Group 5

"Leaf Me Alone!"

The Resistance of Populus Tremuloides to Predation Over Time

Introduction:

A quaking aspen begins its life defenseless from the outside world, performing a delicate balancing act between defending itself and competing with its neighbors. To survive, it must perfectly allocate its resources between growing larger and defending itself from wandering herbivores and marauding fungi.

At different times in the aspen's life, it requires more resources for growth which affect its ability to defend itself. When the tree is young, much of its resources go to growth and development, and its leaves easily fall prey to passing herbivores and other pests. When the aspen is older, its leaves grow out of reach of some pests and, in turn, the leaves will show less damage. (Lindroth, 2013) Because the older tree spends fewer resources on growth, it has more to "wealth" to invest in defense. When the tree reaches a certain age it becomes more susceptible to damage and its leaf health declines. This graph of tree resistance from Donaldson (2005) mirrors this pattern.

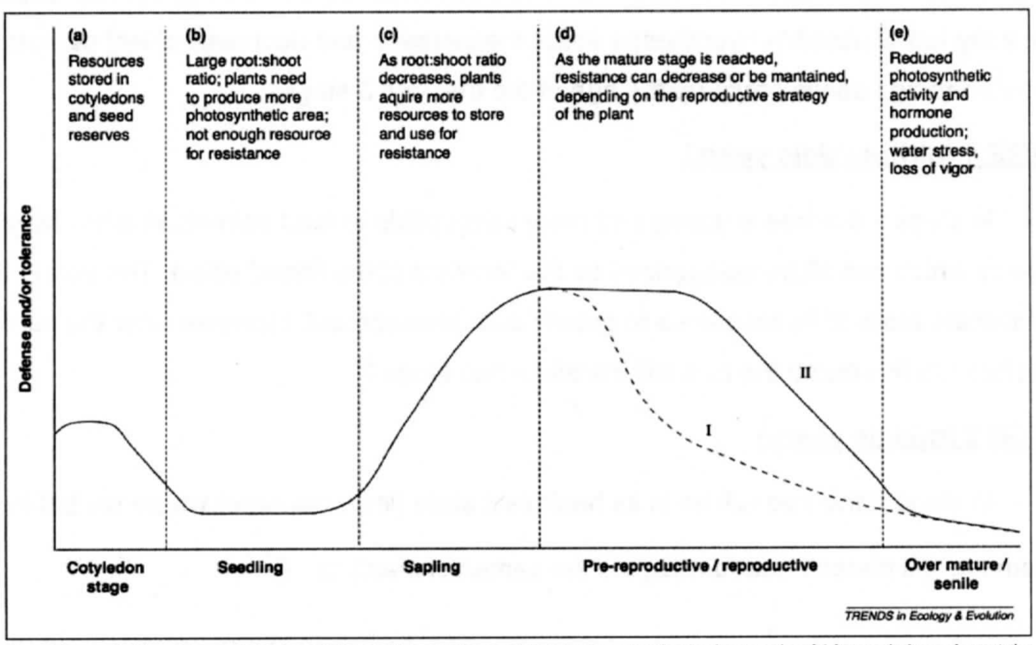


Figure 1. Proposed pattern of changes in defense and tolerance during plant ontogeny. Based on the assumption that the expression of defense and tolerance in vegetative tissues is mainly constrained by resources, the cotyledon stage (a) can be expected to be more resistant than are young seedlings because they have stored resources in seeds and cotyledons that can be allocated to resistance traits. As plants grow and enter the true-leaf seedling stage (b), stored resources are depleted, and a large root:shoot ratio does not enable plants to accumulate resources for functions other than growth. As plants continue to produce photosynthetic area (c), root:shoot decreases and plants are able to develop storage organs. Resources acquired can then be allocated to defensive functions or stored and used to respond to future herbivore attacks. When plants enter the reproductive phase (d), defense and tolerance can either decrease (I), or increase and remain stable (II), depending on the particular reproductive strategies of each plant species. Finally, as plants begin to senesce (e), reduced hormone production, water deficits, decreased growth and an unfavorable photosynthesis–respiration balance promote a decrease in all metabolic functions, including plant resistance, until the plant dies.

Clones are very common in aspen stands, which will allow us to isolate environmental effects when studying them. (Callahan 2013). Because we are able to compare individuals that are genotypically identical, we can determine how much a tree's ability to defend itself depends on its genes and how much it depends on the tree's environment. We will also be able to see if certain clones are more resistant to leaf damage than others.

Another unique quality of aspen trees is their high frequency of triploidy, where each cell has three copies of each chromosome (Mock, 2012). This property makes them an interesting specimen for this study because triploidy is another dimension of differences among clones. Clones with triploidy may exhibit different patterns of resistance than their diploid neighbors.

The clonality of aspen trees, their triploidy, and their pattern of chemical resistance lead us to the following questions:

1. Is there a certain point in an aspen tree's life when it is most resistant to predation by animals, insects, and disease? Is there a pattern of resistance throughout an aspen's life?
2. Does triploidy affect the tree's resistance?
3. Is tree resistance correlated with clonality- does it differ among clones?

Expected Outcomes:

We had two main hypotheses. 1) Either there is no statistically significant correlation between age and amount of leaf damage- which would imply that a tree's age has no effect on its resistance to damage, or 2) there is a correlation between a tree's age and its resistance. We have a myriad of possible hypotheses about the increase and decrease of leaf damage compared to age so we broke up the age of the tree into 3 stages:

STAGE 1 (birth-middle years)

In stage 1 the tree is young and more susceptible to land animals at a low height and diseases which can all be categorized as the "survival of the fittest" effect. The young tree will also allocate more of its resources to growth and development. However after the survival of the fittest has run its course the tree will transition into stage 2

STAGE 2 (middle years)

In stage 2 the tree will be in its healthiest state (least damage) where we believe that

A) damage will plateau (leaf damage is the same/constant) or

B) damage will decrease (regeneration of new healthy leaves). After this middle age we transition into what we call the 3rd stage.

STAGE 3 (middle years- death)

In stage 3 the tree will reach the end of the prime years and begin the transition into old age and death. During this time we predict that the resistance will decrease either rapidly or slowly. Its chemical defense mechanisms may be deteriorating at this stage.

Methods:

Leaves were gathered from an aspen stand surrounding Silver Lake. The damage of each leaf was rated on a scale from 0 to 3. The rating was as follows:

0- no damage

1-minimal damage

2-damaged, but doesn't hinder length/width measurements

3- damaged to the point of no return (no measurements can be made)

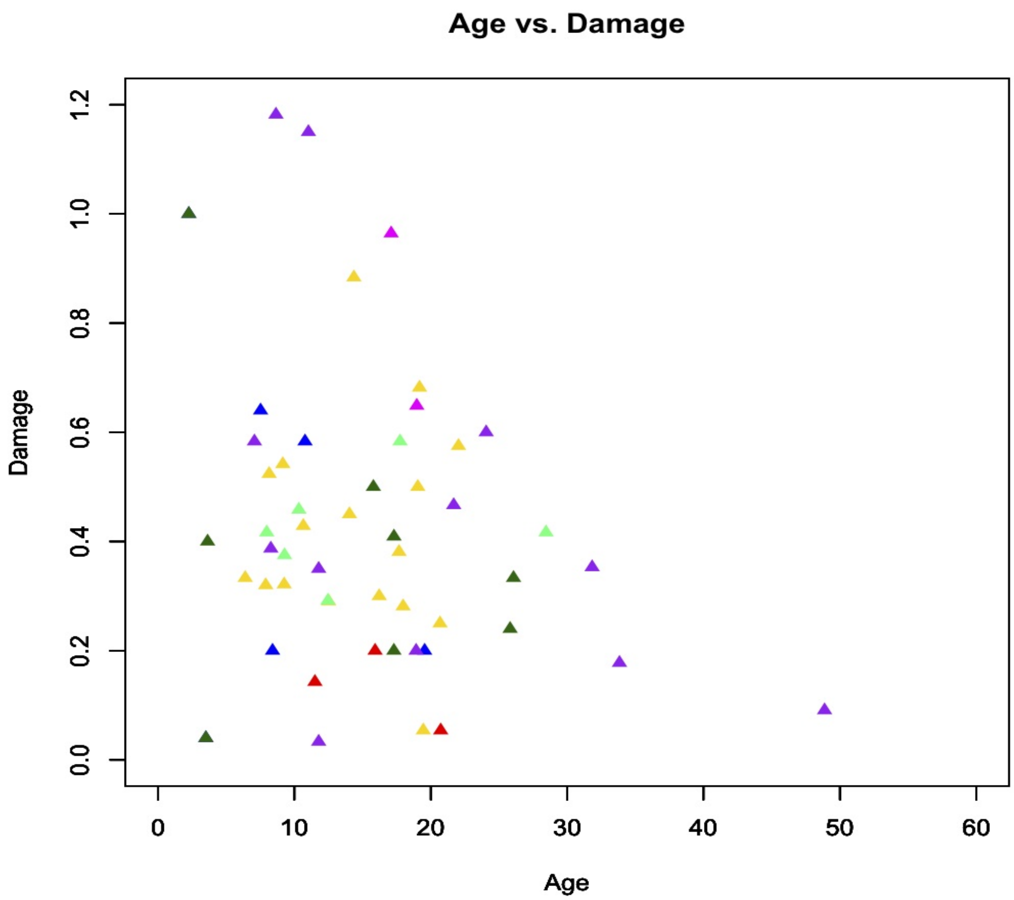
We then used this data to find the average amount of damage for each tree.

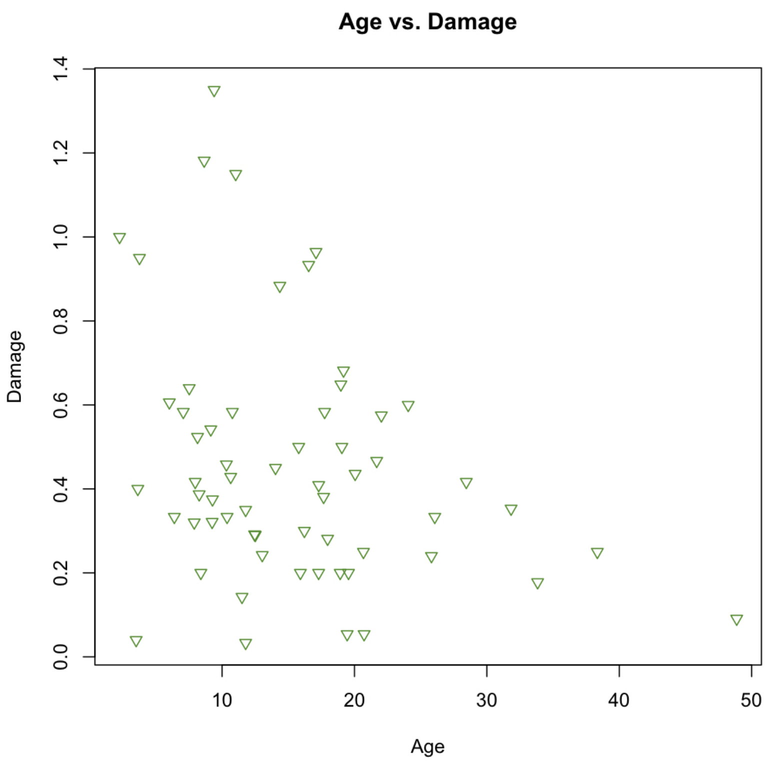
We found the approximate age of each tree using the equation: growth factor x (circumference cm/ (2.54 cm x pi)) = 2 x (circumference cm/ (2.54 cm x pi)) = Age of Tree (Nix, 2013). Leaf samples were genotyped, allowing us to define clonal boundaries and ploidy.

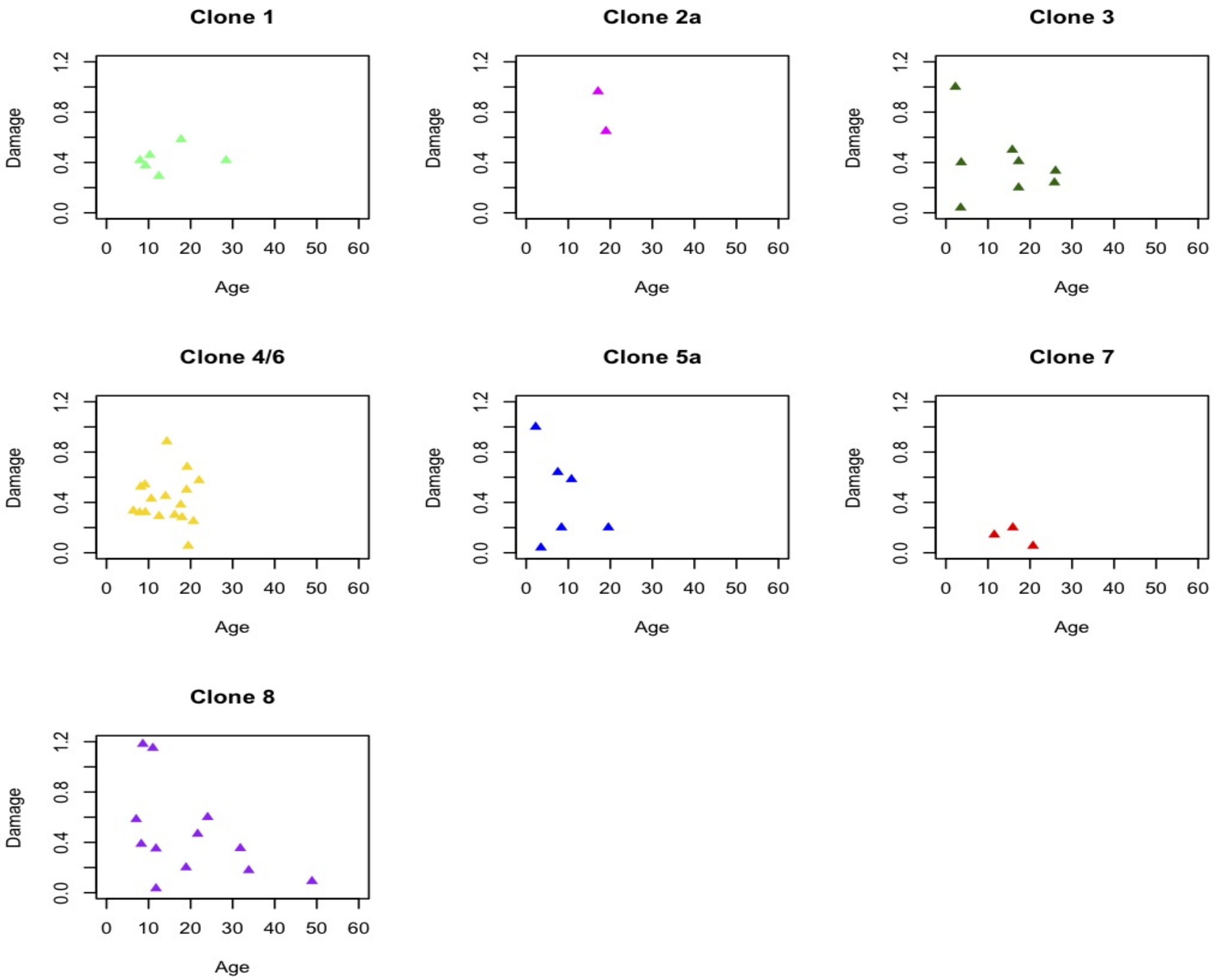
We plotted the data on multiple graphs in R. We also performed further analysis of the data using ANOVA (analysis of variance).

Results:

Clone	Color
4/6 (III)	gold
5a	blue
8 (III)	purple
3	dark green
1 (III)	spring green
2a	magenta
7	red







The three clones on the left are triploid.

Analysis of Variance Table

Response: damage

```
          Df Sum Sq Mean Sq F value  Pr(>F)
age        1 0.4643 0.46428  6.0760 0.01711 *
age:clone  7 0.6261 0.08945  1.1706 0.33602
Residuals 51 3.8970 0.07641
```

Data Analysis:

The three graphs above show the relationship between age and damage for every tree in our sample. There is an interesting pattern in the data. Instead of a "golden age", as mentioned in our hypothesis, the data generally falls below a line with negative slope that the trees do not cross. Because there are so many more young trees than old ones, the data implies that, as time goes on, the trees that have sustained a large amount of damage die leaving behind only those individuals that have little damage. The line of regression also shows the average decrease in damage as age increases. In addition, the clone graphs compare triploidy and diploidy. There is no apparent difference between the defense pattern of triploid and diploid clones.

The graphs appears to show a pattern, and the data after running a nested ANOVA seems to verify this. ANOVA gave a p value of .017 for the effect of age on damage. When a nested ANOVA is performed to determine the effect of age on damage within clones, it returns a p value of .336. So, within clones, age doesn't have any discernable effect. When considering the entire population, however, there is a clear correlation between how much damage a tree has incurred and how old it is.

It is difficult to tell, however, why the older trees survived as long as they did. It could be because they grew out of reach of herbivores faster or they had superior chemical defenses, but none of our data specifically explains it. A one way ANOVA test on the impact of clonality on damage returned a p value of 21.6%. So, according to this data, the average leaf damage does not differ significantly between the clones, implying that they have roughly the same resistance to leaf damage.

Conclusion:

Although the ANOVA test showed that age and damage are correlated, the graph did not match our expectations at all. We were anticipating a graph that peaked, but our graph more closely resembled a straight line of negative slope than any kind of curve. This may be because an individual aspen tree can live up to 100 years (NPS 2013), yet our oldest tree was only 48 years old. With a larger sample size and a wider range of ages, it may still be possible to find a different correlation between age and damage. It would also be interesting to go into further detail with the types of damage on leaves. Are younger trees more likely to be attacked by insects than fungi, do trees near the end of their lives show more damage? These could be extremely interesting studies to pursue.

Sources

Barton KE, Koricheva J. (April 2010), The Ontogeny of Plant Defense and Herbivory: Characterizing General Patterns Using Meta-Analysis. *The American Naturalist* , Vol. 175, No. 4 pp. 481-49

Boege K, Marquis RJ (2005) Facing herbivory as you grow up: the ontogeny of resistance in plants. Trends in Ecology & Evolution. Journal of Chemical Ecology vol 20, issue 8, 441.

Callahan CM, Rowe CA, Ryel RJ, Shaw JD, Madritch MD, Mock KE (2013) Continental-scale assessment of genetic diversity and population structure in quaking aspen (Populus tremuloides) Journal of Biogeography. vol 40, issue 9, 1780.

Donaldson JR, Stevens MT, Barnhill HR, Lindroth RL (2006) Age-related shifts in leaf chemistry of clonal aspen (Populus tremuloides). Journal of Chemical Ecology. vol 32, issue 7, 1415.

Lindroth RL, St Clair SB (2013) Adaptations of quaking aspen for defense against herbivores. Forest Ecology and Management. vol 299, 14.

Mock, K.E. , Callahan, C.M. Islam-Faridi, M.N., Shaw, J.D., Rai, H.S. Sanderson, S.C. Rowe, C.A., Ryel, R.J., Madritch, M.D., Gardner, R.S., Wolf, P.G. (2012) Widespread Triploidy in Western North American Aspen (Populus tremuloides). PLos One. vol 7, issue 10.

National Parks Service (2013) “Quaking Aspen” http://www.nps.gov/brca/naturescience/quakingaspen.htm

Nix, Steve, Jacksonville GA, http://forestry.about.com/od/silviculture/a/Estimating-A-Trees-Age.htm 2013

Porter (1976) Diameter growth not affected in 3 year study.
http://www.uaf.edu/files/ces/aknfc/resources/workshops/06AspenLeafMiner.pdf

Smith EA, Collette SB, Boynton TA, Lillrose T, Stevens MR, Bekker MF, Eggett D, St Clair SB (2011) Developmental contributions to phenotypic variation in functional leaf traits within quaking aspen clones. Tree Physiology. vol 31, issue 1, 68.