Investigation of Rough Periderm
Group 1

Introduction:

Kaufert (1937) stated that rough bark comes from external forces such as lichen, fungi, and mechanical injury afflicting the smooth and thin periderm of quaking aspen (*Populus tremuloides*). This showed that environmental factors influence rough bark formation. However, we observed that the rough bark height of aspens appeared consistent within individual clones, suggesting that genetic factors could also be highly correlated with rough bark formation. This led us to speculate whether trees within a clone actually have similar rough bark height relative to their size. Furthermore, because triploidy and rough bark are considered abnormal traits, we believed a correlation may exist. By analyzing two ratios (rough bark to tree height, and rough bark to circumference) among the different clones at Silver Lake, we were able to determine whether rough bark is actually a clonal trait, and if it is associated with triploidy.

Methods:

Sampling was completed in Brighton, Utah at Silver Lake during early and mid September - 9 and 16. It is alpine climate with moderate temperatures and generally consistent moisture year round including annual snow. Using a satellite image to identify phenotypic similarities, such as leaf color, tree density, etc., we chose what we believed to be clone sites within an aspen population.

We quantified the amount of leaf damage per tree by giving each sampled leaf a rating from 0 to 3, summing the damage of the leaves, dividing my leaf count, and then assigning that value as the tree's damage rating. Regarding the tree trunk, we took the circumference, height, and rough bark height measurements with using a tape measure, sextant, and trigonometric analysis.

We examined rough bark height to total tree height, rough bark height to tree circumference , rough bark height to the tree damage ratings, tree damage, and rough bark height. These factors were processed with respect to clone and ploidy in R software using the Analysis of Variance function. Ratios were established between the desired values within the program. Those ratios were run in ANOVA. The ANOVA determined the significance of the clone and ploidy on the ratios, exponentially factoring the influence of those categories on those ratios to show.

Research Questions (Clone and Ploidy):

I. Is there a correlation between the height and periderm height of the tree?
II. Is there a correlation between the circumference and rough periderm height of the tree?
III. Is there a correlation between rough bark height and damage of the leaf?
IV. Is there a correlation between undesirable phenotypes (i.e. rough bark and leaf damage) and membership in a clone and/or ploidy?

Data and Observations:

```
    # Total Tree Height vs. Rough Bark Height ~ Clone
b = lm(ratio1~clone, data=leaves)
anova(b)
# Analysis of Variance Table
# Response: ratio1
          # Df  Sum Sq Mean Sq F value Pr(>F)
# clone      7  1456.6  208.09   0.325 0.9343
# Residuals 22 14085.7  640.26
```

Fig. 1 No correlation exists between rough bark height and total tree height among clones.

```
    # Total Tree Height vs. Rough Bark Height ~ Ploidy
f = lm(ratio1~ploidy, data=leaves)
anova(f)
# Analysis of Variance Table
# Response: ratio1
          # Df  Sum Sq Mean Sq F value Pr(>F)
# ploidy     1    90.1   90.08  0.1632 0.6893
# Residuals 28 15452.3  551.87
```

Fig. 2 No correlation exists between rough bark height and total tree height among triploid clones.

```
    # Circumference vs. Rough Bark Height ~ Clone
c = lm(ratio2~clone, data=leaves)
anova(c)
# Analysis of Variance Table
# Response: ratio2
          # Df Sum Sq Mean Sq F value Pr(>F)
# clone      9 13.598  1.5109  1.1451  0.359
# Residuals 35 46.180  1.3194
```

Fig. 3 No correlation exists between rough bark height and tree circumference among clones.

```
    # Circumference vs. Rough Bark Height ~ Ploidy
g = lm(ratio2~ploidy, data=leaves)
anova(g)
# Analysis of Variance Table
# Response: ratio2
          # Df Sum Sq Mean Sq F value Pr(>F)
# ploidy     1  0.129 0.12894   0.093 0.7619
# Residuals 43 59.649 1.38718
```

Fig. 4 No correlation exists between rough bark height and tree circumference among triploid clones.

```
    # Leaf Damage vs. Rough Bark Height ~ Clone
d = lm(ratio3~clone, data=leaves)
anova(d)
# Analysis of Variance Table
# Response: ratio3
          # Df    Sum Sq    Mean Sq F value Pr(>F)
# clone      9 0.0018662 0.00020735  1.2815 0.2824
# Residuals 34 0.0055016 0.00016181
```

Fig. 5 No correlation exists between leaf damage and rough bark height among clones.

```
    # Leaf Damage vs. Rough Bark Height ~ Ploidy
h = lm(ratio3~ploidy, data=leaves)
anova(h)
# Analysis of Variance Table
# Response: ratio3
          # Df    Sum Sq    Mean Sq F value Pr(>F)
# ploidy     1 0.0001646 0.00016459  0.9597 0.3329
# Residuals 42 0.0072032 0.00017150
```

Fig. 6 No correlation exists between leaf damage and rough bark height among triploid clones.

```
##### Correlation between damage and rough bark height.
aaa = lm(damage~trbh, data=leaves)
anova(aaa)
# Analysis of Variance Table
# Response: damage
          # Df Sum Sq  Mean Sq F value Pr(>F)
# trbh       1 0.0115 0.011543  0.1487 0.7017
# Residuals 42 3.2606 0.077632
```

Fig. 7 No correlation exists between rough bark height and leaf damage.

```
    # TRBH ~ Clone
fff = lm(trbh~clone, data=leaves)
anova(fff)
# Analysis of Variance Table

# Response: trbh
          # Df Sum Sq Mean Sq F value Pr(>F)
# clone      9   9294  1032.6  0.8808 0.5513
# Residuals 35  41033  1172.4
```

Fig. 8 No correlation exists between rough bark height and clone.

```
    # TRBH ~ Ploidy
ggg = lm(trbh~ploidy, data=leaves)
anova(ggg)
# Analysis of Variance Table

# Response: trbh
          # Df Sum Sq Mean Sq F value Pr(>F)
# ploidy     1    539  538.63  0.4652 0.4989
# Residuals 43  49788 1157.86
```

Fig. 9 No correlation exists between rough bark height and triploid clone

```
    # Damage ~ Clone
a = lm(damage~clone, data=leaves)
anova(a)
# Analysis of Variance Table
# Response: damage
          # Df Sum Sq  Mean Sq F value   Pr(>F)
# clone     10 2.0851 0.208513  3.1253 0.002591 **
# Residuals 65 4.3367 0.066719
```

Fig. 10 A correlation exists between average leaf damage and clone.

```
    # Damage ~ Ploidy
z = lm(damage~ploidy, data=leaves)
anova(z)
# Analysis of Variance Table

# Response: damage
          # Df Sum Sq  Mean Sq F value Pr(>F)
# ploidy     1 0.0184 0.018403  0.2127  0.646
# Residuals 74 6.4035 0.086533
```

Fig. 11 No correlation exists between damage and having a certain ploidy.

Discussion:

The data yielded no correlation between rough bark height and total tree height, rough bark height and circumference, and rough bark height and leaf damage when categorized based on clone or ploidy membership. Based on their P-values, these analyses demonstrate that rough bark height is not a clonal trait. Since trees belonging to the same clone share the same genotype, rough bark height not being a clonal trait suggests that rough bark height is not mainly controlled by genes. This prompts us to question whether there is any genotypic influence, and how much it can account for the rough bark.

Furthermore, the analyses of rough bark height and leaf damage in regards to ploidy was also not statistically significant, showing that our hypothesis of triploidy being linked to the observed undesirable phenotypes is false. Thus, this study showed that triploidy does not heighten the likelihood of aspens having rough bark and damaged leaves.

However, our analysis did suggest that leaf damage could be a clonal trait, as the p-value between leaf damage and clones was significant, suggesting that it is gene controlled. Since this yielded significance, whereas leaf damage vs. rough bark height and rough bark height vs. clones did not, it indicates that genes controlling the resistance of leaves to damage do not also control bark resistance.

One potential technical explanation for our results is that the collected data used in the R program was incomplete. Damage values were inconsistent; leaf sampling was random and potentially biased towards healthy leaves. Many trees lacked rough bark height, total tree height, and/or tree circumference values. As a result, those trees were omitted during the analysis, leading to incomplete ratios, etc. Omission of so many cases from such a relatively small sample could have significantly influenced ANOVA values.

Conclusion:

Despite the data disparity, ANOVA values were still clear enough to analyze, and helped clarify exactly which factors influenced rough bark development and leaf damage. The results also evoked further questions. The p-values suggested that genetic influences were not significant, making us question whether only environment influences rough bark development, or if genetic influences are still present, just smaller than environmental ones. Furthermore, we question whether leaf damage is controlled by R-genes, or by other genes. This additional information, on top of those in this study, could help control and improve the defense mechanisms of aspen trees in the future.

References:

Callahan, C M, Rowe, C A, Ryel, R J, et al. (2013). Continental-scale assessment of genetic diversity and population structure in quaking aspen (populus tremuloides). Journal of biogeography, 40(9), 1780-1791.

Jones, J.R., DeByle, N.V., & Bowers, D.M. (1985). Insects and Other Invertebrates. *Aspen: Ecology and Management in the Western United States. General Technical Report RM-119. USDA Forest Service, Rocky Mountain Forest and Range Experiment Station, Fort Collins*, 107-114.

Kaufert, F. (1937). Factors influencing the formation of periderm in aspen. *American Journal of Botany*, *24*(1), 24-30.

Keigley, R. B., & Frisina, M. R. (2008). Aspen height, stem-girth and survivorship in an area of high ungulate use. Northwest Science, 82(3), 199-210.

Zhang, Q., Zhang, Z. Y., Lin, S. Z., Zheng, H. Q., Lin, Y. Z., An, X. M., ... & Li, H. X. (2008). Characterization of resistance gene analogs with a nucleotide binding site isolated from a triploid white poplar. *Plant Biology*, *10*(3), 310-322.

DeByle, N.V., & Winokur, R.P. (1985). Aspen: Ecology and Management in the Western United States.

Mock KE, Callahan CM, Islam-Faridi MN, Shaw JD, Rai HS, et al. (2012) Widespread Triploidy in Western North American Aspen (*Populus tremuloides*). PLoS ONE 7(10): e48406. doi:10.1371/journal.pone.0048406