



GENETIC INFLUENCE ON DROUGHT TOLERANCE IN QUAKING ASPEN

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Introduction

Climate change poses a threat to quaking aspen (*Populus tremuloides*) in the southwestern U.S. as increases in temperature and decreases in precipitation are predicted to lead to more frequent, severe droughts.¹ While some populations of quaking aspen tolerate drought, the extent of their tolerance is unknown and likely has limitations which future environmental conditions may exceed. Quaking aspen is a clonal species, which provides an ideal system to study genetic contributions to drought-tolerance traits. The clonal ability of aspen allows for comparisons between individuals of the same clone as well as comparisons between clones, particularly between diploid and triploid clones. By quantifying levels of drought tolerance from clones over multiple years, the variability in how one clone responds to changes in climatic conditions can be studied (i.e. phenotypic plasticity). Phenotypic plasticity can allow a species to survive in unfavorable conditions and may allow for acclimation to new climate conditions.² By comparing drought-tolerance traits between nearby clones within the same year, genetic influences on drought tolerance can be detected. Phenotypic plasticity combined with genetic influences on drought-tolerance traits may allow certain clones to tolerate more severe drought than others.³ We hypothesize that all clones will exhibit phenotypic plasticity in drought-tolerance traits. Additionally, we hypothesize that genetic influence will be evident in drought-tolerance traits, and clones that experience the same environment will respond to drought similarly.

Methods

To investigate phenotypic plasticity and genetic influence on drought response in quaking aspen, we investigated the extent to which physiological drought-tolerance traits differ within ramets of a single clone and between clones across an elevation gradient in aspen forest stands in the San Juan National Forest in Colorado during a severe drought year (2018) and a wet year (2019). Sixteen plots were established at three different elevation levels (Fig. 1A). There are 6 low elevation plots (2650-2700m), 5 mid elevation plots (2850-2950m), and 5 high elevation plots (3070-3100m). Trees in the low elevation plots are expected to experience a hotter and drier climate while those in high plots are expected to be cooler and wetter. The following drought-tolerance traits were measured: percent loss of stem hydraulic conductivity (PLC), leaf area-to-sapwood area ratio ($A_L:A_S$), and specific leaf area (SLA). PLC was quantified using the pressure-flow method by Sperry et al. (1988) before and after vacuum infiltration to remove embolisms.⁴ PLC captures the level of drought stress that a

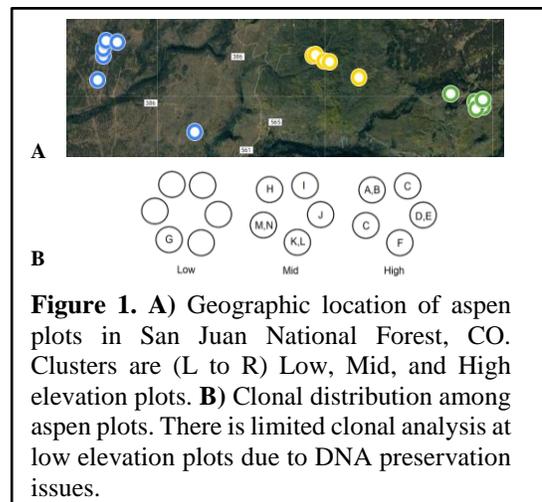
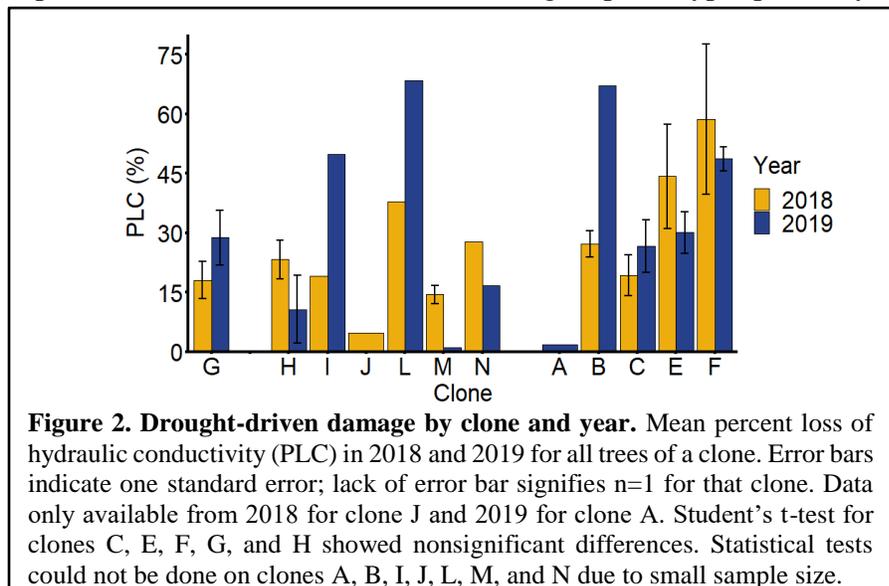


Figure 1. A) Geographic location of aspen plots in San Juan National Forest, CO. Clusters are (L to R) Low, Mid, and High elevation plots. B) Clonal distribution among aspen plots. There is limited clonal analysis at low elevation plots due to DNA preservation issues.

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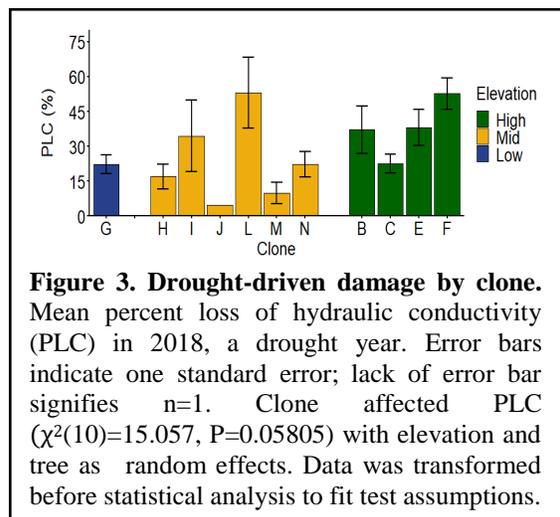
tree is experiencing. Changes in the other traits reflect how a tree might physiologically respond to drought stress. $A_L:A_S$ is calculated by dividing the cumulative leaf area at the end of a branch by the area of a cross-section of sapwood where the branch was cut. $A_L:A_S$ demonstrates the water transport efficiency of a tree, and a decrease in this ratio also decreases the drought stress on the tree. SLA is a calculation of the surface area of leaves divided by their mass, and it provides a measurement of the drought tolerance of the leaves of a tree. A lower SLA can increase tolerance to drought.⁵

To assess clonal structure within the plots, leaf samples were collected from 10 trees in each plot. Leaf samples were stored on silica gel in the field, dried at 50°C for 24-48 hours, and stored in the freezer until DNA extraction. DNA was extracted with a generic spin column protocol modeled on the Qiagen DNeasy Plant Kit and stored at 4°C. Eight microsatellite loci were amplified by PCR and fluorescently labelled. Fragment analysis was completed on a 3730 capillary electrophoresis unit. Trees were assigned to a clone if all 8 loci were identical. Ploidy was determined from fragment analysis, and clones with only 1 locus appearing triploid were treated as triploid. Trait data was analyzed to investigate if clone affected PLC, $A_L:A_S$, and SLA in 2018. PLC was also compared between 2018 and 2019 to investigate phenotypic plasticity.



Results/Discussion

Forty-eight trees were successfully assigned to 14 genetically distinct clones (Fig. 1B). Clones stayed within plot boundaries, except for clone C. In addition, plots commonly contained 2 clones. Clone A was the only diploid clone, and clones B-M were triploid. While this seems unusual, Mock et al. (2012) reported up to 69% of quaking aspen were triploid in the western US.⁶ Given our small sample size, it is possible that we collected mainly triploids and few diploids. Clones exhibited high variation in PLC between 2018 and 2019 in response to different climatic conditions (Fig. 2). The variation in PLC in different years indicates that clones are physiologically experiencing and



responding to drought differently (i.e. a change in phenotype) as environmental conditions change. This is slight evidence for phenotypic plasticity.

Variation in PLC by clone in 2018 is evident (Fig. 3). Clone affected PLC ($P=0.06$) using a mixed effects model where elevation and tree were accounted for as random effects. This indicates slight genetic influence on drought-driven damage, or the amount of drought stress experienced by a clone. Variation in drought tolerance due to clone is present at a low significance level ($P=0.1$) as indicated by $A_L:A_S$ (Fig. 4). This indicates that while water transport efficiency varied somewhat by clone, genetic influence is not strongly supported in this drought-tolerance trait and does not account for all the variation observed. Elevation did appear to influence $A_L:A_S$ as mid elevation clones trended to higher $A_L:A_S$ ratios than both low and high elevation clones. There is no evidence for genetic influence in leaf drought tolerance as SLA did not vary significantly by clone (Fig. 5).

In conclusion, there is slight evidence of genetic influence on the drought stress (PLC) experienced by aspen clones in the San Juan National Forest, but no evidence of strong genetic influence in other traits. These results suggest that while genetic influence may be a factor in drought-tolerance traits, other aspects of each clone, such as location, stand density, or herbivory pressure, could account for variation in drought-tolerance traits.

Future Research

Future research should first aim to continue genotyping additional trees from each plot to expand knowledge of clonal structure and ploidy, particularly in the low elevation plots. These low elevation plots are a gap in our data, and are of interest because they should experience the most drought stress. Trait data should then continue to be compared between clones, but also between diploid and triploid clones. Phenotypic plasticity can continue to be investigated by including additional 2019 data on $A_L:A_S$ and SLA into analyses.

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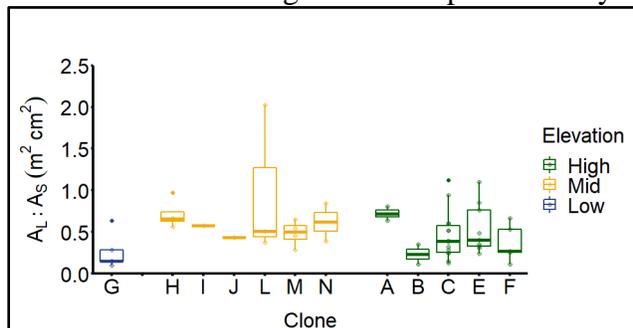


Figure 4. Water transport efficiency by clone. Leaf area-to-sapwood area ($A_L:A_S$) varied between trees within a clone and between separate clones in 2018. Mid elevation $A_L:A_S$ trends higher than both low and high elevation $A_L:A_S$. Clone affected $A_L:A_S$ ($\chi^2(11)=17.577$, $P=0.09193$) with elevation and tree as random effects. Data was log transformed before statistical analysis to fit test assumptions.

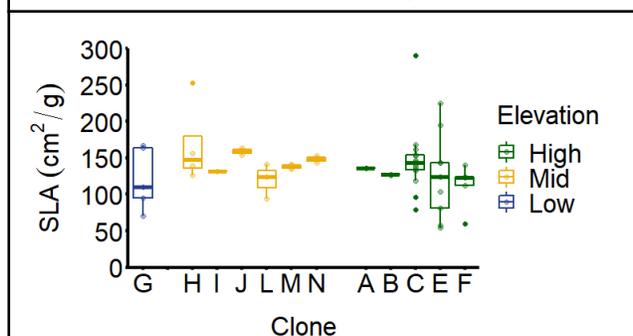


Figure 5. Leaf drought tolerance by clone. Specific leaf area (SLA) in 2018 varied between trees within a clone, but did not vary significantly between separate clones.

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