

The effect of cytotype on radial growth rate in quaking aspen (*Populus tremuloides*) across environmental gradients

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Abstract

Quaking aspen (*Populus tremuloides*) trees are a foundation species that is widely distributed across all North America. Since the early 2000s, high levels of mortality have been observed in these forests. In recent decades, many quaking aspen forests have been experiencing high mortality rates. This large-scale mortality has been the subject of many studies attempting to ascertain the cause and gather knowledge which could aid in future forest management efforts. There have been observed differences between aspen ramets' (individual stems) drought-response at small spatial scales under similar environmental conditions, which suggests intraspecific (within species) variation in drought mortality risk. Aspen ramets grow in genetically identical clones (genets) that have either two (diploid) or three (triploid) copies of each chromosome. My project investigated the effect of ploidy level on drought response in quaking aspen and sought to gather data on aspen cytotype growth rate differences across a larger environmental gradient. I collected stem core samples from both aspen cytotypes, compared their radial growth for each year, and analyzed their responses to drought years using historical drought data for the area. While my data analysis did show cytotype to be a significant factor in aspen radial growth, the diploids sampled were measured to have a wider average ring width than triploids in the same areas, counter to the original hypothesis. Elevation, aspect, and slope were all found to have a significant impact on aspen growth rates for both cytotypes. These findings and future studies into polyploidy's impact on growth rates could prove useful in future forest management efforts through an understanding of which aspen trees may be more vulnerable, or more successful, in future droughts.

Introduction

Quaking aspen (*Populus tremuloides*) is the most widely distributed tree species in North America. It acts as a foundation species that holds high ecological value for the systems it is found in, providing food and habitat for a large variety of insects and ungulates alike, as well as operating as an early successional species that is well adapted to grow in environments following disturbances such as fire. This is a species of enormous ecological, economic, and social value [1]. Ecologically, aspen has the largest distribution of any North American tree, promotes biodiversity, and the quaking leaves for which it's named allow for a more even light distribution which aids a productive understory [2]. Economically, aspen is an important source of fiber, wildlife, and livestock forage and socially it is a beloved species and popular tourism attraction for its vibrant foliage in the fall season [3].

Increased levels of climate-induced mortality within aspen forests have been reported in recent decades, with up to 17% of these forests impacted in Colorado and some areas in the southwestern region of the state seeing 50-60% mortality rates [4,5]. Previous research has linked these large-scale mortality events to extreme 'climate change-type droughts', alongside disease and stand replacement [6]. This research set forth a prediction that coming years will continue to bring more drought stress to areas of the western United States and further impact the aspen forests of Colorado.

However, mortality events have been observed to be patchy at small spatial scales under similar environmental conditions, suggesting there may be within-species variation in drought mortality risk [7]. Aspen ramets grow in genetically identical clones (genets) that have either two (diploid) or three (triploid) copies of each chromosome [8,9]. This instance of ploidy variation (differences in cytotype, or chromosome copies, among individuals) can produce shifts in genetic systems and phenotypes that have the potential to result in increased evolutionary diversification [10]. There have also been research findings within the Gothic, Colorado area which suggest that structural differences between the ploidy levels may lead to differences in plant function in response to the environment [11]. Additionally, a study on the Pando aspen clone and adjacent

stands in central Utah explored how cytotype affected the growth rate of ramets within the clone and found that triploids had a wider average ring width than diploids [12].

Hypotheses

This study expanded on this prior research by collecting data that explored the following hypotheses:

1. Triploid aspen trees were expected to have wider rings than diploid trees across a variety of different environmental conditions, which was investigated using sites of varying slope, elevation, and aspect.
2. Triploid aspen trees were also expected to have wider rings across the sampled environmental gradient as related to the temperature variability, so more radial growth in triploids in areas at lower elevations and south-facing slopes.
3. Diploid aspen trees were expected to have wider rings during drought years due to their more conservative growth patterns.

This investigation used a dendroecological approach to explore these hypotheses. Dendroecology uses tree ring analyses to answer ecological questions. Every tree goes through an annual growing season during which it expands radially, producing a new layer of secondary xylem (wood), its water-conducting tissue. This new layer of xylem can vary in size depending on tree stress and resource availability during that growing season [13]. During the beginning of a growing season in early spring, the wood is often growing quickly and results in a less dense wood termed ‘early wood’. This period of growth is followed by the harsher seasons, during which the tree develops a denser wood termed ‘late wood’. The result of these two distinctive wood types each year leads to visible growth rings which will show how each tree responded to its environment that year. In drought years, it is typical for trees to show narrower growth rings. This study compared both cytotypes’ radial growth during these drought years to determine which was more successful under this environmental stressor. Samples were collected for this study from both diploid and triploid trees across an environmental gradient.

Methods

Study Area: Tree cores were collected from an existing network of quaking aspen demography plots where the cytotype and genotype of each focal tree has been measured (*Figures 1 & 2*). These plots cover a wide range of elevation (2,747-3,309 m), aspect, and slope (0-38°) and represent 73 diploid and 407 triploid focal trees. From these plots a subset of cores was collected from 8 diploids and 8 triploids by selecting for the widest coverage of all variables available from samples collected in time for this project. The subset covers a range of elevation of 2906-3129 meters and slope from 3-27° (*Figure 3*).

Sample Collection: At each plot, one tree was selected to sample based on proximity to the focal tree (no more than 10m away) and a minimum size of over 5cm DBH (stem diameter at breast height). The focal tree was not cored to avoid impacting the ongoing demography study. I assumed that cytotype did not vary within a 10m spatial scale based on prior research which showed strong spatial homogeneity of clones at <50m spatial scales.

Each tree core was extracted using a 5mm increment borer at breast height on the upslope side of the tree to avoid compression artifacts. The increment borer was sterilized and sharpened before each extraction to avoid pathogen cross-contamination between trees and to ensure that a dull cutting edge did not cause cracks or compression in the cores. Once extracted, the core was marked at the top with a pencil to indicate the direction of the axially oriented xylem cells. Once marked, the core sample was placed into a straw for transport, taped shut on both ends, and marked with the date and plot identification number. At the end of each field day, the collected samples were then stored in a cool, dry space in the Blonder lab space in the Gothic Research Center at the Rocky Mountain Biological Laboratory. The core samples were later placed into a drying oven at its lowest setting to help prevent any mold build up and to better preserve them until they could be processed.

Sample Processing: First, each core was glued into an individual wooden core mount to ease in the sanding process and stabilize the samples (*Figure 4*). Next, I

surfaced each core using progressively finer grit sandpaper (80, 120, 220, then 320 grit level) to remove approximately one third of the diameter and provide a flat surface for imaging (*Figure 5*). I then imaged the surfaced cores using a dissecting microscope at 1.25x magnification with a digital camera (*Figure 6*). Once the core was imaged, the ring widths were measured using the free software program, ImageJ. This allowed for multiple measurements of the core images so that an accurate width could be recorded.

Environmental Data: Measurements of the elevation, aspect and slope at each sampling location were collected from the USGS National Elevation Dataset. Environmental conditions were represented using the reconstructed Palmer Drought Severity Index (PDSI), currently available for the period 1858-2021 (*Figures 7 & 8*). PDSI has been shown to be a strong limiting factor for aspen growth [12]. All monthly PDSI values were collected over the available period and the mean, maximum, and minimum from a dataset prepared by TerraClimate. TerraClimate is a dataset of monthly climate and climatic water balance for global terrestrial surfaces. TerraClimate provides a free dataset of climate data from a team of scientists at UC Merced. They combined historic datasets from a WorldClim dataset, with coarser spatial resolution (*Figure 9*), and time-varying data from CRU Ts4.0 and the Japanese 55-year Reanalysis (JRA55). Topography values were extracted at the coordinate of each focal tree and recorded using a Trimble GPS unit in 2018.

Data Analysis: I used a linear effect model to predict annual growth increment (dependent variable) as a function of cytotype and environment (independent variables), with interactions between cytotype and environmental variables. I modeled this function as:

$$\text{RWI} = \text{PDSI Max} + \text{cytotype} + \text{PDSI max*cytotype} + \text{elevation} + \text{aspect} + \text{slope}$$

Where RWI represents the ring width index, our measurement for annual growth increment. Environmental conditions were represented using elevation,

aspect, slope, and reconstructed PDSI. The PDSI value for this model was chosen by testing the PDSI value from each month of the year, as well as the yearly mean, maximum value, and minimum value (*Table 1*). After testing each expression of PDSI value, I selected the maximum PDSI value from each year to represent drought severity for the linear effect model.

Results

Radial growth across environmental gradients

The linear effect model returned the following p values for our variables: PDSI maximum ($1.07e-1$), ploidy level-triploid ($3.59e-3$), elevation ($1.02e-6$), aspect ($4.0e-2$), slope ($8.30e-9$), and the interaction of ploidy level and PDSI maximum ($3.39e-1$). The results of this function showed PDSI max to be mildly significant as a predictor of ring width size, while all three environmental factors returned p values indicating they were highly significant for ring width size.

Aspen cytotype's impact on radial growth rate

Additionally, the results showed a significant p value for cytotype, showing an aspen that is a triploid would be negatively impacted and more likely to have narrower ring widths (*Table 2*). A comparison of ring width sizes for all 16 samples also showed diploids to have a higher average ring width than triploids (*Figure 10*). While the average ring width measurement was higher from the 8 diploid samples, the triploid samples exhibited several ring width measurements that were wider than diploids.

Aspen cytotype's impact on drought response

PDSI returned a mildly significant p value, with the PDSI max value showing a p value of 0.10706 in our linear model. This was the most significant PDSI value found after running the linear model through for the PDSI value from each month, along with some other PDSI variations (minimum, mean, PDSI values

from prior months leading into the next year's spring growing season). Diploids and triploids did not show a conclusive difference in radial growth success during pointer drought years.

Discussion

Aspen cytotype's impact across environmental gradients

After analyzing the core samples and the data output from our linear effect model, we found significant relationships between aspen radial growth rates and the three environmental factors we tested for: elevation, aspect, and slope.

However, the results then diverge from prior research and place diploids above triploids in terms of average yearly radial growth [12]. This can be seen in *Figure 10*, though it also appears from background research and the multiple triploid outliers that these unexpected average values could be the result of a small sample size.

Aspen cytotype's impact on drought response

The PDSI component of this project reflected a significant relationship between drought severity values and radial growth rate success for aspens. There were no conclusive differences found between the success of a diploid or triploid tree under drought circumstances. This may be a combination of sample size and the coarse spatial resolution of the TerraClimate dataset. Due to the proximity of our sites, the PDSI information collected had values that overlapped for many of our study areas. This is an interesting note for future studies in the area and could be resolved by collecting from a more distanced variety of sites or securing a PDSI historic dataset with a finer resolution.

Conclusion

Overall, the results found failed to support my original hypotheses, but they did provide a foundation upon which larger projects could build. The results of this

study were inconsistent with current literature and our expectations for polyploidy in quaking aspens. It would be very beneficial to build upon this study's methods and collect more cores for further exploration of cytotype's impact on aspen growth. One alternate hypothesis may be that the diploids sampled may be better specialized for the conditions in this area of Colorado, as opposed to the conditions experienced by diploid trees in the central Utah area. It's also possible

In addition to increased sample size, a future study could draw more conclusive results through using a linear mixed effects model as opposed to the linear model used in this study's analysis. A linear mixed effects model may provide different insights into the relationship of different variables in aspen growth, while using sites or genotype as random effects. These improvements to the base of this study would provide an opportunity for a more thorough examination into aspen cytotype and its impact on aspen success through future droughts.

This study contributes toward an increased understanding of aspen climate-induced mortality, but also as a basis for exploration for many other plant species that experience polyploidy [11]. Forest management efforts will benefit from more data on which trees may be better equipped to handle drought conditions. A further understanding of cytotype effects on aspen growth will be important to those working with aspen forests given that genetic diversity is necessary for species adaptation to rapidly changing climates. Ideally, a pragmatic approach to in situ forest management will focus on the long-term maintenance of aspen genetic diversity and cytotype diversity (i.e. both diploids and triploids) on the larger landscape [15].

References

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Figure 1: A zoomed in map of the aspen sites we collected our samples from within the Middle East River watershed. Sites are marked by green ticks and assigned a unique acronym for identification.



Figure 2: A zoomed in map of the aspen sites we collected our samples from within the Oh-Be-Joyful Watershed. Sites are marked by green ticks and assigned a unique acronym for identification.

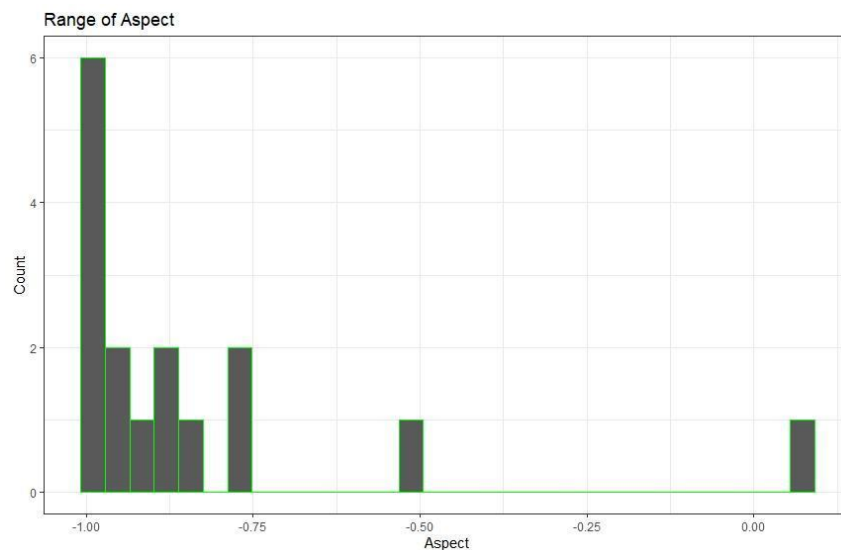
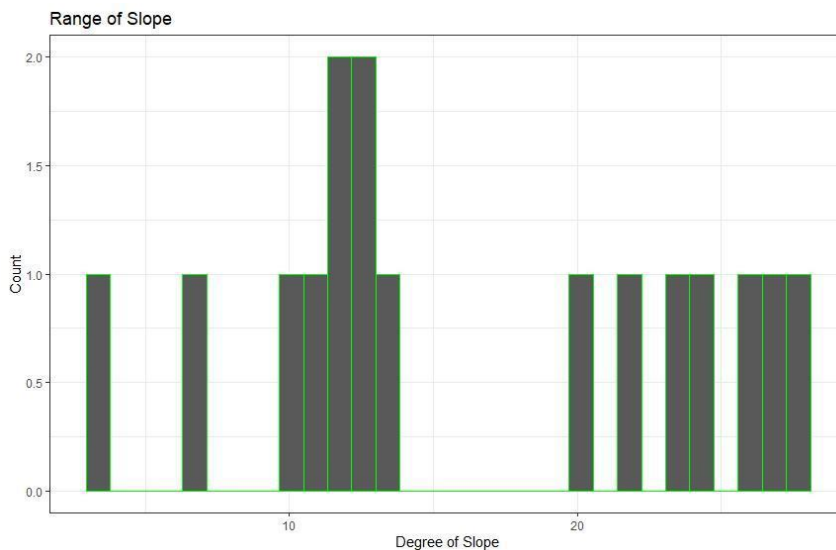
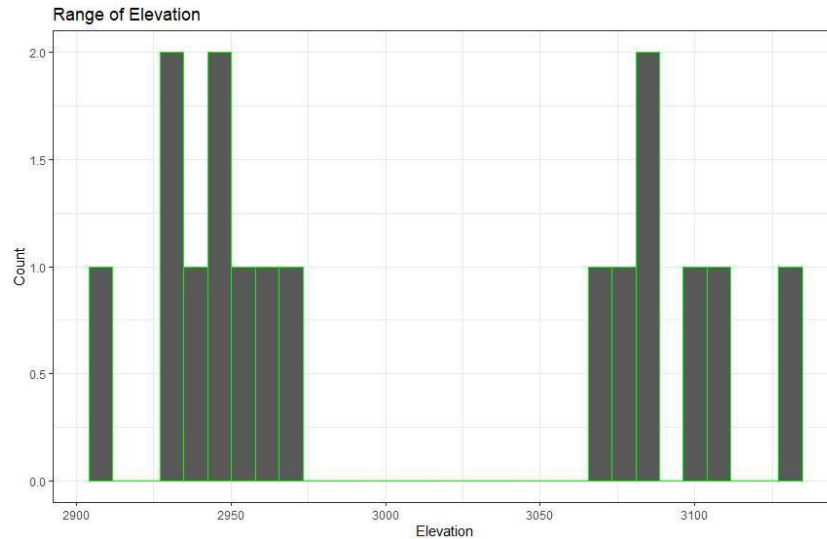


Figure 3: Due to the limited timeframe for collecting samples, there are several gaps in the environmental range. This study and its results would benefit from increased sample size and coverage of a wider array of environmental variables. The range of elevation covered sites from 2906 to 3129 meters, the range of slope 3 to 27 degrees, and the range of aspect showed sites from -0.93 to -0.98.

As the results of the linear effect model show all three of these environmental factors to be significant, it would be a worthwhile follow up to explore an expanded range of sampling sites.



Figure 4: An aspen core sample after being glued into its wooden mount



Figure 5: The same aspen core sample (Figure 1) after sanding using all four levels of grit paper.

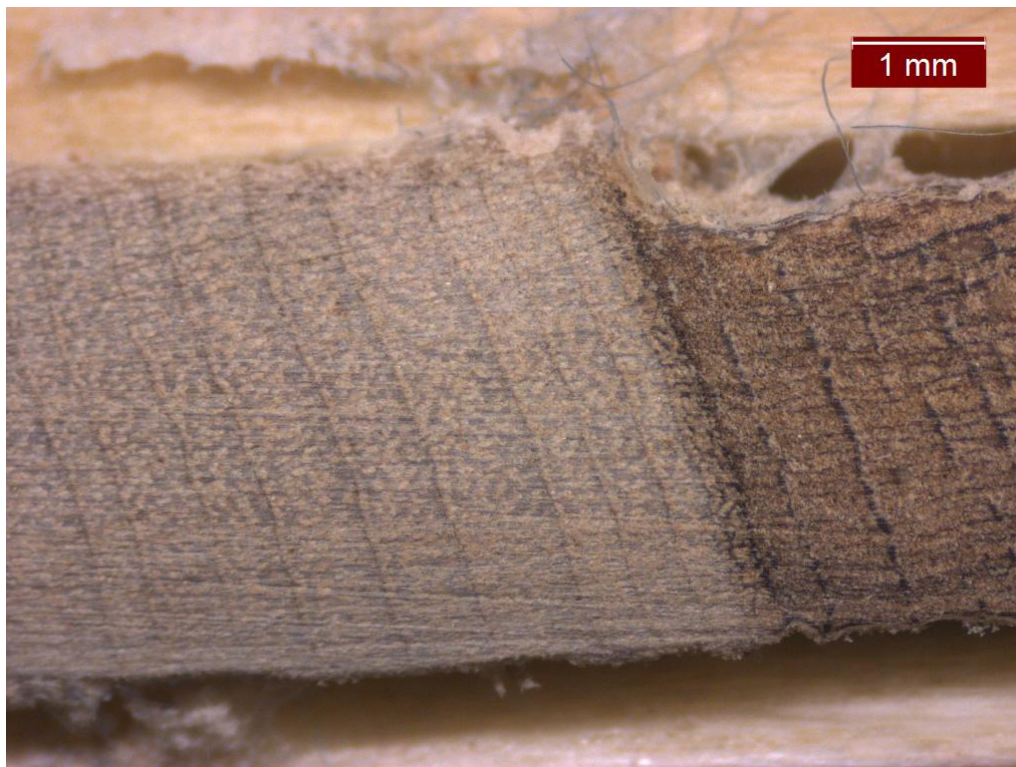


Figure 6: Aspen core sample from site VPBI after processing and being imaged under 1.25x magnification.

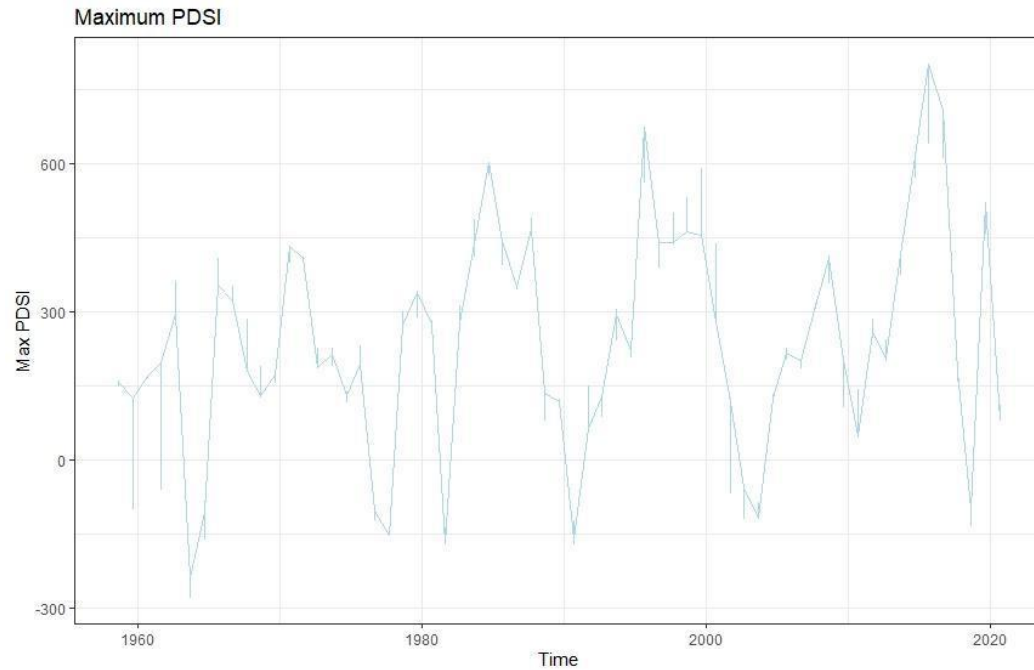


Figure 7: The PDSI value which returned the most significance in the linear model was the max PDSI. The TerraClimate dataset provided PDSI values from 1958 to present, and here the graph demonstrates the increase of drought severity extremes in recent decades.

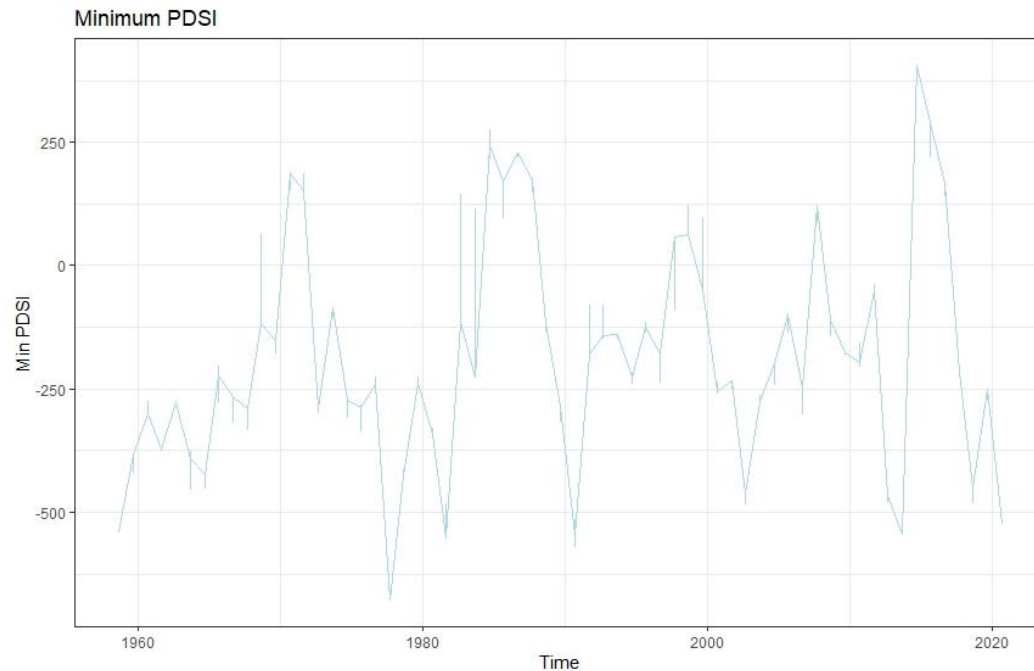


Figure 8: For comparison, this graph shows the trends over the last several decades for PDSI minimum values each year.

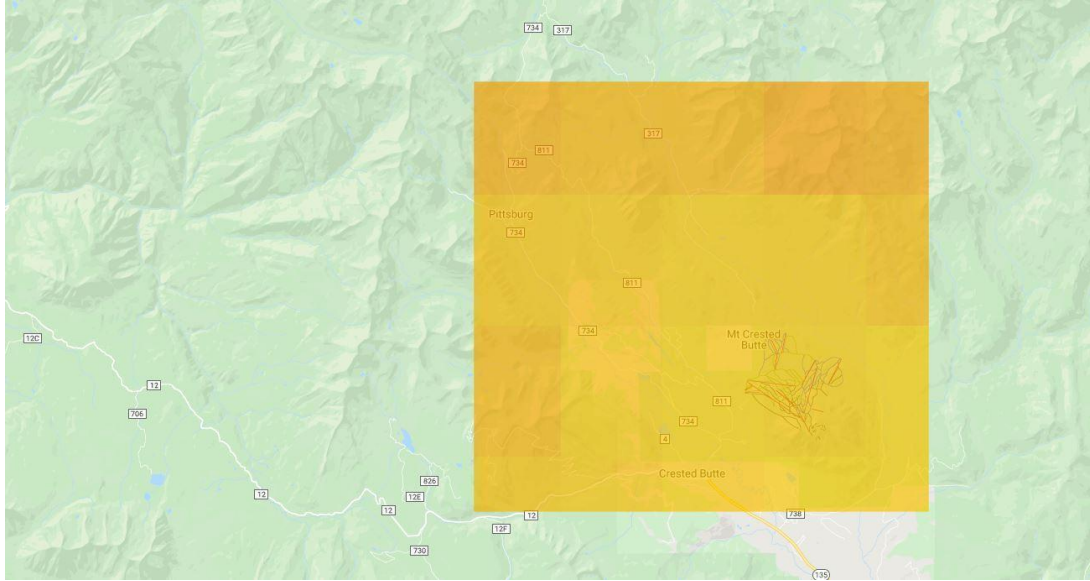


Figure 9: The PDSI information collected from the TerraClimate dataset has a coarse spatial resolution exhibited above. The dataset provides a PDSI value for every 4 km² of area across the world's surface.

PDSI type	P value
min	0.96027
mean	0.78814
max	0.10706
12_prev	0.58841
11_prev	0.55607
10_prev	0.70419
12	0.17437
11	0.45024
10	0.17469
9	0.21265
8	0.36769
7	0.24457
6	0.24193
5	0.48827
4	0.9243
3	0.81597
2	0.83461
1	0.5014

Table 1: All p values from various PDSI values. Minimum, mean, maximum, monthly values (January=1, February=2, etc.), and the PDSI values from the previous year's last three months (10_prev=previous year's October)

	Estimate	Std. Error	T value	Pr (> t)	
PDSI Max	-1.223e-04	7.583e-05	-1.613	0.10706	
Ploidy Level-Triploid	-1.309e-01	4.481e-02	-2.920	0.00359	**
Elevation	-3.577e-03	7.268e-04	-4.922	1.02e-06	***
Cos.aspect	-1.793e-01	8.725e-02	-2.055	0.04016	*
Slope	3.364e-02	5.781e-03	5.189	8.30e-09	***
PDSI Max : Ploidy Level	9.425e-05	1.092e-04	0.863	0.38844	

Table 2: Dataset produced by the linear effect model, run with PDSI maximum values for all years. P values (Pr (>|t|)) are considered significant at 0.1 and below. Based on this data, PDSI was found to be mildly significant, while an aspen's ploidy level, elevation, aspect, and slope were all very significant. Our results showed a triploid to have a negative relationship to an aspen's ring width, but this may also be attributed to the limited sample size.

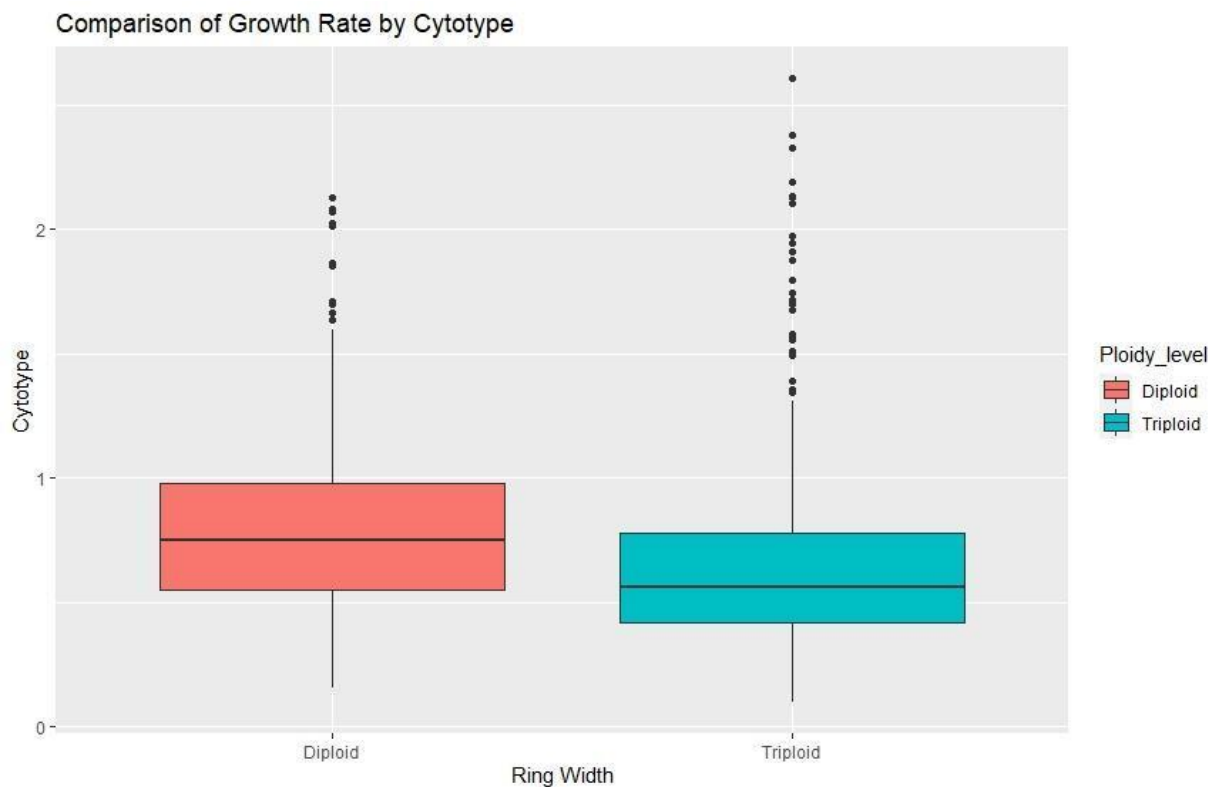


Figure 10: Diploids exhibited higher average ring widths than triploids, which does not align with current literature on diploid/triploid growth rates. However, this boxplot shows that there were several outlier triploid ring widths that were much wider than diploid ring width measurements. This is noteworthy, especially when considered in addition to the limited sample size of this study.