5-2016

Stomatal Differences in Western Aspen and Linkage to Drought Tolerance

Brianne Palmer
Utah State University

STOMATAL DIFFERENCES IN WESTERN ASPEN AND LINKAGE TO DROUGHT TOLERANCE

by

Brianne Palmer

Thesis submitted in partial fulfillment

of the requirements for the degree

of

HONORS IN UNIVERSITY STUDIES

WITH DEPARTMENTAL HONORS

in

Conservation and Restoration Ecology

in the Department of Wildlife Resources

Approved:

Thesis/Project Advisor Karen Mock	**Departmental Honors Advisor** Dave Koons

Director of Honors Program

Kristine Miller

UTAH STATE UNIVERSITY

Logan, UT

Spring 2016

ABSTRACT

Aspen (*Populus tremuloides*) is the most widely distributed broadleaf forest tree in North America. However, aspen are declining rapidly in areas of the Intermountain West. Aspen in this area are prone to experiencing limited moisture and high temperatures. An important aspect of plant physiology when dealing with these stressors is stomatal function. Stomata control the rate of photosynthesis, therefore, the size and frequency of the stomata is likely to influence the survival of the species in this environment.

An unusual feature of aspen is the high frequency of triploidy in the southern portion of its range. Stomatal size and density differences between cytotypes have not been assessed in aspen. The purpose of this study is to evaluate the differences in stomatal length and density between diploid and triploid aspen in Utah. If stomatal differences are pronounced between cytotypes, this could be the basis of a rapid field-based test to distinguish cytotypes without laboratory analyses. To test this, I collected leaves from independent clones in Logan Canyon and Fishlake National Forest in the summers of 2013, 2014, and 2015. Using cellulose acetate impressions of the underside of the leaves, I measured the stomatal size and frequency. The results indicated that triploid aspen have larger and fewer stomata than their diploid counterparts which may influence the ploidy response to drought conditions. Understanding the complexities of the different aspen ploidy levels is essential in future forest management and predicting future vegetation changes in a changing climate.

ACKNOWLEDGEMENTS

I would like to thank my faculty mentor Karen Mock for her support throughout the project. I would also like to thank the Utah State National Aquatic Monitoring Center (Bug Lab) for allowing me to use space in the lab. As well as Dr. Chuck Hawkins for allowing me to use the compound microscope. Additionally I would like to thank the many organizations that provided funding for this project including the Undergraduate Research and Creative Opportunities program, the Honors program, and the Quinney College of Natural Resources Undergraduate Research Program. Finally, I would like to thank the faculty and students in the Quinney College of Natural Resources for their invaluable support of undergraduate research.

TABLE OF CONTENTS

INTRODUCTION

Quaking aspen (*Populus tremuloides*) is the most widely distributed broadleaf forest tree in North America (Little 1971, Jones and DeByle 1985, Perala 1990, Peet 2000). Throughout its range, aspen are regenerated vegetatively or sexually, depending largely on environmental conditions (Barnes 1966, Schier 1973, Kemperman and Barnes 1976). In xeric landscapes, seedling establishment may be uncommon and episodic, and vegetative reproduction can dominate, resulting in large clones (Barnes 1966).

Despite the ability to persist vegetatively, aspen are declining rapidly in some areas of the Intermountain West (Bartos and Campbell 1998, Bartos 2001, Shepperd *et al.* 2006, Rehfeldt *et al.* 2009, Worrall *et. al* 2010). There are many causes of aspen decline, including fire suppression (reducing regeneration by suckering), over-browsing by ungulates, and climate change (Kay 1997, Hanna and Kulakowski 2012). Much of this mortality is described as Sudden Aspen Decline (SAD), and is associated with drought and temperature stress (Allen *et al.* 2010). With increasingly severe drought conditions predicted for many areas of western North America (Seager *et al.* 2007), there is a critical need for improved understanding of the mechanisms of drought sensitivity and resistance in aspen.

In the southern portion of its range where drought stress is common (Worrall *et al.* 2008, Anderegg *et al.* 2012), there is a high frequency of triploid aspen (Mock *et al.* 2012). Triploidy is the state of having three copies of each chromosome, and occurs when unreduced gametes are fertilized (Harlan and deWet 1975). The presence of three or more copies of the genome is common in plants, and can have a range of physiological and morphological effects, including traits that influence drought tolerance (Levin 1983). The reasons for elevated triploidy in the southern range of aspen is not well understood (Mock *et al.* 2012), but may include past selection

for growth rates (De Rose *et al.* 2015) and/or drought tolerance. Physiological and structural differences between diploid and triploid aspen, including drought tolerance differences, have never been directly investigated. Two traits which may vary with ploidy level are stomatal size and density. Because genome size is correlated with nuclear volume and cell volume, polyploid cells are often significantly larger than diploid cells (Mowforth and Grime 1989, Kudo and Kimura 2002). Previous research has also shown a positive correlation between genome size and stomatal size and a negative correlation with stomatal density (Beaulieu *et al* 2008, Hodgson *et. al* 2010), suggesting that stomatal size is inversely related to stomatal density.

Increased stomatal sizes or densities in triploid aspen, if present, may in turn influence drought tolerance due to their roles in gas exchange and water loss (Hetherington and Woodward 2003). Several studies have indicated a positive correlation between drought tolerance and stomatal size and density among species. Aasamaa *et al* (2001) demonstrated a clear, negative relationship between stomatal length and sensitivity to drought in six deciduous temperate trees (*Acer plantanoides* L., *Tila cordata* Mill, *Padus avium* Mill, *Quercus robur* L., *Salix caprea* L., and *Populus tremula* L.). The six species were grown in a greenhouse and exposed to three different growth conditions: nitrogen fertilization, water-stressed, and a control. The results indicated that species with larger stomata experienced slower stomatal closure rates and increased water loss when exposed to water-stressed conditions. Similarly, Carpenter and Smith (1975) assessed stomatal size and density in Appalachian hardwoods and found that between groups of similar species, trees growing on dry sites have smaller stomata and greater stomatal density, suggesting that smaller and denser stomata are more suited for droughty environments. Pearce *et al* (2005) investigated three species of poplars native to southern Alberta, Canada including: *Populus deltoides* Bartr. Ex Marsh common in warm and dry prairie regions, *P.*

balsamifera L. common in cooler and wetter mountainous regions, and *P. angustifolia* James which is occurs in grassland regions. The results showed that (1) stomatal length and density differed among genotypes within a species but that mean stomatal length and density did not differ among species and (2) diverse genotypes of the Poplar species growing in semi-arid environments had small and dense stomata, consistent with observations in other species groups (Pearce *et al* 2005, Dunlap and Stettler 2001). In spring wheat (*Trisetum aestivum*), Baloch (2013) showed that relative water content (an indicator of water stress; Merah (2001)) was lower with larger stomatal size and the yield was reduced in cultivars with large stomata grown under drought stressed conditions. Variation in stomatal size and density has also been studied to a limited extent within other species, but is often confounded with ploidy level variation, which may have other independent effects on drought tolerance. In *Betula papyrifera*, for example, pentaploid (5n) and hexaploids (6n) had greater guard cell length and lower stomatal density, suggesting that diploids were more sensitive to water-deficits and polyploids were more drought tolerant (Li *et. al* 1996). Pallardy and Kozlowski (1979) tested 21 *Populus* clones representing 11 species and hybrids and Ceulemans *et al.* tested 10 *Populus* clones representing 4 species and hybrids, both finding that stomatal characteristics are highly variable among genotypes. Li and Wang (2003) grew three populations of *Eucalyptus microtheca* from different habitats in well-watered, moderately water-stressed, and extremely water-stressed environments. There were significant differences in drought response between the three populations demonstrating that different populations exhibit different survival mechanisms and drought-response characteristics between populations *E. microtheca*. Additionally, differences in drought tolerance has also been recorded in subspecies of *Artemisia tridentata* (spp. *Wyomingensis* and spp. *vaseyana*) indicating that within a species, there are different responses to drought (Kolb and Sperry 1999).

Save *et al* (2000) assessed water relations in *Lotus creticus creticus* and *L. certicus cystoides* and demonstrated clear differences in stomatal density, leaf water potential, and leaf surface water retention between the two subspecies. Previous studies have shown that stomatal density increases with water stress in the studied species (Yang and Wang 2001, Zhang *et. al* 2006). However, the results of a study by Xu and Zhou (2008) with a perennial grass, *Leymus cinerus*, showed that stomatal density increased with increasing water stress but stomatal size decreased with water stress, implying that more and smaller stomata may be beneficial in water-stressed environments. Another study indicates differences in guard cell size and stomatal densities between subspecies of *Phragmites australis*, where the native subspecies has larger guard cells and lower stomatal density (Saltonstall *et al* 2007). The relationships between stomatal characteristics such as stomatal size and density have not been assessed in aspen clones or cytotypes, nor has the relationship between stomatal characteristics and drought tolerance.

In general, smaller stomata are thought to be able to open and close more quickly, allowing maximization of gas exchange during favorable but rapidly changing conditions, and are associated with higher densities (Drake *et al* 2013, Aasamaa *et. al* 2001). Individuals with larger stomata may be capable of efficient photosynthesis (Parkhurst 1994), but may also be at risk of water loss during transpiration in environments with low precipitation and hot temperatures (Baloch 2013). Larger stomata also increased transpiration rates (Baloch 2013). The ability for small stomata to close more rapidly benefits individuals with small, dense stomata during drought conditions (Aasamma *et al* 2001). Additionally, greater stomatal density, correlated with smaller stomatal size, may increase the efficiency of photosynthesis by allowing more surface area for gas exchange (Parkhurst 1994). However, previous studies have demonstrated that the studied polyploid organisms are more likely to be found in and to survive

in arid environments (Smith 1946, Li *et. al* 1996) and diploids are more sensitive to drought conditions (Lewis 1980, Lumaret *et al.* 1987). However, the presence of smaller and denser stomata shown to be associated with diploids (Li *et al*) has been hypothesized to be a beneficial combination for drought tolerance (Baloch 2013, Aasamaa *et al.* 2001, Pearce *et al* 2005, Carpenter and Smith 1975).

The difference in stomatal size and density in aspen ploidy levels has not been assessed. We hypothesized that 1) triploids will have significantly larger stomata with lower density and 2) diploids will have smaller stomata at greater density. If our results support these hypotheses, then these differences may also be indicators of drought susceptibility differences between cytotypes. Further, if stomatal size differences are pronounced between cytotypes, this could be the basis of a field-based diagnostic for ploidy level. A similar field-based technique has been previously demonstrated in *Actinidia delicosa* (Przywara *et al* 1988) and *Acacia mearnsii* (Beck *et al* 2003). Similarly, Sari *et al* (1999) demonstrated in *Citrullus lanatus* that stomatal size measurement is an effective way to distinguish haplotypes from diploids in the field and is not prohibitively labor intensive compared to chromosome counting, and flow cytometry.

METHODS

FIELD METHODS

In the summers of 2013, 2014, and 2015 we collected five leaves from single aspen trees within 44 discrete stands (29 diploid and 15 triploid) in Logan Canyon (northern Utah) and Fish Lake (central Utah) (Figure 1). Sample trees were chosen at intervals greater than 1 km to increase the probability of sampling from a variety of discrete clones. Leaves were collected prior to fall senescence. From each tree, leaves were sampled haphazardly from throughout the canopy. All sampled trees were approximately 2 meters tall. We coated the abaxial surface of the

leaf with clear nail polish (cellulose acetate), creating an imprint of the stomata, which was then carefully peeled from the leave and placed on a microscope slide, as described by Long and Clements (1943) and Payne (1970).

STOMATAL MEASUREMENTS

The stomatal imprints were viewed using a Leica DMLB light microscope and the images captured and measured using a ToupView digital camera and associated software. We measured the guard cell length across the leaf imprint area in random fields of view (Figure 2). Following Beaulieu *et al.* (2008), we measured guard cell length as opposed to guard cell areas to provide a more stable correlate of stomatal size. When stomata are open or closed, the width of the cell can change but the length remains the same. The guard cell lengths were measured in pixels at 400x. Using a calibration slide, the pixel values were converted into micrometers (at 40x, 1187.12px=0.01mm). Five stomatal density measurements were made for each leaf imprint in random fields of view, avoiding obvious veins and obstructions by counting the number of stomata in the field of view (Figure 3). All the stomata in the frame were counted at 10x magnification. To assess the precision of the measurements, the same observer measured all images, and for four samples the observer re-measured the same slide five times over the period of four days to ensure replicability. These replicated measurements of individual leaves were generally consistent (CV<1, Table 1).

CYTOTYPE DETERMINATION

To determine the cytotype, two leaves were collected from one tree in each clone and preserved using silica desiccant. Dried leaves were analyzed using flow cytometry to confirm the

cytotype by determining the amount of DNA in the cells of the leaves (following Mock *et al.* 2012).

STATISTICAL ANALYSES

The statistical tests were computed in R v. 3.2.5. Using the lmerTest package, a nested ANOVA was used to compare the stomatal length and density between triploid and diploid aspen and to account for multiple levels of variation (within trees, within clones and within ploidy) and to determine if there is a significant difference in stomatal length and density between diploid and triploid aspen. Separate ANOVAs were used to assess length and density.

RESULTS

Stomatal length ranged from 15.3-45.2 micrometers in diploids and 19.0-53.1 micrometers in triploids. The density measurements ranged from 14-59 in diploids and 13-34 in triploids (Figure 4). The triploid aspen had larger and fewer stomata than the diploid aspen (Figure 5). Triploid stomata are 16% larger; however, there are 24% more stomata in diploid aspen. The length of triploid aspen (31.820 μm, n=12) averaged 4.481 micrometers longer than the diploid aspen (27.339 μm, n=43) (nested ANOVA, $F= 18.903$, $p < 0.001$). The density of the triploid aspen (23.19, n=12) averaged 5.550 stomata fewer than the diploid aspen (28.74, n=43) (nested ANOVA, $F=9,535$, $p<0.001$) (Table 2).

The stomatal length varied by clone, as shown in previous studies assessing stomatal characteristics in poplars (Pallardy and Kozlowski 1979, Ceuleman *et al.* 1984). However, the clonal effects did not negate the significance of the length difference between the ploidies.

DISCUSSION

The differences in stomatal size and density are important features to determine how a plant responds to drought. Triploid aspen have significantly larger stomata than diploid aspen, indicating that they may be more efficient photosynthesizers due to the greater surface area for gas exchange (Parkhurst 1994); alternatively, greater surface area increases the transpiration rate and may result in higher drought susceptibility (Baloch 2013). However, similar conclusions can be made regarding the stomatal density of the two aspen ploidies. Diploids have a greater stomatal density than triploid aspen, again providing a greater surface area for both gas exchange and photosynthesis (Parkhurst 1994). More research needs to be done to assess the differences in water loss and gas exchange between stomatal length and stomatal density in aspen.

During the field collection, more diploid stands were analyzed through the haphazard sampling method suggesting that diploids are more common on the landscape. This may be evidence that diploids are more adaptable to this environment, perhaps due differences in drought response and stomatal function compared to triploid aspen.

More and smaller stomata may be beneficial in water-stressed environments (Xu and Zhou 2008). Another plausible explanation is simply that triploids are a relatively new evolutionary phenomena and have not yet established to the extent of diploids. Mock *et al* (2012) determined that the highest proportion of triploidy occurs west of the continental divide, south of the last glacial maximum and is particularly high in southern Utah and western Colorado. The majority of the ramets sampled in this study occurred west of the continental divide in northern Utah. Both clones were found on similar sites with similar environmental stressors, however diploids are more common across the landscape. Although research on other *Claytonia perfoliata* demonstrated that diploid and tetraploids occupy a separate niche, though the cytotypes

responded similarly to environmental variation (McIntyre 2012). Such niche differentiation has not been observed in aspen, though triploidy is more common in the southwestern portion of the range (Mock *et al* 2012).

The difference in drought tolerance between the triploid and diploid aspen is particularly important in terms of forest management and restoration. Landhausser *et al* (2009) predicts that the as the current environment will continue to get warmer and drier, and current management practices continue to expose mineral soil substrates, the range of aspen will move to higher elevations where it is cooler and moister. We are currently undergoing a period deemed Sudden Aspen Decline (SAD), which has been attributed to drought conditions and a changing climate (Allen *et al* 2010). However, response of ploidies to the changing conditions may be related to the massive die-offs, and have not yet been assessed. If there is one ploidy susceptible to climate-change type drought, then determining the scope of the ploidy and the restoration potential may help reduce the range of SAD and aid in the restoration of aspen forests. Triploid clones are significantly larger than diploid clones (Mock *et al* 2012), and may be pivotal in the understanding of these die-offs, particularly if the cytotype is more susceptible to drought stress. However, Li *et al* 1996, determined that in *Betula papyrifera,* tetraploids and hexaploids are more commonly found in xeric environments. Triploid aspen are found in warmer environments in the southwestern portion of the range (Mock *et al* 2012) and may be adapted to the warmer and drier conditions. Although, these areas are expected to get warmer and drier, which may surpass a threshold in the effectiveness of stomatal response.

As the climate warms and changes, the ploidy response to drought should be monitored. We might expect diploid aspen stands to be dominant across the landscape due to their current dominance and the prevailing thought that smaller and denser stomata are more beneficial in arid

environments (Xu and Zhou 2008, Drake *et al* 2003, Aasaama *et al* 2001, Baloch 2013, Spence *et. al* 1986). To restore and manage the current aspen forests, managers should consider using the more drought tolerant ploidy for seeding and plantings; this may increase the effectiveness of aspen restoration projects. Current aspen management and restoration practices include but is not limited to and depending on site specificities: clearcutting, burning, selective cuts, and exclosures (O'Brian *et al* 2010, Shirley and Erickson 2001)

However, determining the stomatal differences would be a time consuming and possibly inaccurate test in the field to determine ploidy due to the small differences in stomatal size and density. There is a wide range of both length (15.37- 53.12 μm) and density (13 - 59 stomata) between both ploidies, and a strong correlation to clone. However, other studies, not taking into account field identification of ploidy, support stomatal differences as a valid test to determine the cytotype (Przywara *et al* 1988, Sari *et al* 1999, Beck *et al* 2003). More testing is needed with field microscopes to determine the true effectiveness of this test outside of a laboratory setting.

In order to expand this research, we must begin correlating the stomatal size and density to actual stomatal conductance and water loss/retainment in aspen. We must understand the relationship to the physiological mechanisms to fully understand the scope of the stomatal size and density differences.

TABLES AND FIGURES

Table 1: The results from replicated leaf samples for (a) length and (b) density. The same stomata was measured for each sample and the density was determined for the same section of leaf over a period of four days. The coefficient of variance (CV) indicates little variation between the measurements.

(a) Stomatal Length

	1	2	3	4	5
9/2/2015	33.604	27.438	24.486	35.736	27.161
9/3/2015	34.150	27.353	24.946	35.554	26.208
9/4/2015	32.974	27.494	24.312	36.583	26.218
9/8/2015	33.153	27.488	24.232	36.575	25.971
CV	**0.206**	**0.00317**	**0.0764**	**0.222**	**0.208**

(b) Stomatal Density

	1	2	3	4	5
9/2/2015	23	25	26	24	30
9/3/2015	24	25	25	23	28
9/4/2015	23	26	24	25	30
9/8/2015	25	24	26	23	29
CV	**0.6875**	**0.5**	**0.6875**	**0.6875**	**0.6875**

Table 2: Summary statistics of (a) stomatal length and (b) stomatal density.

(a) Stomatal Length

Ploidy	Mean	SE	Lower CI	Upper CI	p-value
Diploid	27.339	0.604	26.1	28.6	<0.0001
Triploid	31.886	0.840	30.2	33.6	<0.0001

(b) Stomatal Density

Ploidy	Mean	SE	Lower CI	Upper CI	p-value
Diploid	28.74	1.06	26.6	30.9	<0.0001
Triploid	23.19	1.45	20.3	26.1	<0.0001

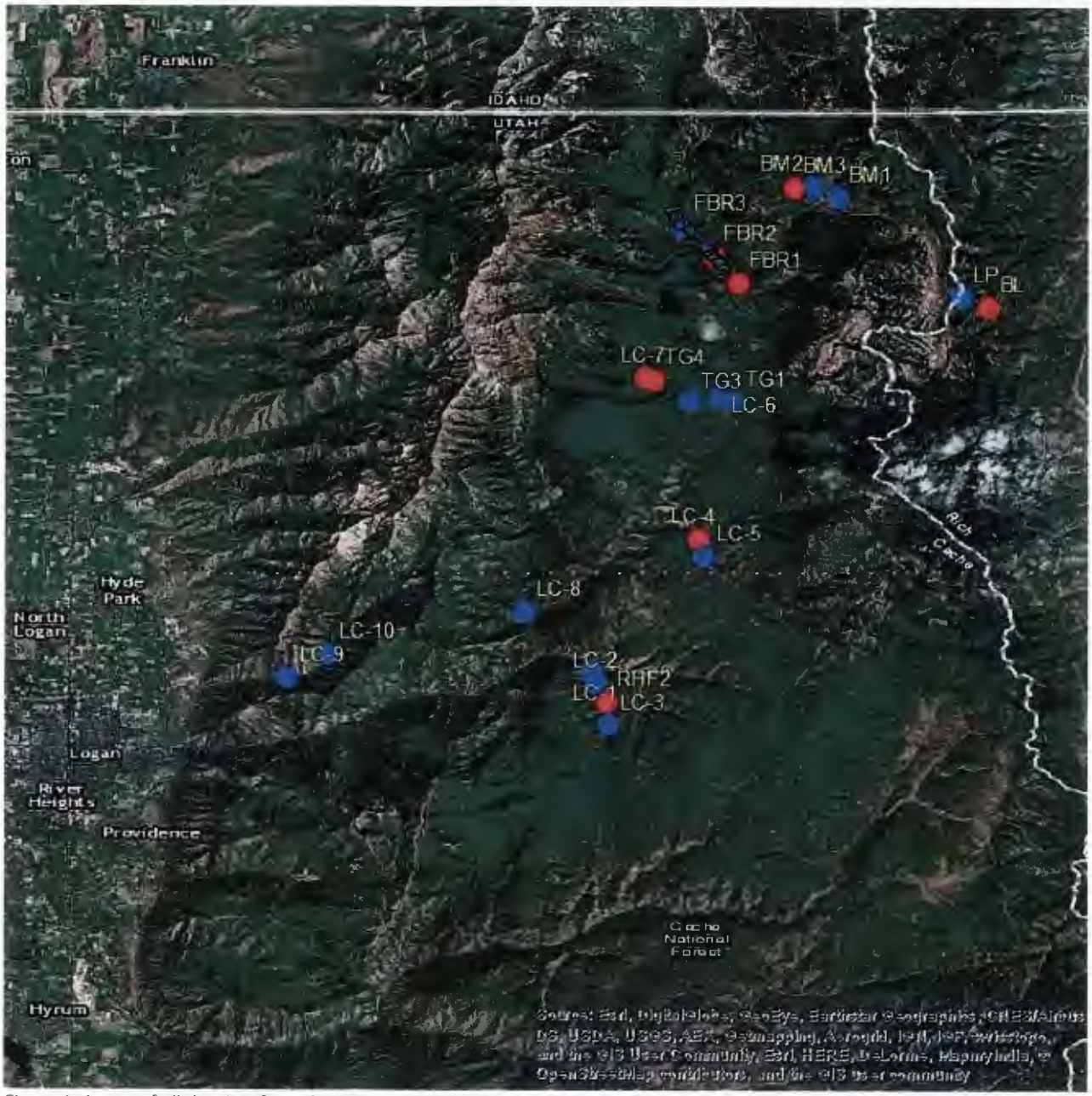


Figure 1: A map of all the sites from the 2014 and 2015 collections in Logan Canyon. Blue circles represent diploid clones. Red circles represent triploid clones.

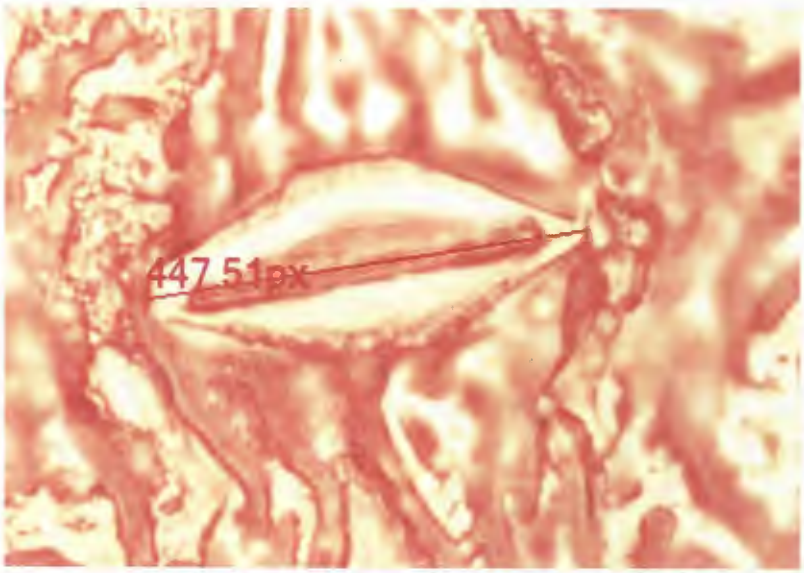


Figure 2: An example of a stomatal length measurement in pixels which was later converted into micrometers. The image was taken with a ToupView microscope camera at 400x magnification.

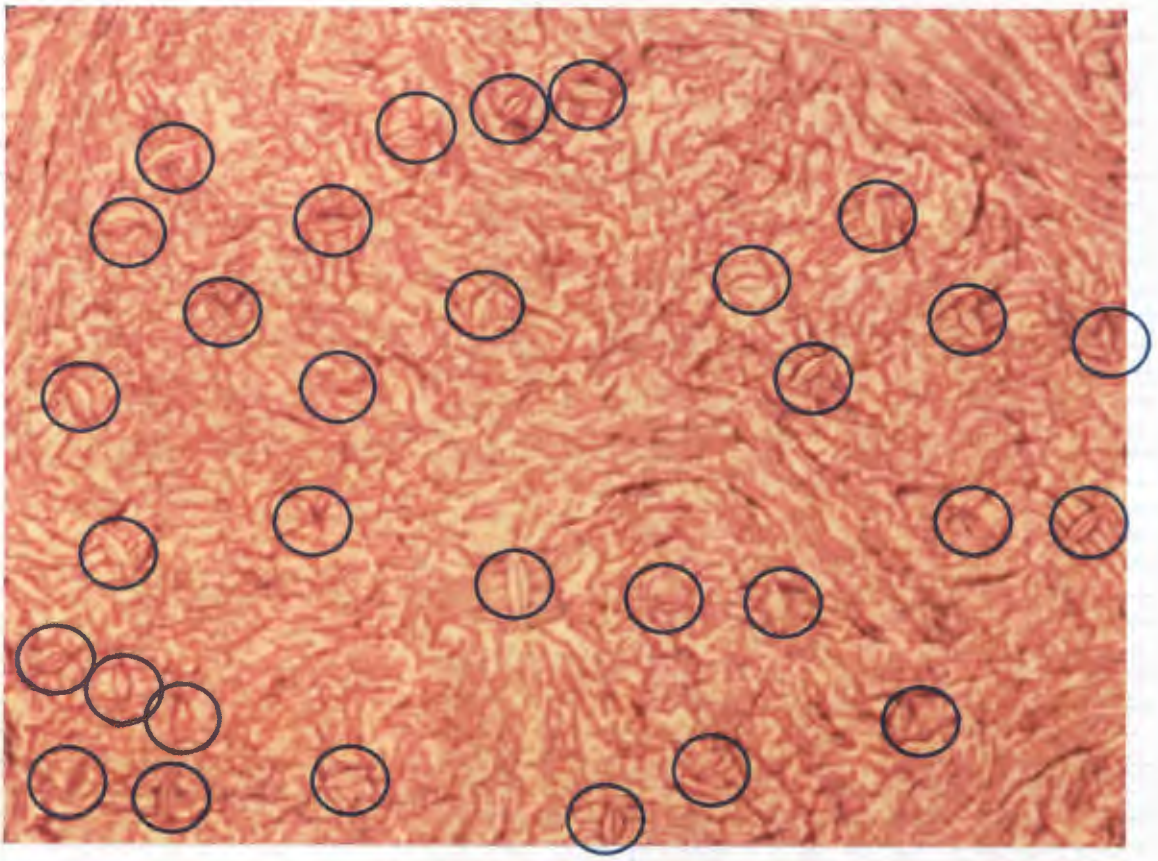
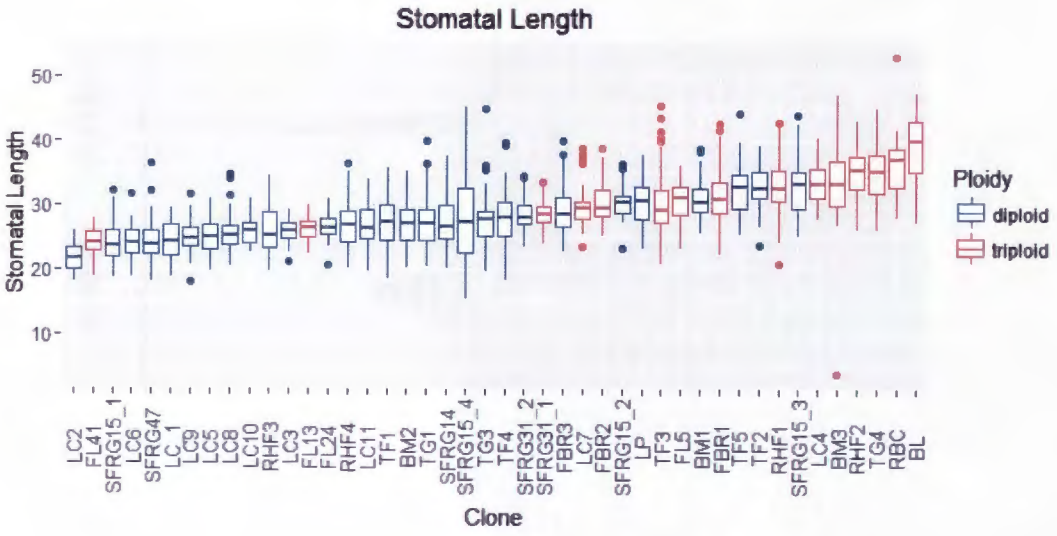


Figure 3: An example of a stomatal density measurement from a picture taken with a ToupView Camera at 10x magnification.

(a)



(b)

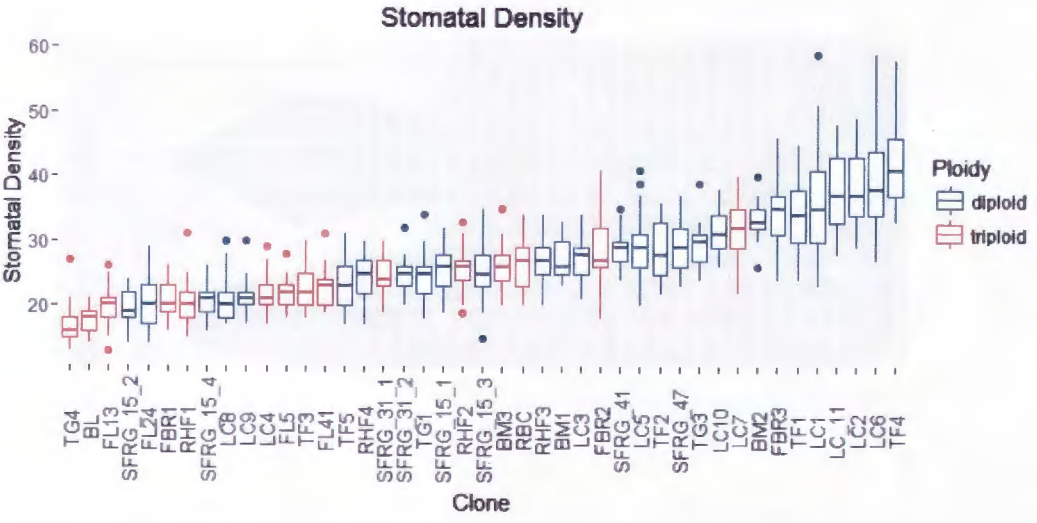


Figure 4: The range and average stomatal length (a) and density (b) for each sample. Blue represents diploid samples and red represents triploid samples. There is a broad range of stomatal density that may be distinct within clone and between cytotype.

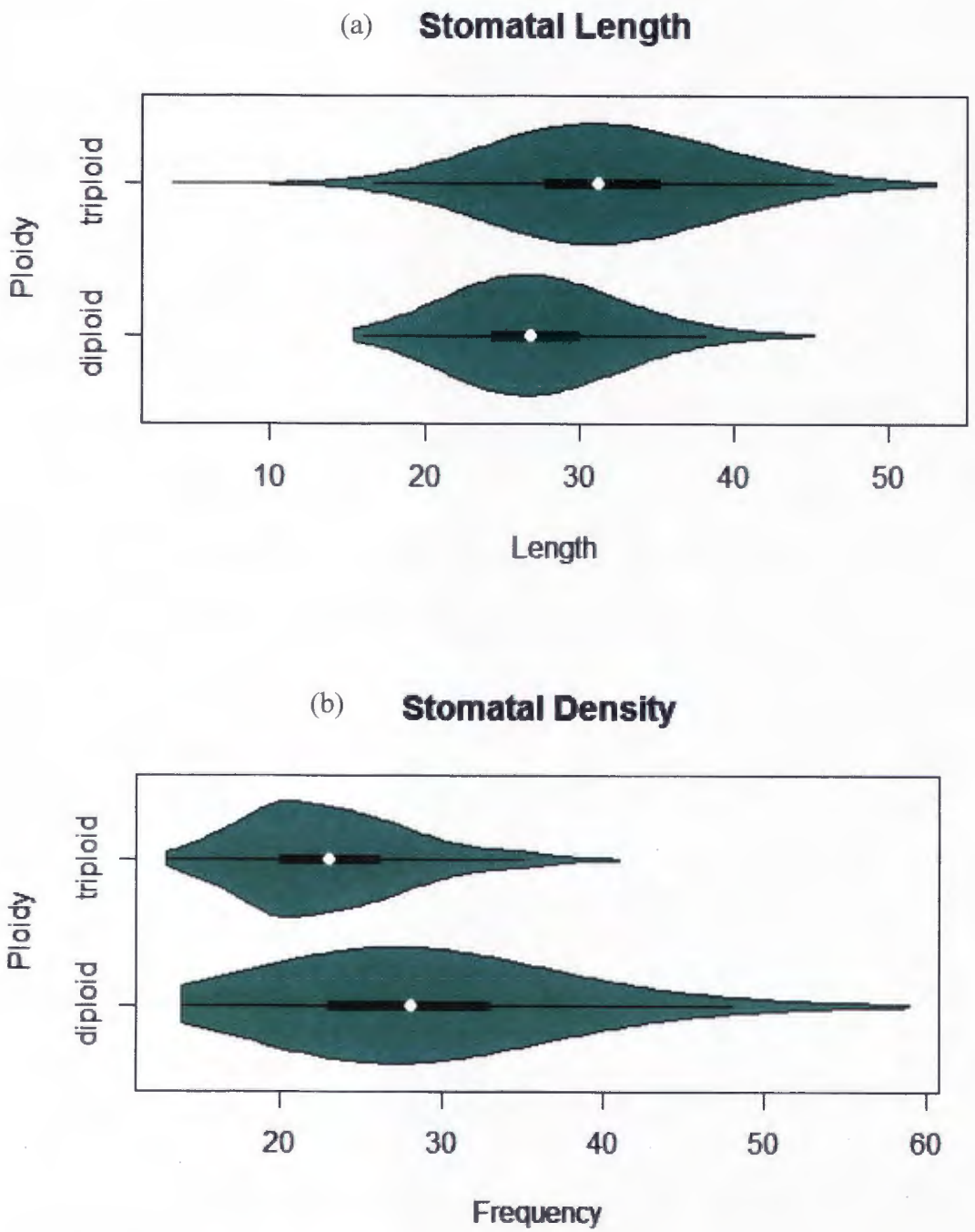


Figure 5: (a) Stomatal length in micrometers. The mean of triploids is larger than the mean of the diploids. (b) Stomatal frequency, the mean number of stomata per field of view at 10x is larger in diploids than in triploids. There is a considerable spread for both measurements (length and frequency), although the means are significantly different for both length ($p<0.0001$) and density ($p<0.001$).

REFLECTION

When I entered USU as an Undergraduate Research Fellow I didn't know how to do research or that undergraduates could do more than just go to class. I didn't know what a thesis entailed but I knew it was daunting. I got some experience lab hopping my freshman year and started on this project in the fall of my sophomore year. I responded to a posting by Dr. Mock asking for someone with an interest in genetics and aspen to help with a new idea she had, a new idea she didn't even know would work.

The first few weeks of the project were spent in the Natural Resources parking lot spraying aspen leaves with Shellac and wood finish and painting them with nail polish to determine which substance produced the best "peel" of the underside of the leaf. I received many strange looks from the proprietors of the parking lot with a couple complaints about the fumes being blown by the wind. Once this was established we collected leaves from the field and I walked around the woods with wet, nail polish soaked aspen leaves for hours. This project was the brainchild of my mentor, but she is a geneticist and I was an undergrad, neither of us were well versed in the next steps. We would determine the ploidy of the leaves using flow cytometry, that part was simple. Then I had to figure out how to peel off the nail polish, make microscope slides that are preserved and don't degrade the leaf peel, and somehow figure out how to measure the stomatal size and density under the microscope when my microscope use was limited to the lab in BIOL 1620.

It was at this point when I finally realized, that unlike my previous high school science classes, where the labs are structured and the lab results are pre-determined, actual research is uncertain and there is no one who knows what the results should be. Despite the social hierarchy of professor to student, research is the great equalizer.

Over the next couple years I spent hours in the lab measuring and counting stomata, and in the summer, going out to collect some more. I reveled in the independence of the project and the idea

that the work I am doing, the work that may seem tedious, will make an impact on the field and further research.

Once the leaves were collected in the field, I thought the difficult part was over however, the process of measuring and counting the stomata took months. I would sit in the lab during breaks between classes and on the weekends, starting at the images so much so that I began dreaming about stomata. My estimate is that I counted about 7,000 stomata for the density measurements.

As the project progressed and I began analyzing the data I learned how unprepared I was in regards to statistical analyses. I had limited knowledge of R and regretted not paying attention in my Friday afternoon Stats class freshman year. I spent countless frustrating hours reading R forums online and trying different lines of code until I could produce an adequate graph.

When I began the project as a sophomore I knew very little about how to design an experiment. As I wrote this thesis, I constantly thought back to those early days and what I would have done differently. 1) I would have started with a randomized ANOVA design instead of just driving down the road and picking leaves off of the trees and 2) I would have collected additional data about the soil and light conditions and stomatal conductance. However, I appreciate my mentor giving me the reins and essentially allowing me to design my own experiment. Learning from my mistakes as an experimental designer has made me a better scientist and better at critiquing other study designs, something I don't know I would have learned if the project was handed to me pre-designed. This project reinforced my convictions that I am in the right field. As I was writing the literature review, I was constantly distracted by other papers that popped up during my literature search. I fueled my passion for my field and got a better idea about what I would want to study late down the road in graduate school.

The most rewarding aspect of this project has been the numerous opportunities I have had to present my findings. This project has taken me to conferences in Kentucky, Washington, California,

Texas, and across the state of Utah with audiences ranging from undergraduate students to professionals in the field. Entering college, I was very timid and terrified of public speaking but throughout the duration of this project I have learned that when I am passionate and knowledgeable about a subject (i.e. aspen), I can easily speak on it with little to no nerves.

To students who are just beginning this process, my biggest piece of advice is do NOT procrastinate and give our mentor ample time to edit each draft. However, on the other side, accept the fact that you will procrastinate. This time last year I told myself I would write my thesis in the summer before the start of my last year. I had the data, I had the drive and it didn't seem that daunting. Alas, that did not happen. Nor did it happen within the first semester. In fact the first draft of the introduction wasn't complete until February and the first draft the entire paper was not completed until the middle of April. But I was anticipating the procrastination and with a dedicated mentor and lots of hard work and just a couple panic attacks, the thesis is complete. It is the longest cohesive piece of writing I have ever written and represents a culmination of data and analyses I have been working on for almost three years. It is a daunting and terrifying task, but in the end, at the very end, I think it was worth it.

I would like to leave you with a poem:

Terrifying yet

Hopeful of publication, an

Enigmatic

Synthesis of my undergrad

Involvement in

Scientific research.

LITERATURE CITED

Aasamaa, K., A. Sõber, and M. Rahi. 2001. Leaf anatomical characteristics associated with shoot hydraulic conductance, stomatal conductance and stomatal sensitivity to changes of leaf water status in temperate deciduous trees. Australian Journal of Plant Physiology. 28 (8) 765-774.

Allen, C.D., A.K. Macalady, H. Chenchoini, D. Bachelet, N. McDowell, M. Vennetier, T. Kitzberger, A. Rigling, D.D.Breshears, E.H. (Ted) Hogg, P. Gonzalez, R. Fensham, Z, Zhang, J. Castro, N. Demidova, J. Lim, G. Allard, S.W. Running, A.Semerci, and N. Cobb. 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. Forest Ecology Management. 259:660–684

Anderegg W., L. Anderegg, C. Sherman, and D. Karp. 2012. Effects of widespread drought-induced aspen mortality on understory plants. The Journal of the Society of Conservation Biology. 26(6): 1082-1090.

Arango-Velez, A. J. Zwiazek, B. Thomas, and M. Tyree. 2011. Stomatal factors and vulnerability of stem xylem to cavitation in poplars. Physiologia Plantarum. 143: 154-165.

Baloch, M.J., J. Dunwell, N.U. Khan, W.A. Jatoi, A.A. Khakhwani, N.F. Vessar, and S. Gul. 2013. Morpho-physiological Characterization of Spring Wheat Genotypes under Drought Stress. International Journal of Agriculture and Biology. 15: 945-950.

Barnes, B. 1966. The clonal growth habit of American Aspens. Ecology. 47(3):439-447.

Bartos, D. L., and R.B. Campbell Jr. 1998. Decline of Quaking Aspen in the Interior West--Examples from Utah. Rangelands. 20(1): 17-24.

Bartos, D.L. 2001. Landscape dynamics of aspen and conifer forests. Pages 5-13 in Shepperd, W.D., D. Binkley, D.L.Bartos, T.J.Stohlgren, and L.G. Eskew, editors. Sustaining Aspen in Western Landscapes. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station Proceedings RMRS-P-18, Fort Collins, CO.

Beaulieu, J.M., I.J. Leitch, S. Patel, A. Pendharkar, and C.A. Knight. 2008. Genome size is a strong predictor of cell size and stomatal density in angiosperms. New Phytologist. 170:975-986.

Beck, S.L., R.W. Dunlop and A. Fossey. 2003. Stomatal length and frequency as a measure of ploidy level in black wattle, *Acacia mearnsii* (de Wild). Botanical Journal of the Linnean Society. 141:177-181.

Carpenter, S.B. and N.D. Smith.1975. Stomatal distribution and size in southern Appalachian hardwoods. Canadian Journal of Botany. 53: 1153-1 156

Ceulemans, R., I. Impens and V. Steenackers. 1984. Stomatal and anatomical leaf characteristics of 10 Populus clones. Canadian. Journal of Botany. 62:513–518.

DeRose, R.J, K.E. Mock and J.N. Long. 2015. Cytotype differences in radial increment provide novel insight into aspen reproductive ecology and stand dynamics. Canadian Journal of Forest Research. 45: 1-8

Drake, P.L., R.H. Froend, and P.J. Franks. 2013. Smaller, faster stomata:scaping of stomatal size, rate of response, and stomatal conductance. Journal of Experimental Botany. 64(2):495-505.

Dunlap, J.M. and R.F. Stettler. 2001. Variation in leaf epidermal and stomatal traits of Populus trichocarpa from two transects across the Washington Cascades. Canadian Journal of Botany. 79:528–536

Hanna, P., and D. Kulakowski. 2012. The influences of climate on aspen dieback. Forest Ecology Management. 274: 91-98.

Harlan, J.R. and J.M.J. deWet. 1975. On O. Winge and a Prayer: The Origins of Polyploidy. Pages 361-390 in Cronquist, A, editor. The Botanical Review. The New York Botanical Garden, Bronx, New York.

Hetherington, A.M. and F.I. Woodward. 2003. The role of stomata in sensing and driving environmental change. Nature. 242: 901-908.

J. G. Hodgson, M. Sharafi, A. Jalili, S. Díaz, G. Montserrat-Martí, C. Palmer, B. Cerabolini, S. Pierce, B. Hamzehee, Y. Asri, Z. Jamzad, P. Wilson, J. A. Raven, S. R. Band, S. Basconcelo, A. Bogard, G. Carter, M. Charles, P. Castro-Díez, J. H. C. Cornelissen, G. Funes, G. Jones, M. Khoshnevis, N. Pérez-Harguindeguy, M. C. Pérez-Rontomé, F. A. Shirvany, F. Vendramini, S. Yazdani, R. Abbas-Azimi, S. Boustani, M. Dehghan, J. Guerrero-Campo, A. Hynd, E. Kowsary, F. Kazemi-Saeed, B. Siavash, P. Villar-Salvador, R. Craigie, A. Naqinezhad, A. Romo-Díez, L. de Torres Espuny, and E. Simmons. 2010. Stomatal vs. genome size in angiosperms: the somatic tail wagging the genomic dog? Ann Bot. 105 (4): 573-584.

Jones J.R and N.V. DeByle. 1985. Morphology. Pages 11-18 in DeByle, N.V. and R.P. Winokur, editors. Aspen: Ecology and Management in the Western United States. General Technical Report RM-119, US Department of Agriculture, US Forest Service, Rocky Mountain Forage and Range Experiment Station, Fort Collins, Colorado

Kay, C.E. 1997. Is aspen doomed? Journal of Forestry. 95: 4-11.

Kemperman, J.A. and B.V. Barnes.1976. Clone size in American aspens. Canadian Journal of Botany. 54:2603–2607.

Kolb, K.J. and J.S. Sperry. 1999. Differences in drought adaptation between subspecies of sagebrush (*Artemisia tridentata*). Ecology. 80(7):2373-2384.

Kudo, N. and Y. Kimura. 2002. Nuclear DNA endoduplication during petal development in cabbage: relationship between ploidy levels and cell size. Journal of Experimental Botany 53: 1017–1023.

Levin, D.A. 1983. Polyploidy and the Novelty in Flowering Plants. The American Naturalist. 122(1):1-25.

Lewis, W.H. 1980. Polyploidy in species populations. Pages 104-144 in W. H. Lewis, editor. Polyploidy, biological relevance. Plenum Press, New York, NY

Li, C. and K. Wang. 2003. Differences in drought response of three contrasting *Eucalyptus microtheca* F. Muell. Populations. Forest Ecology and Management. 179(1-3):377-385.

Li, W. L., G.P. Berlyn, and P.M.S.Ashton. 1996. Polyploids and their Structural and Physiological Characteristics Relative to Water Deficit in Betula papyrifera (Betulaceae). American Journal of Botany. 83(1): 15-20.

Little E.L. Jr. 1971. Checklist of United States Trees (Native and Naturalized). US Department of Agriculture, Agriculture Handbook 541. Washington D.C

Long, F.L., and F.E. Clements. 1934. The method of collodion films for stomata. American Journal of Botany. 21:7–17.

Long, F.L., and F.E. Clements. 1934. The method of collodion films for stomata. American Journal of Botany. 21:7–17.

Lumaret, R. 1988. Adaptive strategies and ploidy levels. Acta Oeco- logial/ecologia Plantarum 9: 83-93.

Merah, O., 2001. Potential importance of water status traits for durum wheat improvement under Mediterranean conditions. J. Agric. Sci., 137: 139–145

Mock, K.E., C.M. Callahan, M.N. Islam-Faridi, J.D. Shaw, H.S. Rai, S.C. Sanderson, C.A. Rowe, R.J, Ryle, M.D. Madritch, R.S. Gardner, and P.G. Wolf. 2012. Widespread Triploidy in Western North American Aspen (*Populus tremuloides*). PLOS One. 7(10):1-10.

Mowforth M.A. and J.P. Grime. 1989. Intra-population variation in nuclear DNA amount, cell size, and growth rate in Poa annua L. Functional Ecology. 3: 289–295.

Pallardy, S.G. and T.T. Kozlowski. 1979. Frequency and length of stomata of 21 Populus clones. Canadian Journal of Botany. 57:2519–2523.

Parkhurst, D.F. 1994. Diffusion of CO2 and other gases inside leaves. New Phytologist 126, 449–479.

Payne, W.W. 1970. Helicocytic and allelocytic stomata: Unrecognized patterns in the Dicotyledonae. American Journal of Botany. 57:140–147.

Pearce, D.W., S. Millard, D.F. Bray, and S.B. Rood. 2005. Stomatal characteristics of riparian poplar species in a semi-arid environment. Tree Physiology. 26:211-218.

Peet, R. K. 2000. Forests and meadows of the Rocky Mountains. Pages 75–122 in M. G. Barbour and W. D. Billings, editors. North American terrestrial vegetation. Cambridge University Press, New York, New York, USA

Perala. D.A. 1990. Quaking Aspen. Pages 555-566 in Burns, R.M. and B.H. Honkala, Technical Coordinators. Silvics of North America. Volume 2, Hardwoods. Agriculture Handbook 654. Forest Service, United State Department of Agriculture, Washington D.C.

Przywara, L., K. Pandey, and P. Sanders.1988. Length of stomata as an indicator of ploidy level in *Actinidia deliciosa.* New Zealand Journal of Botany. 26:179-182.

Rehfeldt, G. D. Ferguson, and N. Crookston. 2009. Aspen, climate, and sudden decline in western USA. Forest Ecology and Management. 258 (11): 2353-2364.

Saltonstall, K., K. Glennon, A. Burnett, R.B. Hunter, and K.L. Hunter. 2007. Rhodora. 109(940):415-429.

Sari, N., K. Abak, and M. Pitrat. 1999. Comparison of ploidy level screening methods in watermelon: *Citrullus lanatus* (Thunb.) Matsum. And Nakai. Scientia Horticulturae. 82(3-4):265-277.

Save, R. C. Biel, and F. de Herralde. 2000. Leaf Pubescence, Water Relations and Chlorophyll Fluorescence in Two Subspecies of *Lotus creticus* L. 2000. Biologia Plantarum. 43(2):239-244.

Schier, G.A. 1973. Origin and development of aspen root suckers. Canadian Journal of Forest Research. 3:45–53.

Seager, R., M. Ting, I. Held, Y. Kushnir, J. Lu, G. Vecchi, H. Huang, N. Harnik, A. Leetmaa, N. Lau, C. Li, J. Velez, and N. Naik. 2007. Model projections of an imminent transition to a more arid climate in southwestern North America. Science 316:1181–1184.

Shepperd W.D, P.C. Rogers, D. Burton, and D.L. Bartos. 2006. Ecology, biodiversity, management, and restoration of aspen in the Sierra Nevada. General technical report RMRS-GTR-178, USDA Forest Service, Rocky Mountain Research

Smith, H.E. 1946. *Sedum pulchellim:* a physiological and morphological comparison of diploid, tetraploid, and hexaploid races. Bulletin of the Torrey Botanical Club. 73: 495-541.

Worrall, J. L. Egeland. T. Eager. R. Mask. E. Johnson. P. Kemp, and W. Shepperd. 2008. Rapid mortality of Populus tremuloides in southwestern Colorado, USA. Forest Ecology and Management. 255(3-4):686-696.

Worrall, J. S. Marchetti, L. Egeland. R. Mask, T. Eager, and B. Howell. 2010. Effects and etiology of sudden aspen decline in southwestern Colorado, USA. Forest Ecology and Management. 260(5):638-648.

Xu, Z. and G. Zhou. 2008. Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. Journal of Experimental Botany. 59 (12): 3317-3325.

Yang, H.M. and G.X. Wang. 2001. Leaf stomatal densities and distribution in Triticum aestivum under drought and CO2 enrichment. Acta Phytoecologica Sinica. 25: 312–316.

Zhang, Y.P, Z.M. Wang, Y.C. Wu, and X. Zhang. 2006. Stomatal characteristics of different green organs in wheat under different irrigation regimes. Acta Agronomica Sinica. 32: 70–75.

AUTHOR BIO

Brianne Palmer graduated Magum Sum Laude with Honors in the spring of 2016 with a Bachelor's of Science in Conservation and Restoration Ecology with an emphasis in vegetation management and with minors in both biology and English. She received the Jardine Juniper Award, Natural Resources Scholar of the Year, and the Department of Wildland Resources Outstanding Senior as a result of her accomplishments in the College of Natural Resources. Over the course of her undergraduate career she was a recipient of numerous scholarships including: the Undergraduate Research Fellowship, the Dean's Scholarship, the Seely-Hinckley scholarship, and the Class of 1950 scholarship. Additionally she has received many grants to support her research and travel such as: the Undergraduate Research and Creative Opportunity Grant, the S.J. Quinney Lawson Travel Grant, the Utah State Student Association Travel Grant, the Quinney College of Natural Resources Undergraduate Research Grant, the Honors Travel Grant, and the Honors Research Grant. She presented her research at both student and professional conferences across the country. She has also received fellowships through the iUTAH program in Salt Lake City, Utah and the DAAD Rise program in Cologne, Germany, worked as GIS Technician, USDA Biological Technician, and writing tutor. After graduation, Brianne plan on working as a research technician and applying to graduate schools to start in the fall of 2017.