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Trunk Curvature in *Populus Tremuloides*

Introduction

Life is stressful for all organisms, trees included. Certain environmental factors such as soil-creep, snow, catastrophic events, and phototropism can cause stress on trees and make them grow curved (Harker 1996). A tree that encounters this bad luck may have to compensate for its deformity for the rest of its life, and it may have adverse affects on the tree, specifically its fitness.

One way to measure the fitness of a tree is to look at its leaves. There is evidence to suggest that larger leaf area is associated with higher nitrogen levels, (Meziane 2001), other nutrient levels (Nicotra *et al.* 2011), and high photosynthetic rates (Meziane 2001), all of which are good things for a tree. Thus, a more fit tree would have a larger leaf area than a less fit tree.

Another way to measure tree fitness is to look at the shape of the leaves. Leaves that are stunted have not grown normally, resulting in shorter lengths or widths than usual. Low nitrogen levels are associated with leaf stunting (Benckiser 2014). A tree that has stunted leaves will have an abnormal length/width ratio, indicating that it is unfit. Nitrogen levels may also have an effect on the symmetry of leaves, making leaves with nitrogen deficiency asymmetrical.

In order to see if curvy trunks indeed have an adverse affect on tree fitness, we have chosen to study aspen trees (*Populus Tremuloides*). P. tremuloides is a clonal species, reproducing asexually for most of its existence, while only reproducing sexually by flowering after severe fires or other similar events (Mock *et al.* 2008). Since these trees are clonal, they are identical to each other in their genetic code, therefore, varying phenotypic traits can likely be attributed to environmental effects. P. tremuloides can grow in diverse environments, from Canada to Mexico, which makes it an ideal species to study the relationships between the environment and phenotypic traits (Meier *et al.* 2015).

Challenge

We have focused our efforts on three questions to look for evidence that curvature is harmful: (1) Does trunk curvature have an effect on leaf area? If so, how and to what extent? (2) Does trunk curvature have an effect on leaf length/width ratio? If so, how and to what extent? (3) Does trunk curvature have an effect on leaf symmetry? If so, how and to what extent?

We hypothesize that trees that have curvature will have a smaller average leaf area than trees with straight trunks. We also hypothesize that trees that have curvature will have a smaller average length/width ratio than straight trunked trees, which would have a ratio closer to one. Finally, we hypothesize that curved trees will have more asymmetrical leaves than those on straight trees.

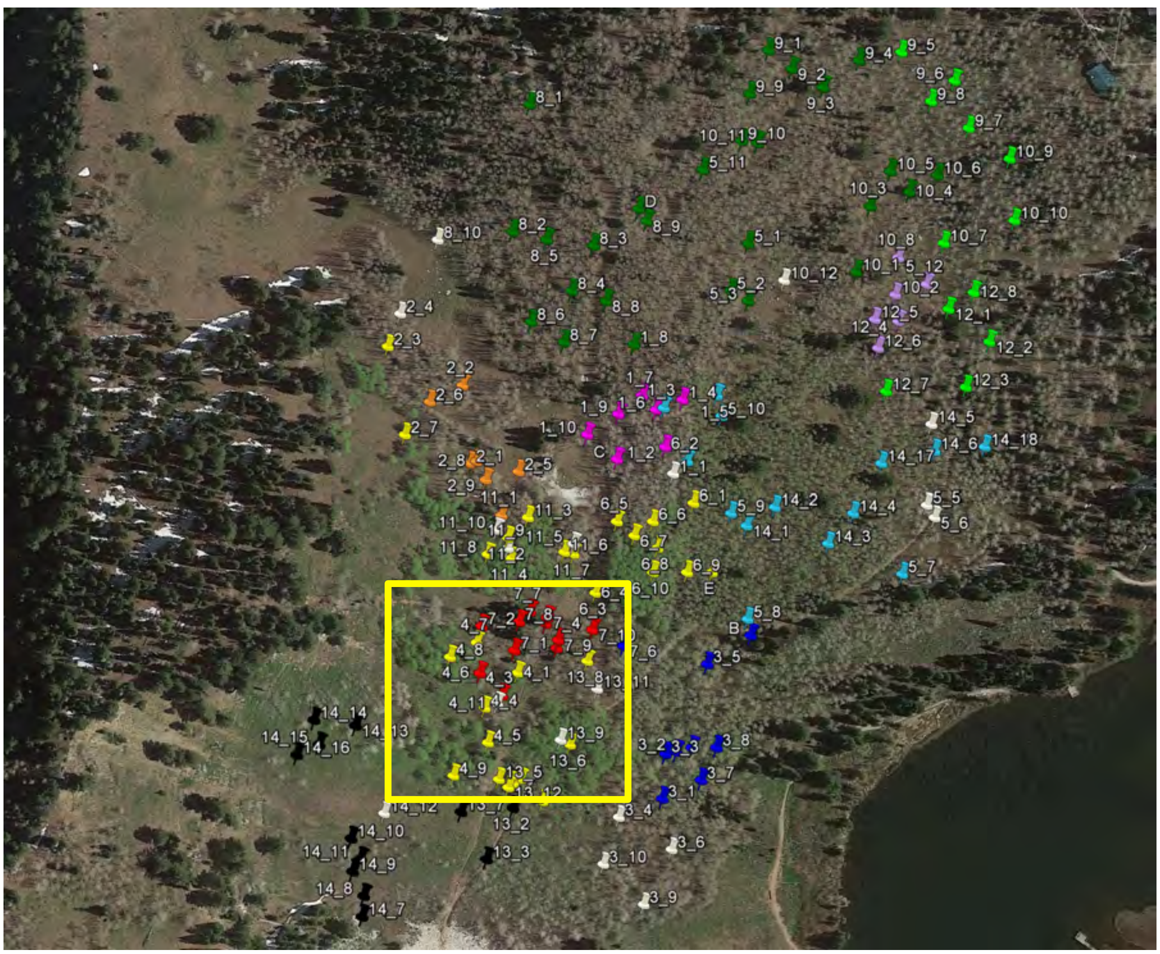
Methods

In our study, a site in northern Utah in Big Cottonwood Canyon was examined. This location contains a large number of accessible aspen as well as a number of different clones, guaranteeing diversity and a large sample size. The site is located on an incline that increases to the west.

To collect data, we chose 11 trees, 6 curved and 5 straight, within Clone c4-6. We sampled approximately 20-40 leaves from each selected tree. In addition to this, we labeled the trees, measured their circumference at an average breast height, and took pictures of the trunks for future reference.

The leaves collected were then be brought back to the lab and placed with labels on leaf sheets. Most leaves were placed on leaf sheets, but a few were saved for genetic testing to determine which clone they belong to. By separating the DNA from the leaf material and running a gel electrophoresis, we determined which of the known clones the tree belonged in.

Leaf sheets were scanned into a computer database, and from here we opened the images using a measurement program called Image J. Every leaf was given both a length and width measurement and a number label for each measurement. Using threshold techniques, we measured the area of each leaf. Running an analysis on a computer program, we rated the symmetry of each leaf on the eleven trees. Using the statistical analysis program R, we compared the area, leaf measurement ratio, and asymmetry value of curved trees to straight trees.



Our trees are all within the yellow box.

Results

Our data is summarized in the following table:

Tree	Curved?	Average Leaf Area	Average L/W Ratio	Average Leaf Asymmetry
13_1	No	2997 mm^2	.8464	0.0230
13_6	Yes	2314 mm^2	.9188	0.0272
13_8	No	2187 mm^2	.9043	0.0210
13_10	Yes	2497 mm^2	.8852	0.0223
13_12	No	2361 mm^2	.8422	0.0242
4_4	Yes	2872 mm^2	.8550	0.0203
4_7	No	1415 mm^2	.8376	0.0248
4_9	Yes	3206 mm^2	.8725	0.0202
4_10	No	2775 mm^2	.8627	0.0221
4_11	No	1567 mm^2	.8670	0.0220
4_12	Yes	1169 mm^2	.8854	0.0237

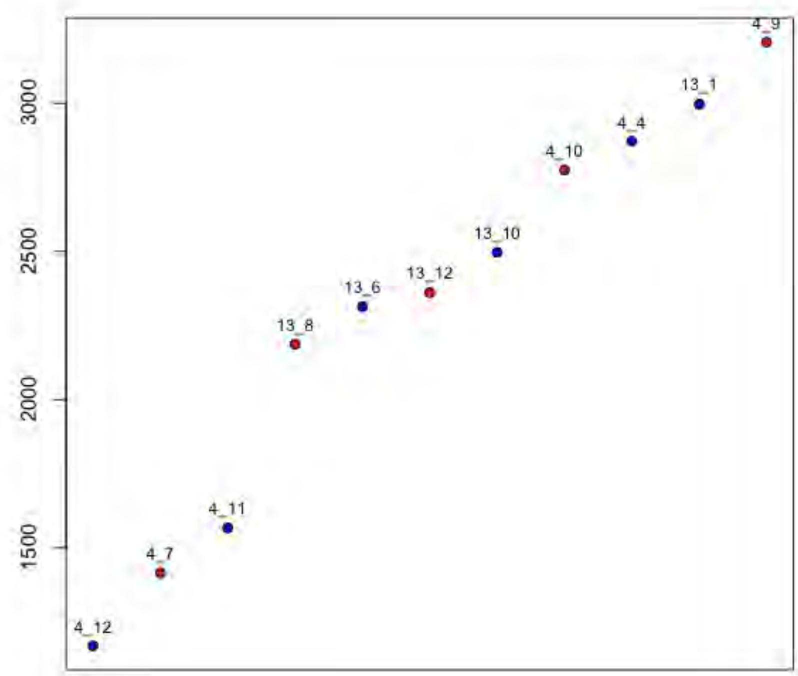
	Average Leaf Area	Average L/W Ratio	Average Leaf Asymmetry
Curved	2412 mm^2	0.8834	0.0227
Not Curved	2217 mm^2	0.8600	0.0229

Leaf Area

First, we compared the areas of leaves on a tree level, comparing the average leaf area of each tree to each other to see if curvature affected the area. We obtained the following output:

```
area <- lm(Area ~ Curve, data = averages)
anova(area)
        Analysis of Variance Table
        Response: Area
        Df  Sum Sq Mean Sq F value Pr(>F)
        Curve    1  103280  103280  0.2107 0.6571
        Residuals  9 4411017  490113
```

Average Tree Area (in mm2)



From this, we can conclude that, at a P value of .6571, the difference between the average leaf area of straight and curved trees is not significant, meaning that leaf area does not depend on trunk curvature in our sample of 11 trees. We decided to take each individual leaf into account to see if this makes a difference and obtained the following output:

```
area2 <- lm(area ~ as.factor(curve)+tree, data=leaves)
anova(area2)
        Analysis of Variance Table
        Response: area
        Df        Sum Sq  Mean Sq F value Pr(>F)
        as.factor(curve)   3   2554083   851361  1.9269 0.1248
        tree               7 132980239 18997177 42.9968 <2e-16 ***
        Residuals        360 159057908   441828
```

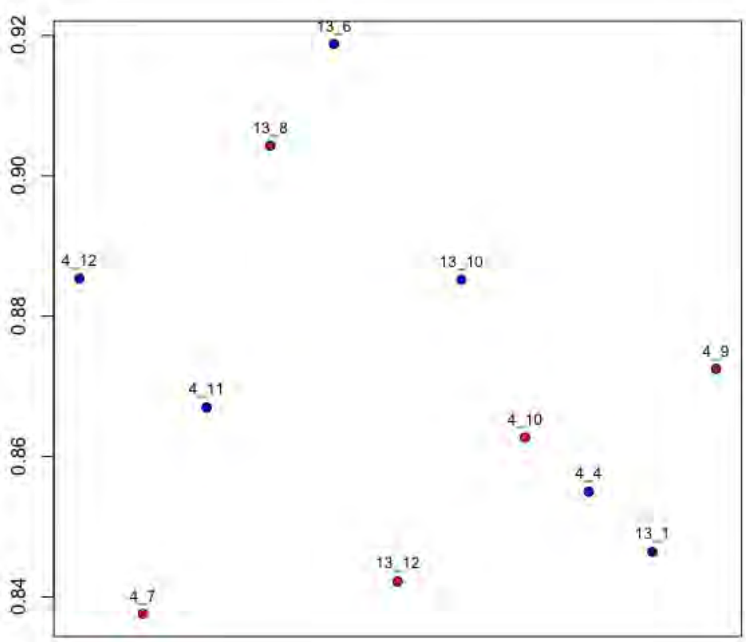
This too tells us that trunk curvature does not have an effect on leaf area.

Leaf Ratio

Next, we compared the L/W ratio on a tree level, taking the average ratio for each of the eleven trees. We obtained the following output.

```
ratio <- lm(Ratio ~ Curve, data=averages)
anova(ratio)
        Analysis of Variance Table
        Response: Ratio
        Df        Sum Sq Mean Sq F value Pr(>F)
        Curve     1 0.0014865 0.00148655  2.5689 0.1434
        Residuals  9 0.0052081 0.00057868
```

Average L/W Ratio



At a P value of .1434, we see that there is not sufficient evidence to conclude that trunk curvature affects the ratio, however it is close to being significant. Because of this, we decided to take individual leaves into account and see if this changed anything. The following output was obtained:

```
ratio2 <- lm(ratio ~ as.factor(curve)+tree, data=leaves)
anova(ratio2)
        Analysis of Variance Table
        Response: ratio
```

```
Df  Sum Sq  Mean Sq F value   Pr(>F)
as.factor(curve)   3 0.12558 0.041861  7.3186 8.94e-05 ***
tree               7 0.11726 0.016751  2.9286  0.00541 **
Residuals        360 2.05913 0.005720
```

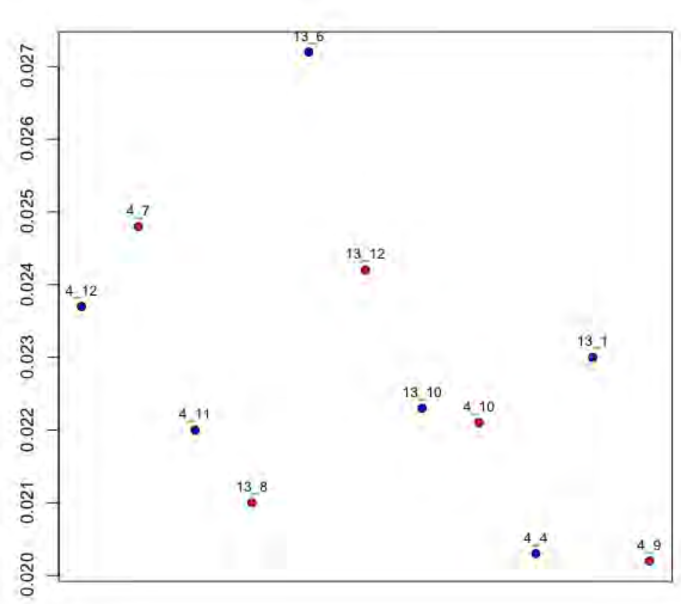
Now, the P value is significant, telling us that curvature does affect the ratio when taking into account all the leaves and not just the average. This is because by taking into account the individual leaves, the degree of freedom is much higher, making the data able to become significant.

Leaf Symmetry

Finally, we compared the symmetry values for straight and curved trunks from our eleven trees. We obtained the following output:

```
symm <- lm(Symmetry ~ Curve, data = averages)
anova(symm)
    Analysis of Variance Table
    Response: Symmetry
    Df         Sum Sq   Mean Sq F value Pr(>F)
    Curve    1 3.3000e-08 3.300e-08  0.0068 0.9362
    Residuals  9 4.3767e-05 4.863e-06
```

Average Symmetry Value



At a P value of .9362, we know that trunk curvature does not have an effect on leaf symmetry for our eleven trees. Since the symmetry score was evaluated on a per tree basis, and not done per leaf, there was no way to take individual leaves into account as we did in the previous two sections.

Conclusion

Through the analysis of the area, leaf ratio, and symmetry value of our eleven trees, we can conclude that all three of our hypotheses were incorrect. We see that trunk curvature does not have an effect on the area or symmetry of leaves for the eleven trees we sampled. Curvature did have an effect on the leaf ratio, but it turned out that curvy trees had a higher ratio - meaning they had longer leaves - than straight trees. We thought the complete opposite. From here, it would be interesting to test nitrogen levels in the leaves to see if it is the nitrogen levels, as Meziane suggests, that affects the shape of the leaves.

One of the theories that could explain the higher leaf ratio could be water stress to the curved trees. Since all of the trees are in a compact location, the curved trees may be in a wetter average location, such as a place with heavy snowpack and late melting, and that could be why they are curved. This is something that could be studied in later research.

One of the major limitations of our study is that we had a very small sample size. Increasing our sample, and therefore increasing the degree of freedom, may show something different, as it is hard to show significance with a small sample size. However, the P Value for the ratio is so small that we are very convinced that curvature does affect it.

These findings shed light not only on aspens, but also on the many other species that obtain curvature deformities early in life. Our findings suggest that curvature does not have an effect on aspects of a trees fitness, and in some cases may even be better for the tree.

Literature Cited

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