graph of all of the trees was also created to determine if there was a similar ratio between all of the trees. Using the analysis of variance function in R, the significance of clones as a factor of the ratio was calculated to determine the p value.

Data
Outcomes and Conclusion

We determined the ratio of the circumference of the quaking aspen trees to their average leaf size. The graphs of circumference to leaf area of the individual clones show little consistency. Clones 2, 4, 5, and 8 had negative correlations and clone 3 showed almost no correlation at all. Looking at the graph of circumference to leaf area of all the trees sampled there are visible clusters of individual clones. Also shown on the overall graph was a positive correlation with high variance. Within the individual clones there is no consistent correlation between tree circumference and leaf size but there is a positive correlation when looking at all of the clones together although there is high variance.

Based on the data from the graphs we were wrong in hypothesizing that the ratio of tree circumference to average leaf size would be a constant, positive, linear correlation. Trend lines across the graphs of the clones are not consistent. Many clones have a negative trend line, while others have positive trend lines and a few have trend lines with a slope close to zero. The p-value that was calculated with r was .0172, which is less than .05 meaning the ratio could be used to differentiate between clones. While the p-value is less than .05, the ratio should be used with caution when trying to determine clones since the p-value is only .04 less than .05.

Three of the eight clones tested were triploid, with the other five being diploid. The leaf sizes of the triploid trees were, on average, larger than the leaf size of the diploid trees. This may be attributed to the fact that triploid cells are generally larger than diploid cells. Another p-value that was calculated in r was 7.04e-12 showing that the ploidy affects the average leaf size.

The data from the last graph with all the clones seems to show that we were correct in several parts of our hypothesis. For one, the graphs showed that different clones have different slopes. Second, plotting the entire sample set produced an overall positive trend with high variance, while plotting each clone individually resulted in a tighter grouping with lower variance than all clones together. Although this is in accordance with our prediction that the variance will be smaller within clones, the groups of clones overlap and the variances do not seem to be consistent enough to be able to predict clonal relations from the leaf to circumference ratio alone.

Something we did not consider is the number of leaves on each tree. It is possible that the larger trees would have a larger number of individual leaves on them in order to support itself, rather than a larger mean leaf size. As we did not have a leaf count for each tree, this was a limitation set by the data before we began analysis of the clones. Finding a way to determine leaf number could be the next step in establishing the relationship between leaves and tree size in Quaking Aspen.
References


