PLASTICITY, GENETIC DIVERSITY, NATURAL SELECTION, AND PHYSIOLOGY OF POLYPLOID *SOLIDAGO ALTISSIMA* (ASTERACEAE) UNDER SIMULATED CLIMATE CHANGE

A THESIS SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF THE UNIVERSITY OF MINNESOTA
BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE

Julie R. Etterson, Adviser

August 2013

Acknowledgements

I would like to thank my adviser, Julie Etterson, for assistance with the design of this project, and advice on project execution and data analysis. I would also like to acknowledge my committee members, Ron Regal for invaluable assistance with data analysis, and Paul Bates for suggestions and additional considerations on project design. Frank Maragi provided assistance and advice for building elevated temperature chambers, Tali Lee provided equipment and technical support for physiological measurements, and Brad Dewey ran samples and advised on procedure for leaf nutrient analysis.

I would like to thank the University of Minnesota Duluth Biology Department for funding through a Teaching Assistantship and partial funding for a Research Assistantship. I would also like to acknowledge the National Science Foundation for two years of support through a GK-12 Fellowship that allowed me to bring aspects of this research into local high school classrooms (DGE-0637027 awarded to Carmen Latterell (PI), Penelope Morton (Co-PI), John Pastor (Co-PI), Bruce Munson (Co-PI) and Cindy Hale (Co-PI)). Funding for this project was provided in part by a National Science Foundation grant awarded to Julie Etterson (DEB-0641285), and the University of Minnesota Duluth Biology Department.

I am also indebted to Jessica Chatterton, Chantal Liepold, Al Pehl, Jessalyn Toldo, Rachel Toczydlowski, Brad Waldorf, Jolene Prochazka, Jared Retka and all other undergraduates, graduate students, and lab technicians who assisted with field work.

Table of Contents

List of Tables

TABLE

List of Figures

FIGURE

CHAPTER 1

PLASTICITY, GENETIC DIVERSITY, AND NATURAL SELECTION: A NATIVE POLYPLOID UNDER SIMULATED CLIMATE CHANGE

SUMMARY

Although plant response to climate change has been observed in many systems, it is unknown whether populations or subpopulations, such as different ploidy levels, within species respond similarly. Ploidy levels may differ in their ability to adapt immediately to changed conditions through phenotypic plasticity. In the longer term, polyploids may evolve faster in response to natural selection than diploids if they harbor greater genetic diversity. The goals of this research were to compare plasticity, genetic diversity, and patterns of selection between diploid and tetraploid genotypes of a native polyploid, *Solidago altissima* grown under conditions simulating climate change (+1.9 ° C, -13% soil moisture). Physiological, morphological, life history traits, and fitness correlates were measured throughout the growing season. Differences in phenology, morphology and physiology suggest that diploids are better adapted to drought than tetraploids. Both ploidy levels had stronger plastic responses to water availability than temperature. For traits that differed between ploidy levels in plasticity under drought, diploids grew taller and developed more rhizomes and thicker leaves, whereas tetraploids flowered earlier, grew taller, and increased stomatal density on the lower leaf surface. Significant selection and heritability were detected for earlier flowering in both ploidy levels. More traits were targets of selection in a hot, dry environment for tetraploids than diploids, whereas more traits were targets of selection in a wet, ambient temperature environment for diploids. Tetraploids may be at a disadvantage in their long-term ability to respond to climate change through evolution due to a lack of heritability.

INTRODUCTION

Anthropogenic climate change has accelerated over the past century, resulting in negative ecological, economic, and social impacts (Field et al. 2007). Models predict that within this century temperature will continue to increase significantly beyond normal fluctuations, likely coupled with changes in precipitation (Ruosteenoja et al., 2003; Galatowitch et al., 2009). A species' ability to respond to climate change by migration may be restricted by anthropogenically fragmented landscapes (Davis and Shaw, 2001), the compound effects of increased temperature and decreased precipitation (Parmesan, 2006), and migration rates insufficient to keep pace with projected warming (McLachlan et al., 2005; Nielson et al., 2005). Thus plant populations that are locally adapted to their current climate may need to respond to climate change in situ (Davis et al., 2005). Responses to increased temperature, including earlier flowering, have already been observed in numerous systems (Menzel et al., 2006; Miller-Rushing and Primack, 2008; Parmesan and Yohe, 2003; Parmesan, 2006; Sparks et al., 2000). However, it is often unclear whether these responses are due to phenotypic plasticity or if adaptive evolution has occurred in response to altered temperature and precipitation. In addition, responses may differ between populations or subpopulations, including diploid and polyploid plants.

Polyploidy is a macromutation resulting in duplication of chromosome number (Leitch and Leitch, 2008). Up to 80% of flowering plants are estimated to be polyploids or have polyploidy in their evolutionary history (Soltis and Soltis, 2000). In many systems, polyploids inhabit different environments than their diploid progenitors (Smith,

1946; Rothera and Davy, 1986; Lumaret et al., 1987; Felber-Gerard et al., 1996; Li et al., 1996, Parisod et al., 2010). Increased chromosome number alone does not confer any specific advantage to polyploids over diploids in certain environments or locations. Success of polyploids is only indirectly related to the increase in chromosome number and depends on novel and adaptive genetic combinations and expression of genes (Stebbins, 1985). Such favorable genetic combinations or expression of genes may afford polyploids an advantage within a population, allow polyploids to colonize different habitats than their diploid progenitors (Thompson and Lumaret, 1992), and potentially benefit polyploids in their effort to respond to the warmer, drier conditions predicted of climate change.

The ability of polyploids to inhabit different environments than diploids suggests that they may possess an enhanced capacity to mount plastic responses (Comai, 2005). Phenotypic plasticity is the ability of plants of the same genotype to produce alternate phenotypes in different environments (Sultan, 2000). If fitness is maintained across contrasting conditions, plasticity is considered adaptive and may allow plants to survive rapid changes to their environment in the short-term (Dudley, 1996; Sultan, 2000). For example, smaller, thicker leaves, increased stomatal density associated with decreased stomatal size, accelerated phenologies, and decreased allocation to above-ground biomass are plant responses associated with escape, avoidance, or tolerance of stress owing to drought or elevated temperature (Dudley, 1996; Reich et al., 1999; Chaves et al., 2002; Xu and Zhou, 2008). Such responses are adaptive if they are correlated with higher fitness under warmer, drier conditions. By growing replicates of diploid and polyploid

genotypes in contrasting water-availability and temperature, we can assess differences between cytotypes in phenotypic plasticity. Analysis of the correlation between fitness and a plastic response in a given environment then indicates whether that response is adaptive.

If plants are unable to respond to climate change through adaptive plasticity, or plastic responses are insufficient to maintain fitness, genetic change may be necessary for survival of plant populations (Etterson and Shaw, 2001). For adaptive evolution to occur, there must be sufficient genetic variation for traits that are targets of selection in a warmer, drier environment. Heritability, or the proportion of phenotypic variation attributable to genotype variation, may differ in contrasting environments (Conner and Hartl, 2004 Pp. 112-114). Stressful conditions may reduce heritability, and thus evolutionary potential, if variation due to the environment overwhelms genetic variation (Hoffman and Merilä, 1999). In addition, there may be little or no heritability for traits that have previously undergone evolutionary change leading to local adaptation to conditions more similar to current climate. In this case, a genetically fixed trait that was important to fitness in a cooler, wetter climate may persist within a population even if it becomes deleterious under changed environmental conditions (Kawecki and Ebert, 2004). Polyploids possess more genetic material than diploids and may harbor greater heritability for natural selection to work with to affect evolutionary change. If this is true, polyploids may have the potential to evolve faster or in novel ways to changing environmental conditions compared to diploids.

Phenotypic selection analysis can be used to analyze differences in patterns of selection between cytotypes. By growing plants under conditions simulating projected climate change, measuring fitness correlates and traits likely to be important in that environment, and regressing measured traits on relative fitness, we can determine which traits may be direct targets of selection in a hotter, drier environment (Lande and Arnold, 1983). Significant partial regression slopes, or selection gradients, for each trait indicate the strength and direction of direct selection, holding all other traits constant. Traits may also be indirectly selected on through phenotypic correlations with other traits. Univariate analyses of the covariance between individual traits and relative fitness in a given environment provide a measure of total selection on traits, taking both direct and indirect selection into account (Lande and Arnold, 1983; Brodie et al., 1995). Predicted response to selection can be calculated as the product of the heritability estimate and the estimate of total selection (the selection differential) on each trait in a warmer, drier environment (Brodie et al., 1995).

In this study, I focused on the responses of diploid and tetraploid *Solidago altissima*, a widespread native perennial, to a simulated hotter, drier climate. The population for this study originates in central Minnesota at the ecotone of three major biomes, partly defined by temperature and water availability, including the prairie biome in the west, the deciduous forest in the center of the state, and the coniferous mixed-forest in the northeast (Tester, 1995). Within the next 30-50 years, these biomes are expected to shift northeast in response to predicted increases in average temperature and decreases in water availability. By 2069, the climate in the north-central portion of the state is

expected to be similar to northwestern Iowa or northeastern Nebraska. Average annual maximum temperature is expected to increase 3.2 °C, and average annual precipitation is expected to increase 0.1 mm per day; however, this increase will still result in reduced soil moisture due to increased evapotranspiration in a hotter climate (Galatowitsch et al., 2009). It is unknown whether plants adapted to cooler, wetter forest conditions will be able to respond to an environment more suitable to prairie species, and significant tree mortality and invasion of exotic species are anticipated (Galatowitsch et al., 2009). The unique assemblage of climate factors meeting at the ecotone of forest and prairie biomes in Minnesota, which is the third fastest warming state in the U.S. over the past 40 years (Tebaldi et al., 2012), make this an ideal location to study the effect of climate change on native plant populations. Further, predictions of increased temperature combined with decreased water availability underscore the importance of studying the combined effect of these climate factors on plant response.

The potential of *S. altissima* cytotypes to respond to climate change through adaptive phenotypic plasticity and evolution was assessed by measuring morphological, physiological, and life-history traits likely to be important in a warmer, drier environment. Differences between ploidy levels in adaptation, plasticity, and evolutionary potential were analyzed to address the following questions; 1) Are diploids and tetraploids currently adapted to different environmental conditions? 2) Do diploids and tetraploids differ in their plastic responses to elevated temperature and drought and are those responses adaptive? 3) Do diploids and tetraploids differ in evolutionary potential taking into account heritability, natural selection, and plasticity?

MATERIALS AND METHODS

These experiments were designed to compare responses of diploid and tetraploid *Solidago altissima* under experimentally manipulated temperature and water conditions in a common garden at the University of Minnesota Duluth Field and Research Studies Center (UMD-RFSC, 46.87˚N, 92.04˚W). To control water availability, the entire experiment was conducted under two 3.7 m by 7.9 m rainout shelters with polycarbonate tops and open-air sides (Appendix 1).

Experimental Design and Treatments

Study System

Solidago altissima L. (Asteraceae) is a long-lived, native perennial that occurs throughout most of North America in dry to damp thickets, often growing along roadsides, disturbed areas, and clearings (Fernald, 1950). It reproduces sexually by seed and asexually by rhizotamous clones (Fernald, 1950). Populations of *S. altissima* may consist of multiple cytotypes, including diploids, tetraploids, and hexaploids, as well as intermediate ploidy levels (triploids and pentaploids) (Halverson et al., 2008; Etterson et al. in preparation). In Minnesota, diploids and polyploids exhibit habitat differentiation. In the relatively dry west, populations have a greater proportion of diploids, and in the relatively wet east, populations have a greater proportion of tetraploids (Etterson et al. in preparation). Plants were originally sampled from a single population in Hubbard Co. MN (47.23 ˚N 94.88 ˚W) in 2008 and were reared in pots at UMD-RFSC. Ploidy level was previously determined using chromosome root-tip squashes and a modified version of Galbraith et al. (1983) flow cytometry protocol.

Genetic composition of experimental populations

Twenty diploid and twenty tetraploid genotypes were cloned on a single day in May 2011 via rhizome cuttings. Clonal propagules were weighed, rhizome diameter was recorded, and propagules were planted into 15.24 cm x 15.24 cm Treepots® filled with PROMIX-BX (Premiere Tech Horticulture, Quakertown, PA) with four pots per 33 cm square crate (TPOT2 and TRAY10, Stuewe & Sons, Inc., Tangent, OR). Clones were watered daily for approximately one week and then as needed for one and a half weeks until established. Plants that died were replaced as described above after one week. All plants were watered to field capacity prior to application of the temperature and water treatments on June 3, 2011. Plants were fertilized once in August (6 mL Miracle-Gro Organic Choice Plant Food, 7-1-2, Scotts Miracle-Gro Products, Inc., Marysville, OH) because plant morphology (short stature, chlorotic foliage) indicated that nutrients were being leeched from the soil by the irrigation treatment.

Temperature treatments

Temperature was manipulated by placing half of the plants in ambient conditions and half in open-top passive elevated temperature chambers with a modified ITEX Hexagon Design (Marion, 1996; Marion et al., 1997) (Appendix 1). To accommodate the taller *S. altissima*, hexagon angles were maintained, but the entire structure was elevated with a 72 cm vertical base. Chambers were constructed with Sun-Lite® HP fiberglass solar glazing (85-90% transmission, Solar Components Corp, Manchester, NH).

Temperature and relative humidity were logged hourly in elevated temperature chambers and the ambient temperature treatment from July to the end of September using

iButtons® (DS1923-F5#, Maxim Integrated Products, Inc., Sunnyvale, CA). iButtons® were hung at plant level within each temperature chamber and above corresponding plants in the ambient treatment. Average temperature in the elevated temperature chambers at 14:00 was 1.93 °C ± 0.20 °C warmer than the ambient treatment ($F_{1,1420}$ = 84.03, $P < 0.0001$, Figure 1.1c). This difference is within the range of expected temperature increase for north-central Minnesota of 1.7 °C – 3.5 °C in the next 25-50 years (Galatowitsch et al., 2009). Although mean relative humidity was also higher (+2.39% ± 0.06%, t = -3.96, $P < 0.0001$) in the elevated versus ambient temperature treatment, the temperature treatment did not have a significant effect on relative humidity ($F_{1,1409} = 0.49$, $P = 0.49$).

To determine whether the rainout shelters or the elevated temperature chambers altered light availability, photosynthetically active radiation (PAR) was measured in the out-of-doors, under the rainout shelters, and within the open-top elevated temperature chambers on a full-sun day hourly between 10:00 and 15:00. Plants in the elevated temperature treatment received 93% of light plants in the ambient treatment received, and this difference was not significant between measurements taken inside of temperature chambers and outside of chambers under the shelters ($t_{103} = -0.72$, $P = 0.47$).

Watering treatments

Water was delivered to plants under rainout shelters in the well-watered treatment for two hours semiweekly via an automated drip irrigation system (Series 500 Battery Operated Controller, DIG Corporation, Vista, CA). Plants in the drought treatment were hand watered when 10% or more were visibly wilted. On average, plants in the drought

treatment experienced a reduction of 13.35% soil moisture compared to the well-watered treatment (Delta Soil Moisture Meter, $F_{1,709} = 741.11$, $P < 0.0001$, Figure 1.1a), accounting for plant height which significantly impacted percent soil moisture ($F_{1,710} = 14.91$, $P = 0.0001$).

To ensure the drought treatment affected plants physiologically, predawn water potential was measured using a PMS Model 600 Pressure Chamber (PMS Instrument Co., Albany, OR) at the end of July when at least 10% of the plants in the drought treatment were wilted. Measurements were taken between 3:30 and 5:00 am on the uppermost fully expanded leaf of 10 randomly selected plants from each ploidy-treatment combination (80 plants total). Plants in the drought treatment showed a 156% reduction in water potential compared to plants in the well-watered treatment ($F_{1,70} = 61.08$, $P < 0.0001$, Figure 1.1b).

The non-significant results for humidity and PAR, and highly significant difference in average temperature between ambient and elevated treatments and water availability between wet and dry treatments suggest that the overwhelming treatment effects were due to experimentally manipulated climate factors.

Experiment layout

Plants were organized in a fully randomized split plot design under two rainout shelters with two blocks per shelter. A single block consisted of 20 crates (four pots per crate) within open-top elevated temperature chambers and 20 crates in ambient temperature conditions. Due to space constraints under rainout shelters, crates in the ambient temperature treatment were arranged in rows two crates deep, whereas crates in the

elevated temperature chambers were arranged in four rows; a row of two crates on each end with two rows of four crates between them. The watering treatment was randomized by crate within each temperature treatment. Each crate of four pots contained two diploid and two tetraploid plants. Each block contained the same set of 20 diploid and 20 tetraploid genotypes randomly assigned to specific positions (4 blocks x 2 temperature treatments x 2 watering treatments x 2 ploidy levels x 20 genotypes = 640 plants).

Plants were placed under the rainout shelters at least 76 cm from the edges, and tarps on the sides and ends of the shelter were lowered during rainy, windy conditions. To prevent experimental plants from escaping and obtaining groundwater, weed cloth was laid under all pots in the experiment, and each pot in the drought treatment was lifted weekly.

Measurements

Traits expected to impact fitness in a warmer, drier environment were measured throughout the growing season, with reproductive biomass measured as a fitness correlate. Measurements taken on the date of first flower include height, leaf number, basal branch number, stem branch number, reproductive branch number, and stem diameter at the soil line. Height was also measured at one point in mid-June, and growth rate was calculated as the change in height (cm/day) between this date and the date of first flower. To measure leaf attributes and specific leaf area, the uppermost fully expanded leaf subtending the reproductive branches and a mid-stem leaf from the reproductive branches were collected and immediately pressed on the date of first flower and dried at 70 °C for 72 hrs. WinFolia Pro (v. 2006a) was used to determine leaf area,

and leaves were weighed, to calculate specific leaf area (SLA) as dry area divided by dry weight. Epidermal peels were taken from the adaxial (upper) and abaxial (lower) surfaces of the uppermost fully expanded leaf below reproductive biomass in early August. Stomata were counted from an area of each peel for which the field of view was completely filled with cells at 40X.

Plants that did not flower were measured as above at the end of the growing season (mid-October), and all plants were harvested. Above-ground biomass was separated into reproductive and vegetative biomass, dried, and subsequently weighed.

Several measured traits will not be further considered in the results or discussion for a variety of reasons. Basal and stem branch number were analyzed, but violated the model assumptions of normality. SLA is discussed rather than leaf area, as it is a more integrated measure of leaf response to environmental conditions. The results for vegetative biomass were similar to height, and height, leaf number, and stem diameter are discussed as more specific indications of plant morphological response to temperature and water availability. Reproductive biomass was highly correlated with reproductive branch number ($r = 0.83$, $P < 0.0001$); however, I focus on reproductive biomass because it is likely a better measure of fitness than branch number. For example, reproductive biomass may better represent seed number, although this was not analyzed in this study.

Statistical Analyses

Differences between ploidy levels and plasticity

Diploid and tetraploid responses and plasticity in response to treatments for all traits were analyzed in SAS (Version 9.2, SAS Institute Inc. 2008) using PROC MIXED

with the REML method. Initial clone weight was log transformed and used as a covariate for analyses of each measured trait. An interaction between initial clone weight and watering treatment was also included as a covariate. Data were log transformed and, when necessary, 1 was added to values to meet the model assumptions of normality and equal variance. Fixed effects included ploidy, watering treatment, temperature treatment, and all two-way interactions between these effects. The three-way interaction between ploidy, watering treatment, and temperature treatment was not significant for any measured traits and was excluded in final analyses. Block, genotype(ploidy), watering treatment x genotype(ploidy) and temperature treatment x genotype(ploidy) were considered random effects. Significance of random effects was tested using likelihood ratio tests, removing each random effect in the model with replacement; the difference between -2 log likelihood values for the full and reduced models was used as the Chi-squared test statistic (1 df).

Heritability

Data were split into subsets by each ploidy, temperature treatment, watering treatment combination to estimate broad-sense heritability (H^2) in each simulated environment. Each trait within each subset was reanalyzed using REML, with log clone weight as the only fixed factor remaining in the model, and genotype and block the random effects. Heritability was estimated as the genotype variance estimate divided by the sum of the estimates of genetic variance, block variance, and residual variance. The significance of genetic variance for each ploidy, watering treatment, temperature

treatment subset was determined by likelihood ratio tests. If the genotype effect was statistically significant in a data subset, heritability was also considered significant.

Phenotypic Selection Analysis

Phenotypic selection analysis was done with PROC REG to compare patterns of selection on traits for diploids and tetraploids in each water-availability, temperature treatment combination (Lande and Arnold, 1983; Brodie et al., 1995). Using data subsets as above, traits expected to be important for fitness in a warmer, drier environment were regressed on relative fitness to estimate selection gradients (β_i) for each trait in each ploidy-treatment combination and to determine whether or not each trait has a significant relationship with relative fitness while holding all other traits constant (Lande and Arnold, 1983). Traits included in phenotypic selection analysis were date of first flower, height, leaf number, stem diameter, stomatal density of upper and lower leaf surfaces, specific leaf area (SLA) for leaves from the middle of the plant and reproductive branches, and rosette number. All trait values were log transformed and then standardized by subtracting the mean and dividing the difference by the standard deviation within each data subset to yield trait values with a mean of 0 and standard deviation of 1, which can be compared across traits (Conner and Hartl, 2004 P. 191). Relative fitness was calculated as reproductive biomass divided by mean reproductive biomass in each data subset and was also log transformed.

The selection differential (S), or total selection on a trait including both direct and indirect selection due to phenotypic correlations, was also estimated for each trait in each ploidy-treatment data subset with PROC CORR. S was estimated as the covariance

between relative fitness and each trait. The significance level of the Pearson correlation coefficient between relative fitness and each trait was used as the significance level for *S*. The product of H^2 and *S*, the predicted response to selection (*R*), for each trait in each ploidy, treatment combination was also calculated and was considered significant if both H^2 and *S* were significant.

RESULTS

Differences between diploids and tetraploids

Diploids and tetraploids differed for seven of the nine traits measured (Appendix 2a). Compared to tetraploids, diploids flowered an average of 16.8 days earlier (Figure 1.2a), had thinner (-18%), more numerous (+37%) leaves, and thinner stem diameters (-18%) (Figure 1.2b, c, g). Physiological traits differed among ploidy levels as well: on average, diploids had 42% greater stomatal density than tetraploids on lower leaf surfaces while tetraploids had 178% greater stomatal density than diploids on upper leaf surfaces (Figure 1.2e, f). Stomatal density was also analyzed using leaf area from a middle leaf as a covariate to ensure that the differences observed were attributable to ploidy or treatments, rather than leaf size; however, the covariate was not significant and its inclusion did not alter results. Diploids and tetraploids differed in both asexual and sexual reproduction, with diploids having 58% more rosettes and 16% greater reproductive biomass (Figure 1.2d, h).

Plasticity

Phenotypic plasticity was observed in response to water availability for more traits, and water availability had a stronger effect, than the temperature treatment (Appendix 2a).

Plants grown in drought conditions had 643% more leaves, 85% greater stem diameters, and 225% greater upper stomatal density (Figure 1.3a, b, c) on average compared to high water availability. Plants in the drought treatment were more fit overall, having twice as much reproductive biomass on average than plants in well-watered conditions (Figure 1.3d). Elevated temperature produced plants that were taller (+16%), had thinner leaves (-7%), and grew 34% faster compared to plants in the ambient treatment (Figure 1.4a, b, c).

Significant interactions indicated differences in plasticity between ploidy levels in response to water availability, with each ploidy level exhibiting plasticity for three traits. Tetraploids flowered 8.8 days earlier on average in the drought treatment than in the well-watered treatment, while diploids flowered relatively early regardless of watering treatment (Figure 1.5a). Earlier flowering was correlated with greater fitness ($r = 0.47$, $P < 0.0001$) for tetraploids in low water availability. Diploids and tetraploids both grew faster under drought; however, in well-watered conditions diploids grew faster than tetraploids (Figure 1.5d). Both ploidy levels exhibited plasticity in physiological responses to water availability, but for different traits. While diploids had relatively high stomatal density on lower leaf surfaces regardless of water availability, tetraploids developed 17% greater stomatal density in response to drought (Figure 1.5e). Tetraploids had relatively thick leaves regardless of water availability, while diploids developed 13% thicker leaves in response to drought (Figure 1.5f). Neither of these responses was correlated with fitness. Both diploids and tetraploids grew taller in low water availability (95% and 130% respectively) (Figure 1.5b), and this response was associated with higher

fitness for both ploidies (diploids: r = 0.51, $P < 0.0001$; tetraploids: r – 0.47, $P < 0.0001$). Diploids also displayed plasticity in rosette number, having 23% more rosettes under drought conditions than under high water availability (Figure 1.5c). This was the only plastic response associated with lower reproductive biomass in the drought treatment (r = -0.20, $P < 0.01$). Diploids had 25% greater reproductive biomass in low water availability than tetraploids, although the interaction between ploidy and watering treatment itself was marginally significant for this trait (Figure 1.5g).

Differences in plasticity were also observed between the ploidy levels in response to temperature for one trait, and in the responses of plants to the combination of altered temperature and water availability for two traits (Appendix 2a). Tetraploids had relatively few rosettes regardless of temperature, while diploids developed 24% more rosettes in the ambient temperature than in the elevated treatment (Figure 1.6a). While tetraploids generally had higher upper stomatal density, there was also a marginally significant interaction for this trait in which stomatal density appears to decrease for tetraploids and increase for diploids between ambient and elevated temperature, although the difference in stomatal density between temperature treatments was not significant for either ploidy level (Figure 1.6b). Plants in the well-watered treatment were similarly small across temperature treatments. Low water availability produced taller plants regardless of temperature; however, these plants were more sensitive to temperature, growing 25% taller in elevated compared to ambient conditions (Figure 1.7b). Rosette number was similar across watering treatments regardless of temperature, except for

plants grown in ambient temperature under drought conditions; these plants had 25% more rosettes than plants in any other treatment combination (Figure 1.7a).

Significant genotype variation was detected for days to first flower, rosette number, and stomatal density on both upper and lower leaf surfaces; however, significant variation for plasticity, or genotype x environment interactions, was not detected for any trait (Appendix 2b). Days to first flower and vegetative biomass were the only traits with significant block effects (Appendix 2b).

Heritability

Significant broad-sense heritability was detected for more traits for diploids than tetraploids, especially for plants exposed to low water-availability (Table 1.1). In hotter, drier conditions, both ploidy levels had significant heritability for days to first flower and stomatal density on lower leaf surfaces. In addition, diploids had significant heritability for leaf number, rosette number, and stomatal density on upper leaf surfaces, whereas tetraploids had significant heritability only for stem diameter. Significant heritability was detected for fewer traits in the dry-ambient treatment, with both ploidy levels having significant heritability for days to first flower and reproductive biomass. Under these conditions, diploids also had significant heritability for rosette number and SLA of apical leaves, and tetraploids again had significant variation for stem diameter.

Under high water availability, each ploidy level had significant heritability for two traits, although these traits differed depending on temperature treatment (Table 1.1). Significant genetic variation was detected for stomatal density under well-watered conditions for both ploidy levels regardless of temperature. Diploids had significant

variation for stomatal density on the upper leaf surface in ambient temperature and the lower leaf surface in elevated temperature, while the reverse was true for tetraploids. In addition to stomatal density, there was significant heritability under ambient temperature for rosette number for diploids, and days to first flower for tetraploids.

Phenotypic selection analysis

Phenotypic selection analysis indicated differences in patterns of selection between diploids and tetraploids exposed to conditions simulating climate change (Table 1.2). Compared to diploids, tetraploids had more traits that were targets of selection in the dry-elevated temperature treatment, while more traits were targets of selection in the wet-elevated temperature treatment for diploids. In hot, dry conditions, six traits were under selection for tetraploids and four for diploids. Both ploidy levels had significant direct selection (β) for earlier flowering, which was greater for tetraploids; however, when phenotypic correlations were taken into account, selection (S) was of similar magnitude for both ploidy levels. Plants with more leaves were directly favored, and taller plants with thicker stems were selected for, likely due to phenotypic correlations with earlier flowering. For diploids, earlier flowering is marginally correlated with taller growth ($r = -0.21$, $P = 0.07$), and both traits are correlated with wider stems (early flowering: $r = -0.46$, $P < 0.0001$; height: $r = 0.52$, $P < 0.0001$). Similarly for tetraploids earlier flowering is correlated with taller growth ($r = -0.30$, $p < 0.05$), which is correlated with wider stems ($r = 0.68$, $p < 0.0001$). In addition, selection favored tetraploids with denser stomata and thicker leaves.

Differences were observed in the traits and magnitudes of selection between the dry-elevated temperature treatment and dry-ambient temperature treatment combinations (Table 1.2). Overall four traits were selected on for tetraploids and five for diploids in the dry-ambient temperature treatment combination. These traits were similar to those in the dry-elevated treatment combination, except the physiological traits (SLA and stomatal density) were no longer under selection for tetraploids, while thicker stems were indirectly selected for for diploids. Earlier flowering was not directly favored for either ploidy level in dry-ambient temperature conditions but still had significant selection through phenotypic correlations. Height, on the other hand, was not directly favored in elevated temperature but was in ambient temperature. When phenotypic correlations were accounted for, selection was stronger in the ambient compared to the elevated temperature treatment.

Selection was generally stronger in the well-watered compared to drought treatment combinations (Table 1.2). In the wet-elevated treatment, seven traits had significant selection gradients or selection differentials for diploids and four for tetraploids. Four traits were under selection under wet-ambient conditions for both ploidy levels.

Comparison between β and S showed that indirect selection due to trait correlations was more often responsible for significant selection on a trait than direct selection (Table 1.2). In most cases, the selection differential was in the same direction as the selection gradient and of similar or greater magnitude, indicating that selection due to trait correlations is reinforcing direct selection. There were two exceptions; shorter

diploids were directly favored by selection in high water availability and elevated temperature, while taller plants were favored due to phenotypic correlations. Similarly, in tetraploids more slender stems were directly favored under wet-ambient conditions, while there was significant indirect selection for thicker stems. In both of these cases, phenotypic correlations were antagonistic in high water availability, while indirect selection was always in the reinforcing direction under low water availability.

Predicted Response to Selection

Response to selection (*R*) was predicted for two traits for each ploidy level, but these traits differed between watering treatments (Table 1.3), and the traits predicted to respond depended on water availability. Under low water availability, both cytotypes had significant heritability and selection differentials for days to first flower regardless of temperature treatment. However, tetraploids are expected to evolve earlier flowering time faster than diploids due to greater heritability for this trait under elevated temperature, as well as both greater heritability and stronger selection in ambient temperature compared to diploids. Tetraploids are also expected to evolve thicker stem diameters in response to low water availability regardless of temperature; however, evolution of this trait is expected to proceed more rapidly under elevated temperature due to greater heritability in warmer conditions. In addition to flowering time, diploids are expected to evolve thicker apical leaves in response to drought under ambient temperature, while plant height is expected to increase under elevated temperature. In contrast to the drought treatment, under well-watered conditions tetraploids had no traits for which there was a predicted response to selection, and in diploids only stomatal

density on the lower leaf surface was predicted to increase under wet-ambient temperature conditions.

DISCUSSION

Diploid and tetraploid *S. altissima* showed marked differences in phenology, morphology, and physiology, suggesting that diploids are currently better adapted to dry conditions than tetraploids. Most importantly, diploids had greater reproductive biomass, which was used as a fitness correlate in this study. In addition, diploids had fewer traits under selection in drought conditions and more traits under selection in wet conditions, suggesting that they are currently better adapted to drought than tetraploids. For several traits, diploids did not exhibit plasticity in response to drought; however, their static responses appear to already be in the direction of selection in a dry environment. For example, earlier flowering was selected for in drought for both ploidy levels. Diploids were not plastic for this trait but flowered significantly earlier than tetraploids regardless of water availability, which may allow them to reproduce before drought stress accumulates or avoid drought conditions when they are most acute in late summer (Chaves et al., 2002; Franks et al., 2006). Diploids also had greater stomatal density on the lower leaf surface regardless of water availability, which may allow greater control over patterns of stomatal opening across the leaf surface (Bosabalidis and Kofidis, 2002) or be associated with a decrease in stomatal size or pore aperture, allowing stomata to stay open under drought stress (Clay and Quinn, 1978; Xu and Zhao, 2008). Either response may help maintain CO_2 diffusion and photosynthesis under low water availability, increasing water use efficiency (WUE) under drought conditions (Xu and

Zhao, 2008; Fraser et al., 2009). Tetraploids generally appear larger than diploids, and their thicker leaves and stems may or may not be adaptations to water availability. Tetraploids also had greater upper stomatal density, which may be an adaptation to cooler, shadier forest environments in which less water loss through upper leaf surfaces occurs. Although *S. altissima* populations can be polymorphic for ploidy level, and the plants from this experiment originate from the same population, it is still possible that they exhibit microhabitat differentiation. This was shown by Richardson and Hanks (2011), where in mixed populations of diploid, tetraploid, and hexaploid *S. altissima*, diploids were found in open, grassy areas, hexaploids in forested areas, and tetraploids at the margin between these two habitats.

Plasticity was observed for the same number traits for each ploidy level; however, the specific traits that were plastic differed between cytotypes. Furthermore, plasticity was not uniformly adaptive. Both ploidies exhibited the adaptive plastic response of taller growth under drought, and earlier flowering of tetraploids was also found to be adaptive. Diploids grew thicker leaves in drought, a trait associated with drought tolerance (Chaves et al., 2002); however, this response was not associated with higher fitness in drought in this experiment. Diploids also seemed to adjust their mode of reproduction depending on water availability, developing more rhizomes in drought conditions, which was associated with lower reproductive biomass in that treatment. This response suggests that diploids may "hedge their bets" under low water availability by sacrificing sexual reproduction to some extent in favor of increasing the likelihood of survival through drought via asexual reproduction or by using a combination of

reproductive strategies. Tetraploids increased stomatal density on the lower leaf surface in response to drought, which was not associated with higher fitness; however, if this response increases WUE, even if transpiration increases, it may facilitate maintenance of photosynthesis under decreased water availability. For example, Xu and Zhao (2008) found that stomatal density of the perennial grass *Leymus chinensis* increased under moderate drought and this response was positively correlated with both photosynthesis and WUE.

Plants of both ploidy levels grown in well watered conditions exhibited overall plastic responses that appeared to be maladaptive including shorter stature, smaller leaves, lower stomatal density, and significantly lower fitness. This may be due to the well-watered treatment leaching nutrients from the growing media at a greater rate than the drought treatment. Even though all plants in the experiment were fertilized once a nutrient deficiency was suspected, the plants probably lacked the nutrients necessary to maintain sufficient photosynthetic machinery during development. The high water availability treatment may have also simulated flood conditions, creating a stressful environment too distant from the drier conditions *S. altissima*, especially diploids, appear to be adapted to (Yordanov et al., 2003). In addition, the high and low water availabilities simulated in this experiment may not accurately reflect current levels of water availability or changes to future patterns of precipitation. This may influence the interpretation of the results of this study as plant response to water deficit depends on the amount, timing, and duration of drought plants experience (Bray, 1997; Winkel et al., 1997). In spite of these potential difficulties, changes in precipitation are predicted to be

the overwhelming factor influencing plant populations as climate changes (Fitzpatrick et al., 2008), which is consistent with the greater effect of watering treatment compared to temperature treatment observed here.

Plastic responses were also observed in response to temperature, although for fewer traits. Plants in the elevated temperature treatment were taller and had thinner leaves. Relative growth rate (RGR), the product of leaf area ratio and net assimilation rate, is generally correlated with thinner, more productive leaves (Lambers et al., 2008). In this experiment, RGR was not measured; however, growth rate based on height was greater in elevated temperature, which may have resulted in the growth of thinner leaves. Alternatively, subtle differences in relative humidity between temperature treatments, although not significantly different in this experiment, may have impacted traits such as SLA. Height may have been affected by reduced light within the chambers; however, when tested there was no difference in PAR or R:FR inside and outside of elevated temperature chambers. Greater height in the elevated temperature treatment, which had a different pot layout than the ambient treatment, could also be due to competition with neighboring plants that may have been more intense within the chambers. In this case greater height may be an adaptive plastic response to crowding (Dudley and Schmitt, 1996).

For two traits, the effect of temperature depended on water availability, underscoring the importance of studying the effects of these climate variables together. Plants in drought were more sensitive to temperature, growing taller with fewer rosettes in the elevated temperature treatment. This may reflect a tradeoff between allocation of

resources to vegetative biomass and asexual reproduction. It is also possible that plants in the hot, wet treatment were unable to grow taller or grow additional rhizomes because of a nutrient deficiency.

Observed plant responses to altered temperature and precipitation related to climate change may not be due to plasticity alone. In this experiment, heritability and significant selection were detected for several traits, including flowering time, in a warmer, drier environment. While genetic change was predicted for traits within each cytotype, patterns of selection and heritability differed between diploids and tetraploids. In particular, tetraploids had more traits that were direct targets of selection in warmer or drier conditions. Tetraploid *S. altissima* may face a greater challenge adapting to a warmer, drier climate if it is currently better adapted to cooler, wetter forest conditions. In this experiment, diploids had more traits under selection in a hot, wet environment, and tetraploids had more traits under selection in a hot, dry environment, further suggesting that diploids are currently better adapted to drought and tetraploids to wetter conditions. Although more traits were targets of selection for tetraploids, their ability to evolve in response to climate change may be limited due to a lack of heritability for those traits. Further, evolution of earlier flowering time may be inhibited in tetraploids occurring in populations polymorphic for ploidy level, due to triploid block potentially reducing fitness in populations where the flowering times of diploids and tetraploids overlap (Comai, 2005).

Response to selection was predicted for two traits within each cytotype. Both ploidy levels are predicted to evolve earlier flowering time in response to warmer, drier

conditions. Tetraploids flower significantly later than diploids and are expected to evolve earlier flowering faster due to greater heritability for this trait. In addition, diploids are expected to evolve a taller growth habit, and tetraploids are expected to evolve thicker stems. Although these traits are predicted to respond to selection, the rate of genetic change may not keep pace with climate change. *S. altissima* is a long-lived perennial making it difficult to estimate whether evolution has the potential to track climate change in this population. Even in an annual species, calculated rates of evolution have not been sufficient to keep pace with climate change (Etterson and Shaw, 2001), and genetic change within populations may occur even more slowly for a species such as *S. altissima* with longer generation times. In addition, antagonistic or reinforcing genetic correlations between traits may hinder or facilitate evolutionary change respectively (Etterson and Shaw, 2001); however, this was not taken into account in these analyses.

The importance of studying the effects of temperature and precipitation in conjunction with one another is further emphasized by differences in heritability, patterns of selection, and predicted response to selection within different treatment combinations. For example, four traits had significant broad sense heritability under dry conditions for diploids in both temperature treatments. Except for flowering time, these traits differed between ambient and elevated temperature. For both ploidy levels, the number of traits with significant heritability was reduced in the well-watered treatment, which may be due to the overwhelming effect of a stressful environment on genetic variation. With a few exceptions, patterns of selection were similar across temperature treatments within the drought treatment, and differences were observed in strength rather than direction of

selection. For example, earlier flowering was a direct target of selection for both ploidy levels, with stronger selection for earlier flowering of tetraploids, within the dry-elevated temperature treatment combination, but was not a target of selection in ambient temperature. When phenotypic correlations between traits were taken into account, however, selection was stronger in ambient than elevated temperature and was similar between diploids and tetraploids in elevated temperature. Strong selection was detected for several traits within the well-watered treatment, including greater leaf number for upper stomatal density for both ploidies, and earlier flowering and shorter growth for diploids in wet-elevated conditions. The combination of water availability and temperature ultimately affected which traits are predicted to respond to selection within ploidy levels.

If climate change proceeds directionally (i.e. consistently warmer and drier), the adaptive plastic response of diploids for greater height and responses of tetraploids for earlier flowering and greater height may maintain fitness in a new environment. Such responses may "buy time" for adaptive evolution to occur by allowing a population to persist while genetic variation increases through mutation or recombination. Once genetic diversity has increased, traits beneficial in the new environment may be integrated into the population through natural selection (Pigliucci, 2005). Alternatively, if environmental change is more variable, a more generalist strategy may be advantageous. In this case, adaptive phenotypic plasticity itself may be selected for and evolve (Thompson, 1991); however, no evidence for heritability for plasticity in the form of significant genotype x environment interactions was detected in this study.

It is expected that plant communities and species will react differently to projected climate change and this has been observed (Walther et al., 2001; Neilson et al., 2005). The results presented here suggest that within a species, even within a population, such as different ploidy subpopulations, short and long term responses to climate change may be complex. Depending on the importance of individual traits to fitness in each ploidy level under altered climate conditions, the observed plasticity and predicted response to selection in this study may be beneficial in the short and long term respectively. However, in the long term tetraploids may be at a disadvantage compared to diploids if they are unable to maintain fitness through adaptive plasticity and are unable to evolve due to a lack of heritability for traits selected on in a hot, dry environment.

The complex responses of diploid and tetraploid plants to conditions simulating climate change observed in this study and the fact that polyploidy is a widespread phenomenon across plant species underscore the importance of understanding how different ploidy levels within species will respond to climate change. This issue warrants further study especially for ecologically important polyploid species that may be used in restoration efforts or other conservation strategies such as assisted migration.

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Table 1.1. Broad sense heritabilities (H^2) for morphological, phenological, and physiological traits of diploid and tetraploid *S. altissima* calculated as the genotype variance estimate divided by the sum of the estimates of genetic variance, block variance, and residual variance. -2 Log Likelihood ratio $\chi 2$ test statistics are for genotypic variation in each ploidy-treatment combination.

	Heritability Analysis							
	Diploid Treatment Combination				**Tetraploid Treatment Combination**			
	Dry-Ambient	**Dry-Elevated**	**Wet-Ambient**	**Wet-Elevated**	**Dry-Ambient**	**Dry-Elevated**	**Wet-Ambient**	**Wet-Elevated**
Trait	H^2 ($\chi 2$)	H^2 ($\chi 2$)	H^2 ($\chi 2$)	H^2 ($\chi 2$)	H^2 ($\chi 2$)	H^2 ($\chi 2$)	H^2 ($\chi 2$)	H^2 ($\chi 2$)
Julian Days to First Flower	**0.47*** (18.60)**	**0.45** (14.84)**	0.09 (0.05)	0.00 (0.00)	**0.48*** (19.67)**	**0.57*** (26.94)**	**0.52* (6.96)**	0.47 (2.54)
Height	0.11 (0.63)	**0.21* (6.45)**	0.18 (2.57)	0.05 (0.31)	0.00 (0.00)	0.09 (0.29)	0.02 (0.00)	0.02 (0.05)
Leaf Number	0.18 (0.00)	0.18 (0.00)	0.18 (0.00)	0.18 (0.00)	0.18 (0.00)	0.18 (0.00)	0.18 (0.00)	0.18 (0.00)
Rosette Number	**0.21* (4.14)**	**0.16† (3.24)**	**0.25* (5.92)**	0.09 (0.53)	0.03 (0.05)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Stomatal Density - Adaxial	0.15 (1.12)	**0.58*** (17.84)**	**0.35* (5.97)**	**0.48** (11.82)**	0.13 (2.08)	**0.22† (3.76)**	0.17 (2.02)	**0.46*** (17.54)**
Stomatal Density - Abaxial	0.07 (0.35)	**0.35* (8.94)**	0.15 (1.78)	0.00 (0.00)	0.06 (0.17)	0.03 (0.01)	**0.22† (3.29)**	0.06 (0.24)
SLA - Apical	**0.34*** (8.22)**	0.16 (1.40)	0.21 (0.04)	0.00 (0.00)	0.00 (0.00)	0.34 (1.96)	0.02 (0.00)	0.00 (0.00)
SLA - Mid	0.06 (0.18)	0.07 (0.14)	0.00 (0.00)	0.00 (0.00)	0.08 (0.45)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Stem Diameter	0.03 (0.00)	0.14 (1.41)	0.07 (0.33)	0.00 (0.00)	**0.23* (4.59)**	**0.36* (7.95)**	0.00 (0.00)	0.09 (0.66)
Reproductive Biomass	**0.23* (4.15)**	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	**0.31* (8.20)**	0.06 (0.12)	0.05 (0.27)	0.01 (0.00)

† $p < 0.10$; * $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$

Table 1.2. Selection gradients (β) (SE) and selection differentials (S) for morphological, phenological, and physiological traits of diploid and tetraploid *S. altissima* in each permutation of water-availability and temperature treatments. All traits were log transformed and standardized within each treatment combination by subtracting the mean from each trait value and dividing the difference by the standard deviation.

		Phenotypic Selection Analysis							
		Diploid Treatment Combinations				**Tetraploid Treatment Combinations**			
Trait		**Dry-Ambient**	**Dry-Elevated**	**Wet-Ambient**	**Wet-Elevated**	**Dry-Ambient**	**Dry-Elevated**	**Wet-Ambient**	**Wet-Elevated**
Julian Days to First Flower	β *(s.e.)*	-0.05 (0.05)	**-0.09* (0.04)**	0.52 (0.54)	**-2.19* (0.67)**	-0.07 (0.04)	**-0.11* (0.05)**	-0.11 (0.69)	-1.35 (0.88)
	S	**-0.19*** **	**-0.14*** **	0.23	**-0.90***	**-0.23*** **	**-0.14***	-0.36	-0.46
Height (cm)	β *(s.e.)*	**0.25*** ** (0.07)**	-0.05 (0.04)	1.19 (1.31)	**-6.00* (1.92)**	**0.21* (0.06)**	-0.06 (0.09)	1.91 (1.74)	**4.32† (2.06)**
	S	**0.26*** **	**0.11***	**1.16*** **	**0.94*** **	**0.23*** **	**0.20*** **	**0.86*** **	**0.96*** **
Leaf Number	β *(s.e.)*	**0.16* (0.05)**	**0.17*** ** (0.04)**	**1.48* (0.59)**	**3.94* (0.92)**	**0.14* (0.05)**	**0.14* (0.05)**	**2.58* (0.81)**	**4.18* (1.33)**
	S	**0.27*** **	**0.18*** **	**1.27*** **	**0.59*** **	**0.25*** **	**0.21*** **	**1.29*** **	**1.00*** **
Rosette Number	β *(s.e.)*	-0.02 (0.05)	0.02 (0.04)	0.43 (0.41)	-0.53 (0.85)	-0.03 (0.04)	-0.03 (0.05)	-0.39 (0.53)	-0.46 (0.81)
	S	-0.08	-0.06	0.00	**-0.50***	-0.03	-0.04	-0.31	-0.32
Stomatal Density - Adaxial	β *(s.e.)*	0.02 (0.05)	0.02 (0.04)	1.05 (0.43)	**1.66* (0.56)**	0.03 (0.04)	0.05 (0.05)	**1.36* (0.53)**	0.46 (0.81)
	S	0.11	0.08	**0.83*** **	0.13	**0.08†**	0.04	**0.41†**	0.01
Stomatal Density - Abaxial	β *(s.e.)*	0.06 (0.05)	0.00 (0.04)	-0.03 (0.33)	0.14 (1.02)	0.02 (0.04)	**0.11* (0.04)**	0.54 (0.47)	0.80 (0.85)
	S	0.05	0.02	-0.43	**0.24***	-0.00	**0.10***	0.25	**0.45***
SLA - Apical	β *(s.e.)*	-0.08 (0.05)	0.01 (0.05)	0.14 (0.43)	**-2.00† (0.84)**	-0.02 (0.05)	0.00 (0.06)	-0.78 (0.58)	-1.01 (0.54)
	S	**-0.11***	0.04	-0.13	-0.18	-0.06	**-0.14***	0.70	-0.23
SLA - Mid	β *(s.e.)*	0.03 (0.04)	0.02 (0.04)	-1.06 (0.62)	-0.86 (1.09)	0.05 (0.05)	0.05 (0.06)	0.35 (0.60)	-0.08 (0.59)
	S	-0.06	-0.03	-0.10	0.00	0.07	0.06	0.16	-0.06
Stem Diameter	β *(s.e.)*	-0.04 (0.08)	0.04 (0.05)	0.31 (0.88)	-0.84 (1.12)	0.09 (0.06)	0.09 (0.06)	**-2.36* (1.03)**	-2.97 (1.71)
	S	**0.17****	**0.16*** **	**1.03*** **	**1.06*** **	**0.23*** **	**0.20****	**0.71****	**1.00*** **

† $p < 0.10$; * $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$

FIGURE LEGENDS

Figure 1.1. Least squares means (SE) for temperature at 14:00 from July through September within ambient and elevated temperature treatments: a) average percent soil moisture and b) leaf water potential for plants in well-watered vs. drought treatments, c) temperature within and outside elevated temperature chambers at 14:00 from July through September.

Figure 1.2. Least squares means (SE) for traits that differed between diploid and tetraploid *S. altissima*: a) Julian days to first flower, b) leaf number, c) SLA – mid leaf, d) rosette number, e) adaxial stomatal density, f) abaxial stomatal density, g) stem diameter, h) reproductive biomass.

Figure 1.3. Least squares means (SE) for traits that differed between *S. altissima* grown in contrasting water availability treatments: a) leaf number, b) stem diameter, c) adaxial stomatal dentisy, d) reproductive biomass.

Figure 1.4. Least squares means (SE) for all traits that differed between *S. altissima* grown in ambient and elevated temperature treatments: a) Julian days to first flower, b) SLA – mid leaf, c) growth rate.

Figure 1.5. Least squares means (SE) for all traits for which plasticity differed between diploid (closed circle, solid line) and tetraploid (open circle, dashed line) *S. altissima* grown in contrasting water availability treatments: a) Julian days to first flower, b) height, c) rosette number, d) growth rate, e) abaxial stomatal density, f) SLA – apical leaf, g) reproductive biomass.

Figure 1.6. Least squares means (SE) for rosette number of diploid (closed circle, solid line) and tetraploid (open circle, dashed line) *S. altissima* grown in ambient and elevated temperature treatments.

Figure 1.7. Least squares means (SE) for responses of *S. altissima* grown under well-watered (closed circle, solid line) and drought (open circle, dashed line) conditions that depended on temperature treatment: a) rosette number, b) height.

Figure 1.1

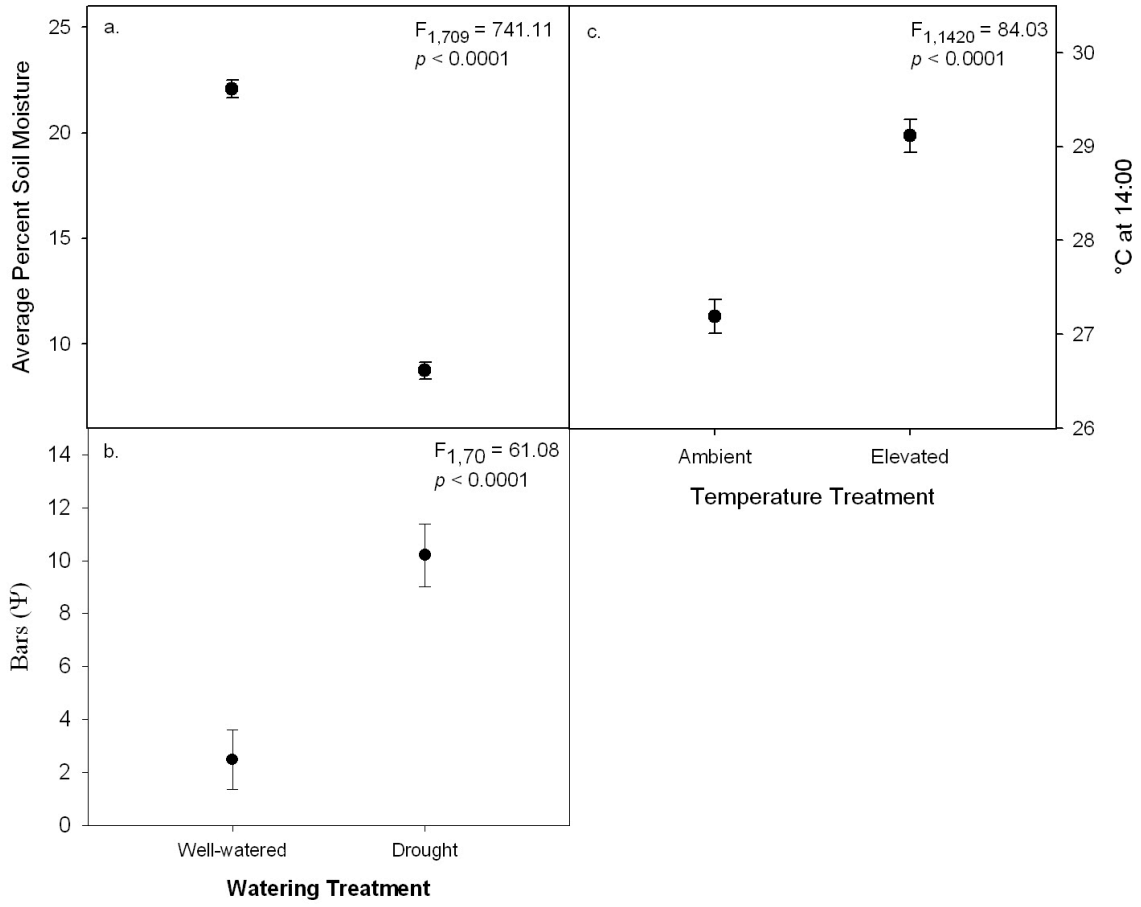

a.
$F_{1,709} = 741.11$
$p < 0.0001$
Average Percent Soil Moisture
25
20
15
10
c.
$F_{1,1420} = 84.03$
$p < 0.0001$
30
29
28
27
26
°C at 14:00
Ambient
Elevated
Temperature Treatment
b.
$F_{1,70} = 61.08$
$p < 0.0001$
Bars (Ψ)
14
12
10
8
6
4
2
0
Well-watered
Drought
Watering Treatment

Figure 1.2

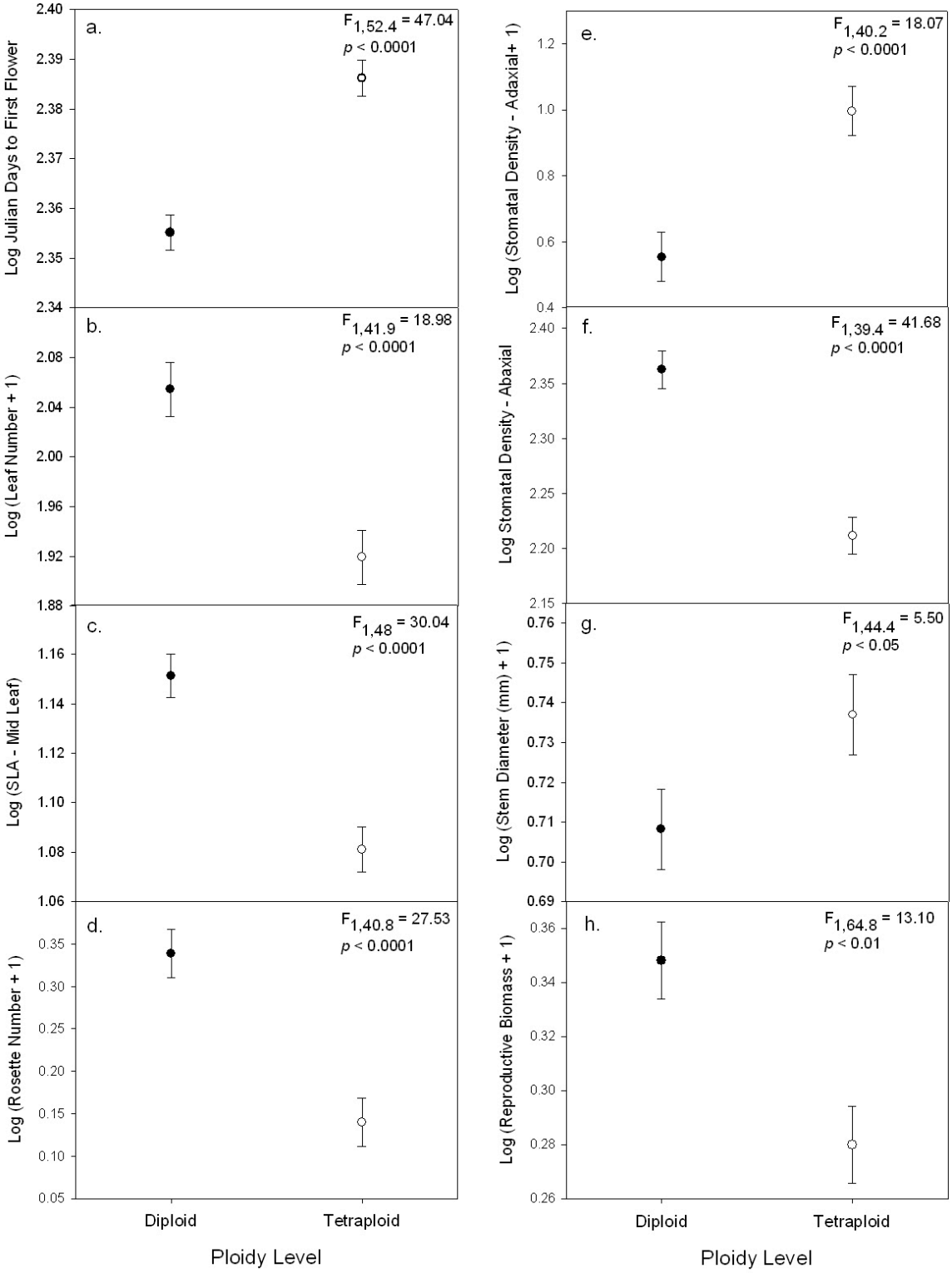

a.
$F_{1,52.4} = 47.04$
$p < 0.0001$
Log Julian Days to First Flower
b.
$F_{1,41.9} = 18.98$
$p < 0.0001$
Log (Leaf Number + 1)
c.
$F_{1,48} = 30.04$
$p < 0.0001$
Log (SLA - Mid Leaf)
d.
$F_{1,40.8} = 27.53$
$p < 0.0001$
Log (Rosette Number + 1)
e.
$F_{1,40.2} = 18.07$
$p < 0.0001$
Log (Stomatal Density - Adaxial+ 1)
f.
$F_{1,39.4} = 41.68$
$p < 0.0001$
Log Stomatal Density - Abaxial
g.
$F_{1,44.4} = 5.50$
$p < 0.05$
Log (Stem Diameter (mm) + 1)
h.
$F_{1,64.8} = 13.10$
$p < 0.01$
Log (Reproductive Biomass + 1)
Diploid
Tetraploid
Ploidy Level

Figure 1.3

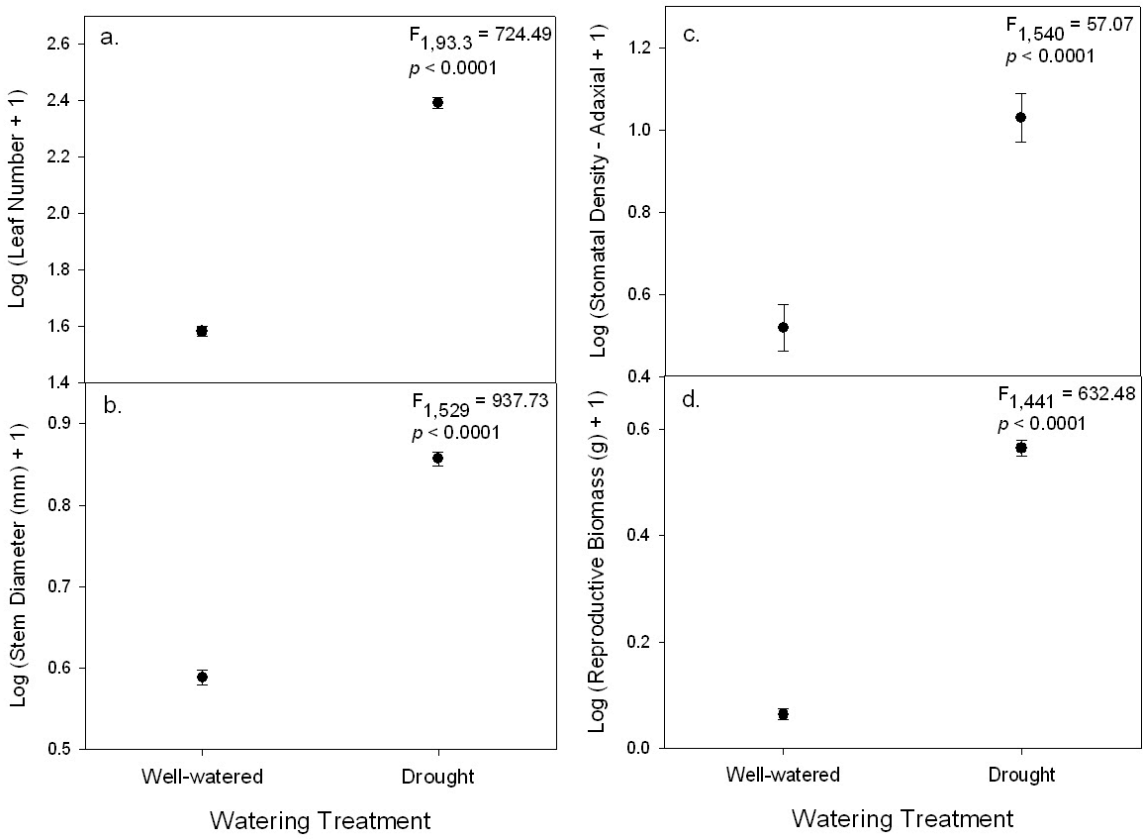

a.
$F_{1,93.3} = 724.49$
$p < 0.0001$
Log (Leaf Number + 1)
b.
$F_{1,529} = 937.73$
$p < 0.0001$
Log (Stem Diameter (mm) + 1)
Well-watered
Drought
Watering Treatment
c.
$F_{1,540} = 57.07$
$p < 0.0001$
Log (Stomatal Density - Adaxial + 1)
d.
$F_{1,441} = 632.48$
$p < 0.0001$
Log (Reproductive Biomass (g) + 1)
Well-watered
Drought
Watering Treatment

Figure 1.4

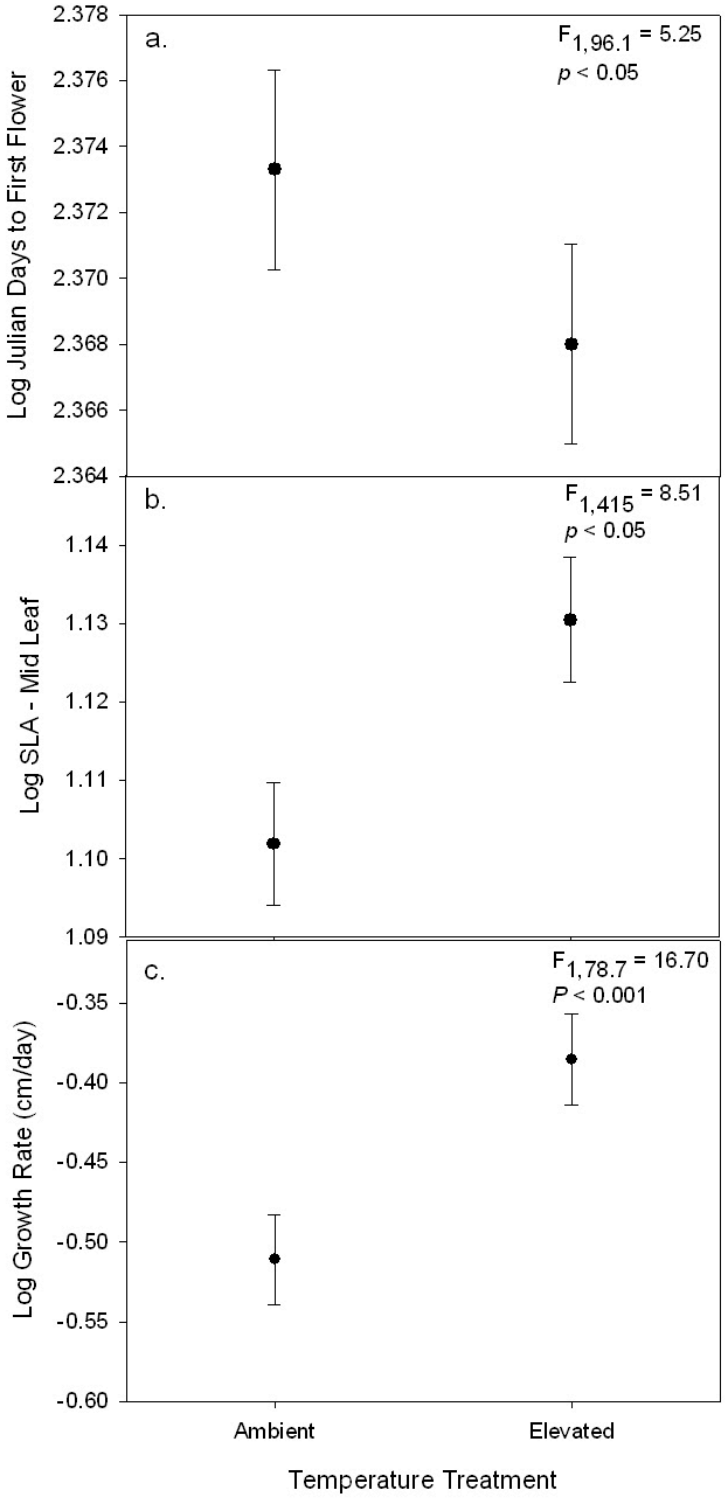

a.
$F_{1,96.1} = 5.25$
$p < 0.05$
Log Julian Days to First Flower
2.378
2.376
2.374
2.372
2.370
2.368
2.366
2.364
b.
$F_{1,415} = 8.51$
$p < 0.05$
Log SLA - Mid Leaf
1.14
1.13
1.12
1.11
1.10
1.09
c.
$F_{1,78.7} = 16.70$
$P < 0.001$
Log Growth Rate (cm/day)
-0.35
-0.40
-0.45
-0.50
-0.55
-0.60
Ambient
Elevated
Temperature Treatment

Figure 1.5

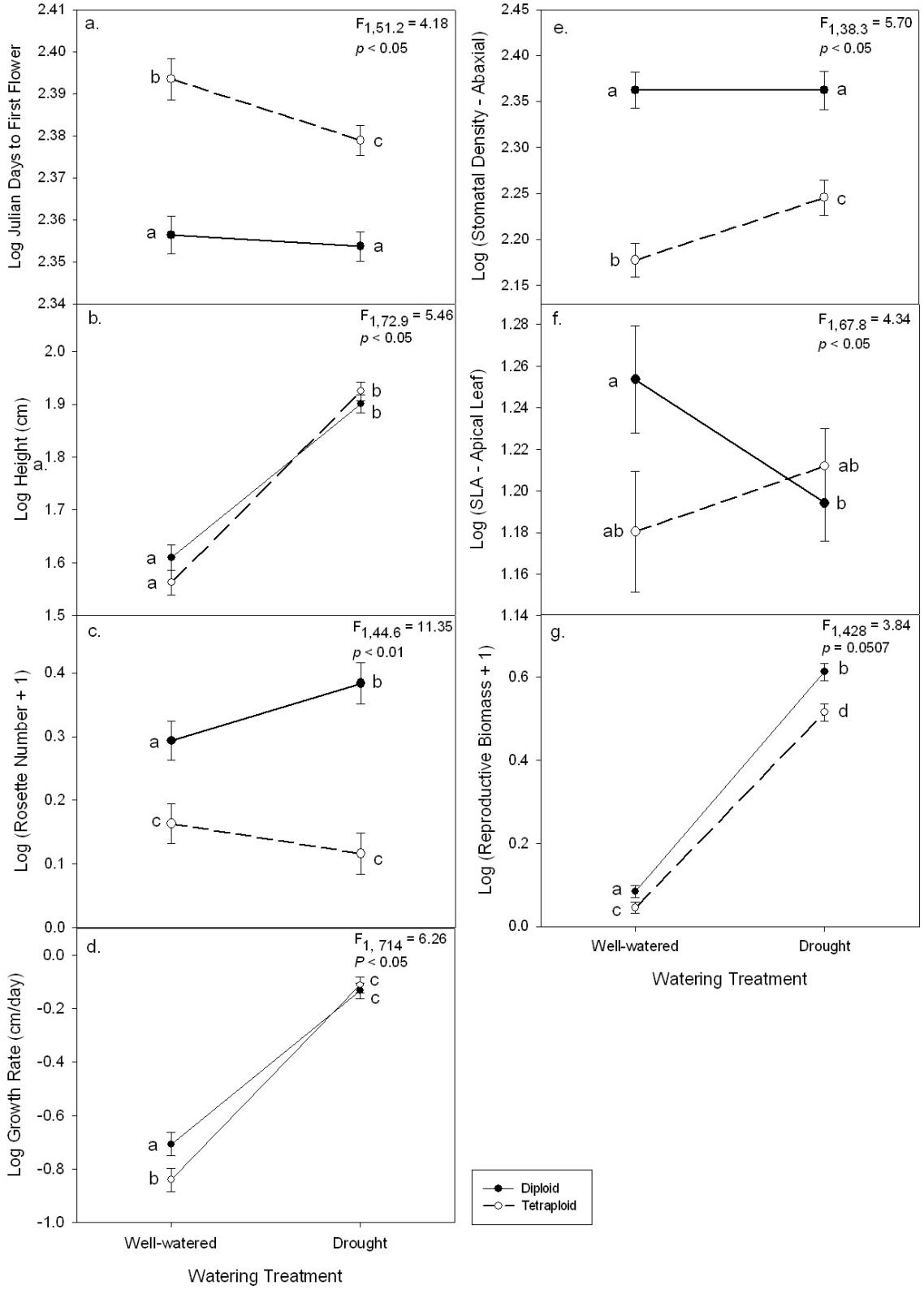

a.
$F_{1,51.2} = 4.18$
$p < 0.05$
Log Julian Days to First Flower
b.
$F_{1,72.9} = 5.46$
$p < 0.05$
Log Height (cm)
c.
$F_{1,44.6} = 11.35$
$p < 0.01$
Log (Rosette Number + 1)
d.
$F_{1, 714} = 6.26$
$P < 0.05$
Log Growth Rate (cm/day)
Well-watered
Drought
Watering Treatment
e.
$F_{1,38.3} = 5.70$
$p < 0.05$
Log (Stomatal Density - Abaxial)
f.
$F_{1,67.8} = 4.34$
$p < 0.05$
Log (SLA - Apical Leaf)
g.
$F_{1,428} = 3.84$
$p = 0.0507$
Log (Reproductive Biomass + 1)
Well-watered
Drought
Watering Treatment
Diploid
Tetraploid

Figure 1.6

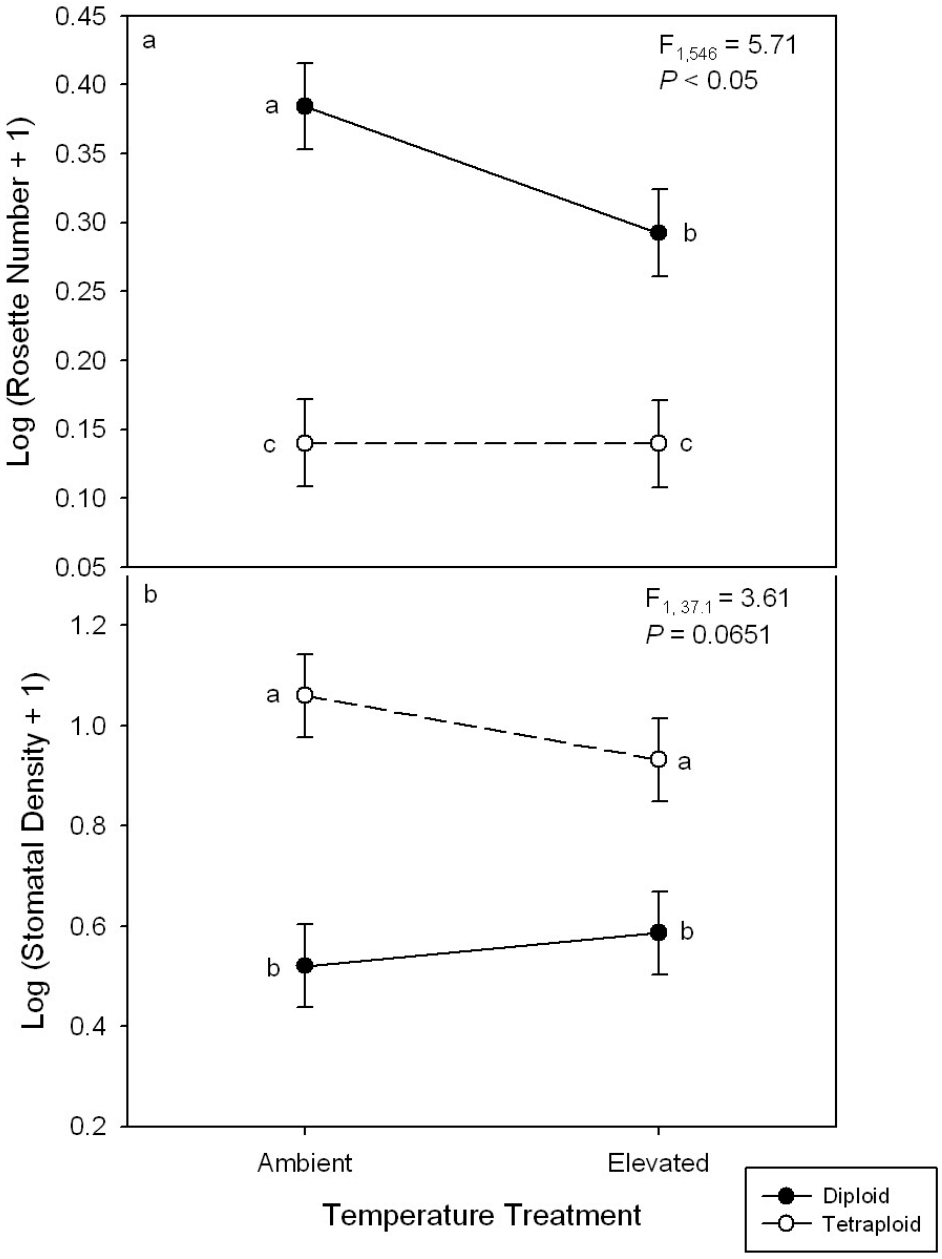
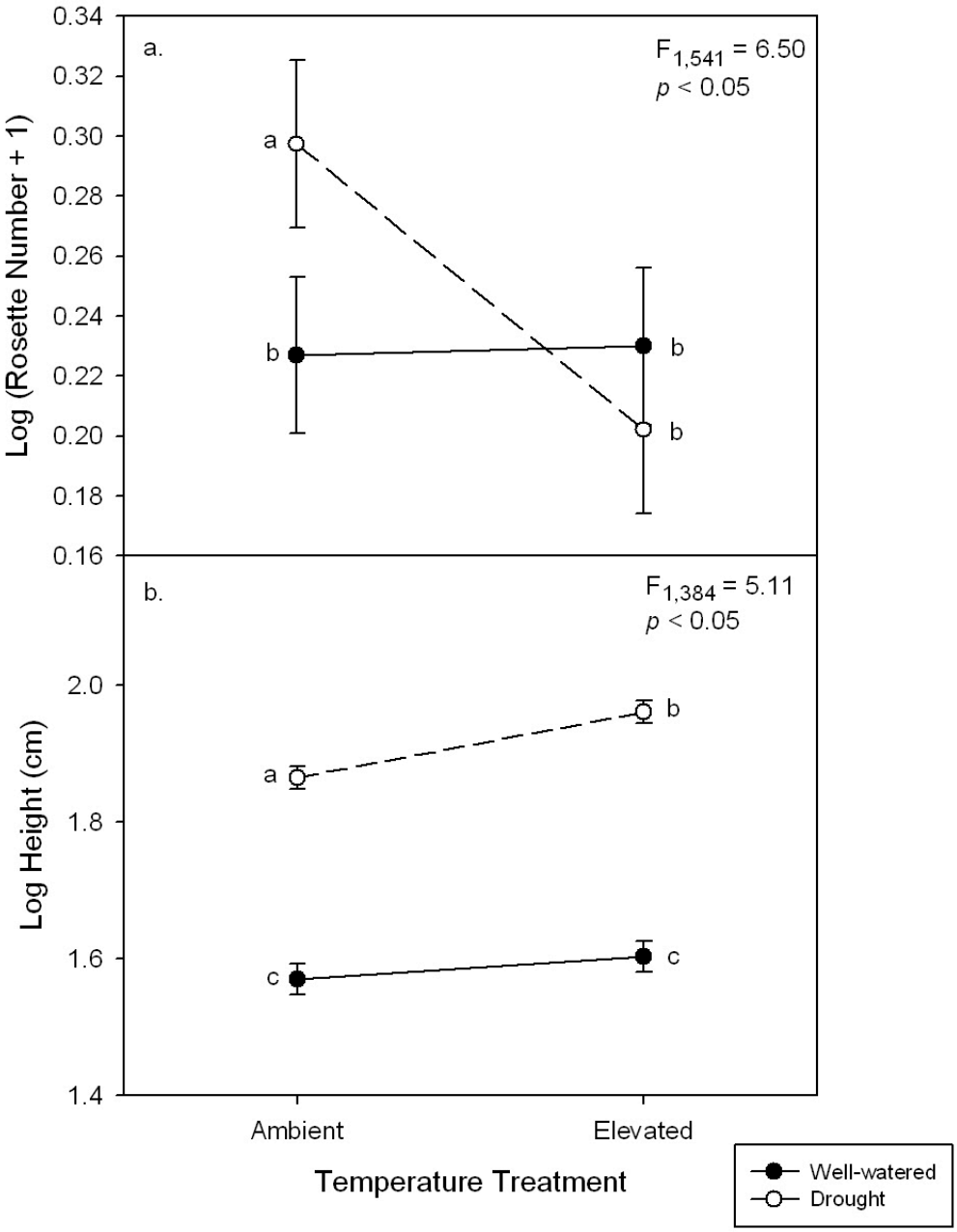

a
$F_{1,546}$ = 5.71
P < 0.05
Log (Rosette Number + 1)
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
a
b
c
c
b
$F_{1, 37.1}$ = 3.61
P = 0.0651
Log (Stomatal Density + 1)
1.2
1.0
0.8
0.6
0.4
0.2
a
a
b
b
Ambient
Elevated
Temperature Treatment
Diploid
Tetraploid

Figure 1.7


a.
$F_{1,541} = 6.50$
$p < 0.05$
Log (Rosette Number + 1)
0.34
0.32
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
a
b
b
b
b.
$F_{1,384} = 5.11$
$p < 0.05$
Log Height (cm)
2.0
1.8
1.6
1.4
a
b
c
c
Ambient
Elevated
Temperature Treatment
Well-watered
Drought

CHAPTER 2

PHYSIOLOGICAL RESPONSE AND LEAF NUTRIENT COMPOSITION OF POLYPLOID *SOLIDAGO ALTISSIMA* (ASTERACEAE) UNDER SIMULATED CLIMATE CHANGE

SUMMARY

In many systems, polyploids inhabit distinct environments compared to their diploid progenitors. Physiological differences between diploid and polyploid plants may play a role in habitat differentiation and may have important implications for plant response to climate change. In addition, cytotypes may differ in their ability to acquire and utilize nutrients vital for physiological performance. In this study, physiological performance and leaf nutrient composition were examined for diploid and tetraploid genotypes of *S. altissima* grown under conditions simulating climate change (+1.9 ° C, -13% soil moisture). The watering treatment had the strongest impact on photosynthesis, but this effect depended on temperature. Both photosynthetic rate and transpiration were relatively low under high water availability regardless of temperature but decreased 40% and 34% respectively under elevated compared to ambient temperature under drought. Physiological responses were similar between ploidy levels; however, differences between diploids and tetraploids were observed in leaf nutrient composition. Total %N of both diploid and tetraploid leaves increased under low water availability, and this effect was more pronounced for diploids. Diploids also had a significantly lower C:N under drought, while tetraploids had similar and relatively high C:N regardless of water availability. These results suggest that diploid and tetraploid *S. altissima* may employ alternate strategies to maintain physiological performance under drought and elevated temperature. The importance of studying these climate variables together is emphasized by the significant effect of their interaction on photosynthesis and transpiration.

INTRODUCTION

Polyploidy has played a prominent role in the evolutionary history of many, if not most plants (Soltis et al., 2003) and may have important implications for plant response to global climate change. This macromutation, which results in chromosome doubling, may affect plant physiological processes such as photosynthesis and water use efficiency (WUE) through changes in enzyme activity and hormone levels (Levin, 1983; Warner and Edwards, 1993). In addition, polyploidy may influence physiological traits such as transpiration and WUE through changes to guard cell size and geometry and xylem elements, which may be a direct result of polyploidization (Ramsey, 2011). For example, in *Achillea borealis*, neohexaploids enjoyed a 70% fitness advantage over tetraploids in dry environments, which may account for the past hexaploid colonization of Mediterranean habitats on the U.S. Pacific Coast (Ramsey, 2011). Genetic evolution may further diverge polyploids from their progenitors following polyploid formation (Adams and Wendel, 2005; Ramsey, 2011), and their physiological traits may undergo adaptation to different environments than diploids (Maherali et al., 2009).

Inherent or evolved differences in physiological traits between diploids and polyploids may enhance polyploids' ability to cope with conditions that are predicted with future climate change, such as decreased water availability (Li et al., 1996; Ramsey, 2011). In *Betula papyrifera*, for example, populations of diploids are restricted to relatively moist sites and are more sensitive to drought than pentaploids and hexaploids, as evidenced by their decreased photosynthetic rate in response to water deficit (Li et al., 1996). However, there are also examples where there is no difference in physiological

performance between diploids and polyploids. In both native and invasive populations of *Solidago gigantea*, Hull-Sanders et al. (2009) found no difference in photosynthetic rate or SLA between diploids and tetraploids, which often co-occur in populations polymorphic for ploidy level. The effect of polyploidy on physiological response clearly depends on the system considered, and mode of polyploidization may also impact physiological traits (Warner and Edwards, 1993). Yet little research has been done specifically addressing differences in ploidal response to conditions simulating climate change. Considering the profound effect polyploidy may have on plant physiological response, this warrants further study.

In addition to potential differences in physiological capacities, ploidy levels may differ in their ability to acquire or assimilate nutrients, such as nitrogen, that are important for physiological processes. At high light availability, the strong correlation between photosynthesis and leaf nitrogen concentration is often explained by limitation of photosynthesis by RuBP (Hirose and Werger, 1987) or chlorophyll content (Evans, 1989), which are comprised of a large proportion of leaf nitrogen. Although higher photosynethtic rates are typically associated with higher leaf nitrogen content, this relationship may also be influenced by leaf morphology, especially specific leaf area (SLA) (Reich et al., 2002). Low SLA indicates more conservative resource use and better adaptation to low water availability, whereas leaves with higher SLA tend to be more productive, acquire more nutrients, and have higher rates of photosynthesis rather than conserving resources (Poorter & de Jong, 1999; Wilson et al., 1995). If differences in nutrient acquisition or assimilation exist between ploidy levels, this may have

important implications for the capability of different cytotypes to respond physiologically to predicted climate change.

In order to elucidate the role of polyploidy in plant physiological response to climate change, it is imperative to study this issue in the context of the climate variables that are predicted to change. In Minnesota, where this study took place, climate is expected to become warmer and drier over the next 30-60 years, accompanied by changes in patterns and severity of precipitation events (Galatowitsch et al., 2009). Both of these climate variables and their interaction may affect plant physiological traits that impact photosynthesis and plant-water relations. For example, in drought-adapted plants, photosynthetic machinery is typically robust to low water availability; however, photosynthesis may be down-regulated when drought conditions are combined with high temperature. Further, plant response to drought stress in the field is usually accompanied by response to other stressors, including elevated temperature (Chaves et al., 2002). This underscores the importance of studying plant physiological response to projected climate change in the context of the multiple stressors plants are predicted to encounter.

In this study, samples from a larger experiment of diploid and tetraploid *S. altissima* exposed to contrasting water availabilities and temperature treatments were analyzed to assess differences in photosynthetic rate, transpiration, WUE, SLA, and leaf nutrient composition to answer the following questions: 1) Do diploids and tetraploids differ in their physiological responses to contrasting water availability and temperature? 2) Do diploids and tetraploids differ in the nutrient composition of their leaves? 3) Does

physiological response or leaf nutrient composition depend on the combination of water availability and temperature?

MATERIALS AND METHODS

These experiments used samples from a larger experiment (refer to Chapter 1 for experimental design) to compare the physiological responses and leaf nutrient content of diploid and tetraploid *Solidago altissima* exposed to simulated climate change (+1.9 ° C, -13% soil moisture).

Gas Exchange Measurements

Gas exchange measurements were taken on one block of plants (160 plants total), which included 20 genotypes of diploids and 20 genotypes of tetraploids. Plants within this block were arranged in ambient and elevated temperature treatments (inside or outside of passive elevated temperature chambers), with well-watered and drought treatments (irrigated vs. hand watered when >10% of plants were wilted) applied randomly to crates containing four pots each; two random diploid pots and two random tetraploid pots (1 block x 2 temperature treatments x 2 watering treatments x 2 ploidy levels x 20 genotypes = 160 samples).

CO_2 gas exchange rates were measured using the LI-6400 and LI-6400XT (LI-COR Biosciences, Inc., Lincoln, NE) over three days in mid-August between 9:00 and 15:00, at the height of flowering. A random selection of plants was measured with each machine. Flow rate of air entering the leaf chamber was set to 400 μmol s^{-1}, mixed at 400 μmol CO_2 mol^{-1} air. Block temperature was controlled at 24° C and quantum flux at 1500 μmol m^{-2} s^{-1}. For each plant, the healthiest uppermost, fully expanded leaf below

reproductive biomass was measured. Three measurements were simultaneously recorded for each leaf when the photosynthetic rate and stomatal conductance appeared steady for 1.5-2 minutes, or at 8 minutes if readings did not stabilize. If leaves did not completely fill the chamber, they were trimmed, scanned, and actual leaf area within the chamber was analyzed using WinFolia Pro (v. 2006a) on the same day. All leaves were dried and weighed to calculate specific leaf area (SLA) as the ratio of fresh area to dry biomass.

Photosynthetic rate and transpiration rate were recalculated for leaves that did not completely fill the chamber using the actual leaf area within the chamber (Using the LI-6400/LI-6400XT Portable Photosynthesis System Version 6.1). Water use efficiency (WUE) was calculated using these adjusted measurements as:

$$\%WUE = A/10^4 * E$$

where A is the adjusted photosynthetic rate and E is the adjusted transpiration rate (Using the LI-6400/LI-6400XT Portable Photosynthesis System Version 6.1). For each of these traits, the average of the three measurements taken on each leaf was used for analysis.

Leaf Nutrient Analysis

Leaf nutrient analysis was performed on a random selection of 60 well-watered plants and 60 plants in low water availability, all from the ambient temperature treatment. Half of these plants were diploids and half tetraploids (2 watering treatments x 2 ploidy levels x 30 plants = 120 samples). A leaf collected from the middle of each plant on the date of first flower for plants that flowered between 7/27/2011 and 9/18/2011 was analyzed. In several cases leaves collected in mid-October from plants that did not flower were substituted due to an insufficient amount of leaf material. Genotype was not considered

in plant selection, because an insufficient number of plants flowered for several genotypes within the ploidy–watering treatment combinations.

Leaves were dried for 72 hours, milled, and 5-7 mg samples were weighed out. Samples were analyzed with a Flash EA 1112 CHNS Analyzer (Thermo Scientific, Waltham, MA), calibrated with orchard leaves standard (LECO Corporation #502-055, C= 50.7%, N=2.39%). Samples were run in duplicate for each plant, and check standards were run after every 12 samples. Average total %N, total %C, and relative percent difference (RPD) were calculated from the duplicate runs. If the RPD was less than 10%, the mean value was used. In a few cases the RPD was greater than 10%, with one sample judged to have unreasonable C or N values, and the value judged to be correct was used.

Statistical Analyses

Gas Exchange Measurements

Gas exchange measurements and SLA were analyzed in SAS (Version 9.3, SAS Institute Inc.) using PROC MIXED with the REML method. The initial rhizome weight of each clone was log transformed and used as a covariate. An interaction between initial rhizome weight and watering treatment was included to account for different variances within the watering treatments. Photosynthetic rate, transpiration rate, WUE and SLA were all log transformed. Fixed effects included ploidy level, watering treatment, temperature treatment, and all two-way interactions between these main effects. Genotype within ploidy and date of measurement were considered random effects. The significance of random effects was determined using -2 log likelihood ratio tests, removing each random effect in the full model with replacement. The Chi-squared test

statistic (1 df) was obtained by calculating the difference between -2 log likelihood values of the full and reduced models.

Leaf Nutrient Analysis

Analysis of leaf nutrient composition was also conducted using the REML method in PROC MIXED (SAS Version 9.3, SAS Institute, Inc.). Initial rhizome weight and the interaction between rhizome weight and watering treatment were included as above. Percent C, %N, and C:N were all log transformed prior to analysis. Watering treatment, ploidy, and the interaction between watering treatment and ploidy were considered fixed effects, and block was considered a random effect. Since these plants were fertilized at the end of July, it was expected that date may have an impact on nutrient content of the leaves collected between the end of July and mid-September; however, date was excluded from the model because it was not significant and the model with date included had a higher AIC. The significance of block was tested in the same way as random effects for gas exchange measurements.

RESULTS

Physiological Measurements

Photosynthetic rate, transpiration rate, WUE, and SLA were all significantly affected by water availability (Table 1a). Plants exposed to conditions simulating drought had a 167% higher photosynthetic rate, a 72% greater transpiration rate, a 19% higher WUE (Figure 2.1a), and 2% thicker leaves than those in the well-watered treatment (Figure 2.1b). Only photosynthesis depended on temperature, with plants in ambient temperature experiencing a 30% greater photosynthetic rate on average. The

combination of water availability and temperature treatment had a significant effect on both photosynthetic rate and transpiration rate. Plants under high water availability had a similarly low photosynthetic rate and transpiration rate across temperature treatments. Plants exposed to drought, on the other hand, were sensitive to temperature, and their photosynthetic and transpiration rates increased 65% and 51% respectively in ambient compared to elevated temperature (Figure 2.2a and b).

Significant variation between genotypes within the ploidy levels was detected for all measured physiological traits. Date of measurement was significant for transpiration rate and WUE (Table 1b).

Leaf Nutrient Analysis

In terms of %N and C:N, the nutrient composition of leaves within the ploidy levels depended on water availability. Tetraploids' responses were less plastic than diploids'; they had a similar C:N regardless of watering treatment, while diploids had a 30% decreased C:N under drought compared to high water availability (Figure 2.3b). Total %N was greater for both ploidy levels under drought conditions than well watered; however, this response was stronger for diploids, which exhibited a 40% increase in %N (Figure 2.3a). On average, plants exposed to low water availability had 2% more C compared to plants in high water availability overall.

DISCUSSION

The striking difference in photosynthetic rate between high and low water availability may be explained in part by the ability of *Solidago altissima* to maintain physiological performance under drought conditions. In this study, WUE was higher regardless of

ploidy level in low compared to high water availability. Drought adapted plants may be able to maintain photosynthesis through manipulation of the carbon gradient between internal and external leaf C concentrations (Lambers et al., 2008 P. 53-54). This may be accomplished through greater allocation of resources to photosynthetic machinery, which manufactures a high demand for C. When stomata close under low water availability to limit evapotranspiration, the leaf internal-external C gradient becomes steep, counteracting the decrease in stomatal conductance resulting from stomatal closure. Thus a higher rate of photosynthesis may be maintained relative to water loss under low water availability, maximizing WUE (Hirose and Werger, 1987), potentially at the expense of nitrogen use efficiency (NUE) (Field et al., 1983; Sheng et al., 2001).

Such manipulation of the C gradient across the leaf surface requires a greater investment in N-demanding photosynthetic machinery and may explain why there was an increase in total leaf %N for both diploids and tetraploids in the drought treatment. Further, the higher concentration of N and lower C:N of diploid leaves exposed to drought suggest that they may have employed this strategy to a greater extent than tetraploids. Interestingly, although diploids and tetraploids differed in leaf N composition, they did not have significantly different photosynthetic rates. One explanation for the difference in leaf nutrient composition between diploids and tetraploids may lie in their contrasting life histories. In *Solidago altissima,* diploids flower earlier than tetraploids (Etterson et al. in preparation), and plants that employ a strategy of early flowering to avoid drought maximize their use of available resources to complete their life cycles before more extreme late season drought (Chaves et al., 2003).

As a result, higher leaf N concentrations are associated with plants that have a shorter growing season (Lambers et al., 2008 P. 303).

In wet conditions, nutrients may be leached from the soil at a greater rate; in this experiment high water availability negatively impacted both leaf nutrient composition and physiological performance. Plants in the well-watered treatment had reduced leaf nitrogen content, and their morphology (plants in the well-watered treatment were stunted with fewer leaves and less reproductive biomass (Chapter 1) and had yellowish leaves (personal observation)). When plants lack sufficient N or P, they allocate more resources to plant parts that acquire the lacking resource, such as roots, and less to plant parts that heavily demand the resource, such as chloroplasts (Lambers et al., 2008 P. 349). In this experiment, plants in high water availability presumably allocated the limited N available to structures that would help acquire additional resources and allocated available resources efficiently at the expense of WUE. Since leaf N is often correlated with Amax (Reich et al., 2002), the capacity to photosynthesize would likely be reduced as a result. While separate leaves were used for measurements of photosynthetic rate and leaf nutrient composition, it seems clear that plants in the well-watered treatment had lower N content than those in drought and that this negatively impacted photosynthesis. In addition, Abrahamson and McCrea (1985) found that leaves and inflorescences are major sinks of N in *S. altissima*, and some N from leaves is reallocated to inflorescences with the difference made up from the soil. This tradeoff between leaf and inflorescence suggests that there was a deficit of N in the soil in the well-watered treatment since in the drought treatment leaf %N was higher and reproductive biomass greater (Chapter 1),

while plants in the well-watered treatment had low leaf %N, yet less reproductive biomass.

The significant effect of the interaction between temperature and drought on photosynthesis in this experiment underscores the importance of studying the combined effects of these climate variables. While a relatively high photosynthetic rate was observed under drought conditions, when combined with high temperature both photosynthesis and transpiration decreased. Similarly, in wheat (*Triticum aestivum*) the combination of increased temperature and drought caused photosynthesis to decrease more than either climate variable alone (Shah and Paulsen, 2003). This is likely due to different mechanisms affecting physiological processes under drought and high temperature. Plant response to drought typically involves stomatal closure, while high temperature can result in increases of photorespiration versus photosynthesis via increased fixation of O_2 versus CO_2 by Rubisco, decreased electron transport, or even damage to Photosystem II (Lambers et al, 2008 P. 60; Shah and Paulsen, 2003; Taiz and Zeiger, 2006 P. 210).

Significant genetic variation was detected for all physiological traits in this study, suggesting that each of these traits has the potential to evolve. If any of these traits are targets of selection or indirectly selected on through correlations with other traits, then in a hotter, drier environment, they may be able to evolve provided this genetic variation is heritable in the new environment.

On a larger scale, models show that net primary production (NPP) will likely be limited by the combined effects of climate factors, especially temperature and water

availability, in areas with extreme summer heat and drought (Churkina and Running, 1998; Ciais et al., 2005; Zhao and Running, 2010). This has implications for the ability of vegetation to sequester CO_2, ecosystem processes, and the global food supply (Zhao and Running, 2010). Considering the effects of these climate variables' interaction on plant physiology and the resulting widespread impact on terrestrial ecosystems, it is important to identify climate factors that are likely to impact species in a particular area and study them in conjunction with one another.

Environmental stress limits the distribution of plant species, and understanding the underlying physiology of stress injury and tolerance will be necessary to elucidate the potential effects of climate change on plants (Taiz and Zeiger, 2006 P. 671). The results of this study, taken in conjunction with the previous chapter, illustrate the importance of an integrated approach to understanding how plants will respond to environmental change. Physiological differences between ploidy levels that impact their ability to survive and reproduce in a warmer, drier environment will have important implications for the many agricultural crops and ecologically important species that are polyploids. In addition, studying the combination of climate variables, especially those predicted to change, is essential as interactions between changing climate factors may have a greater or different impact than the effect of individual factors alone.

LITERATURE CITED

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Table 1.3. Predicted response to selection (*R*) for diploids and tetraploids calculated as the product of the heritability estimate and selection differential for each trait. Predicted response to selection is considered significant for a trait if there is significant heritability (H^2) and significant selection (*S*) for that trait (refer to Table 1.1 and Table 1.2).

	Analysis of Predicted Response to Selection							
	Diploid Treatment Combinations				**Tetraploid Treatment Combinations**			
	Dry-Ambient	Dry-Elevated	Wet-Ambient	Wet-Elevated	Dry-Ambient	Dry-Elevated	Wet-Ambient	Wet-Elevated
Trait	*R*	*R*	*R*	*R*	*R*	*R*	*R*	*R*
Julian Days to First Flower	**-0.09***	**-0.06***	0.02	0.00	**-0.11***	**-0.08***	-0.19	-0.22
Height (cm)	0.03	**0.02***	0.21	0.05	0.00	0.04	0.02	0.02
Leaf Number	0.05	0.03	0.23	0.11	0.05	0.04	0.23	0.18
Rosette Number	-0.02	-0.01	0.00	-0.05	0.00	0.00	0.00	0.00
Stomatal Density - Adaxial	0.02	0.05	**0.29***	0.06	0.01	0.01	0.07	0.00
Stomatal Density - Abaxial	0.00	0.01	-0.06	0.00	0.00	0.00	0.06	0.03
SLA - Apical	**-0.04***	0.01	-0.03	0.00	0.00	-0.05	0.01	0.00
SLA - Mid	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00
Stem Diameter	0.01	0.02	0.07	0.00	**0.05***	**0.07***	0.00	0.09

* Signifcant predicted response to selection for traits with significant heritablity and selection differential.

Table 2.1a. Mixed linear model test statistics (*F*) for physiological measurements taken 8/10/2011 - 8/12/2011 on diploid and tetraploid *Solidago altissima* subjected to drought or well-watered treatment and ambient or elevated temperature treatment. All values were log transformed, including covariates.

	Fixed Effect in Model												**Covariate**			
	Water		Temp		Ploidy		Temp x Water		Ploidy x Water		Temp x Ploidy		Clone Weight		Water*Clone Weight	
Factor	*df*	*F*	*df*	*F*	*df*	*F*	*df*	*F*	*df*	*F*	*df*	*F*	*df*	*F*	*df*	*F*
Photosyntheic Rate	1,111	**39.07*** **	1,103	**6.26***	1,42.1	1.12	1,106	**4.12***	1,105	0.00	1,91.5	0.09	1,107	**5.27***	1,116	0.21
Transpiration Rate	1,120	**10.69***	1,109	**4.79***	1,43	0.46	1,113	**4.08***	1,109	0.34	1,102	0.00	1,131	**3.09**†	1,126	0.29
WUE	1,99.1	**43.32*** **	1,101	0.11	1,98.6	1.39	1,109	0.62	1,98.4	0.34	1,105	0.16	1,99.8	2.61	1,99.8	**3.67**†
SLA	1,125	**7.82***	1,109	0.68	1,42.2	1.16	1,110	1.30	1,110	0.67	1,109	0.14	1,129	**8.66***	1,129	**9.02***

† $p < 0.10$; * $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$

Table 2.1b. Log-likelihood ratio statistics (χ^2) for full model including genotype(ploidy) and date vs reduced model excluding each of these effects individually with replacement. All values were log transformed.

	Random Effect in Model	
	Genotype(Ploidy)	Date
Factor	*χ^2 (df =1)*	*χ^2 (df =1)*
Photosynthetic Rate	**4.08***	2.35
Transpiration Rate	**12.29****	**5.46***
WUE	**6.95***	**6.95***
SLA	**7.00***	1.93

† p < 0.10; * p < 0.05; ** p < 0.001; *** p < 0.0001

Figure 2.1 Least squares means (SE) for traits that differed between *S. altissima* grown in contrasting water availability treatments: a) WUE, b) SLA.

Figure 2.2 Least squares means (SE) for physiological traits of *S. altissima* grown under well-watered (closed circle, solid line) and drought (open circle, dashed line) conditions that depended on temperature treatment: a) photosynthetic rate, b) transpiration rate.

Figure 2.3. Least square means (SE) for traits that differed in plasticity between diploid (closed circle, solid line) and tetraploid (open circle, dashed line) *S. altissima* grown in contrasting water availability treatments: a) leaf total %N, b) leaf C:N.

Figure 2.1

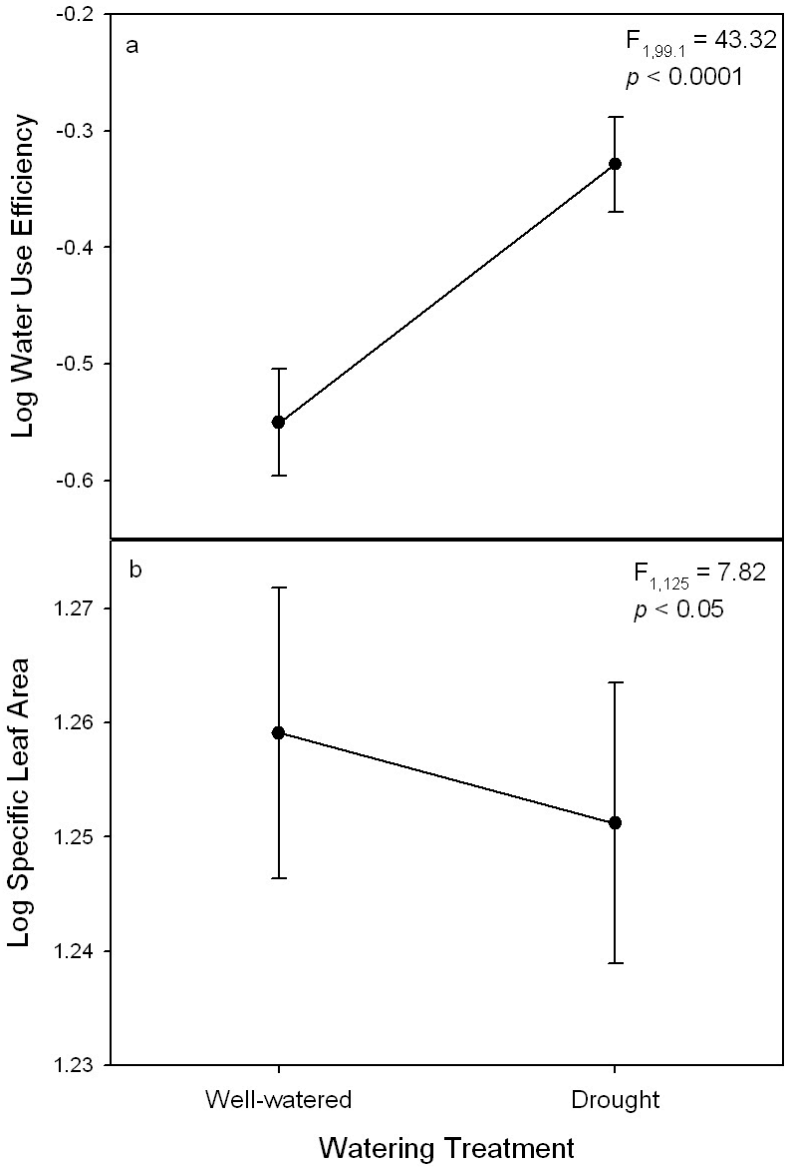

a
$F_{1,99.1}$ = 43.32
p < 0.0001
Log Water Use Efficiency
-0.2
-0.3
-0.4
-0.5
-0.6
b
$F_{1,125}$ = 7.82
p < 0.05
Log Specific Leaf Area
1.27
1.26
1.25
1.24
1.23
Well-watered
Drought
Watering Treatment

Figure 2.2

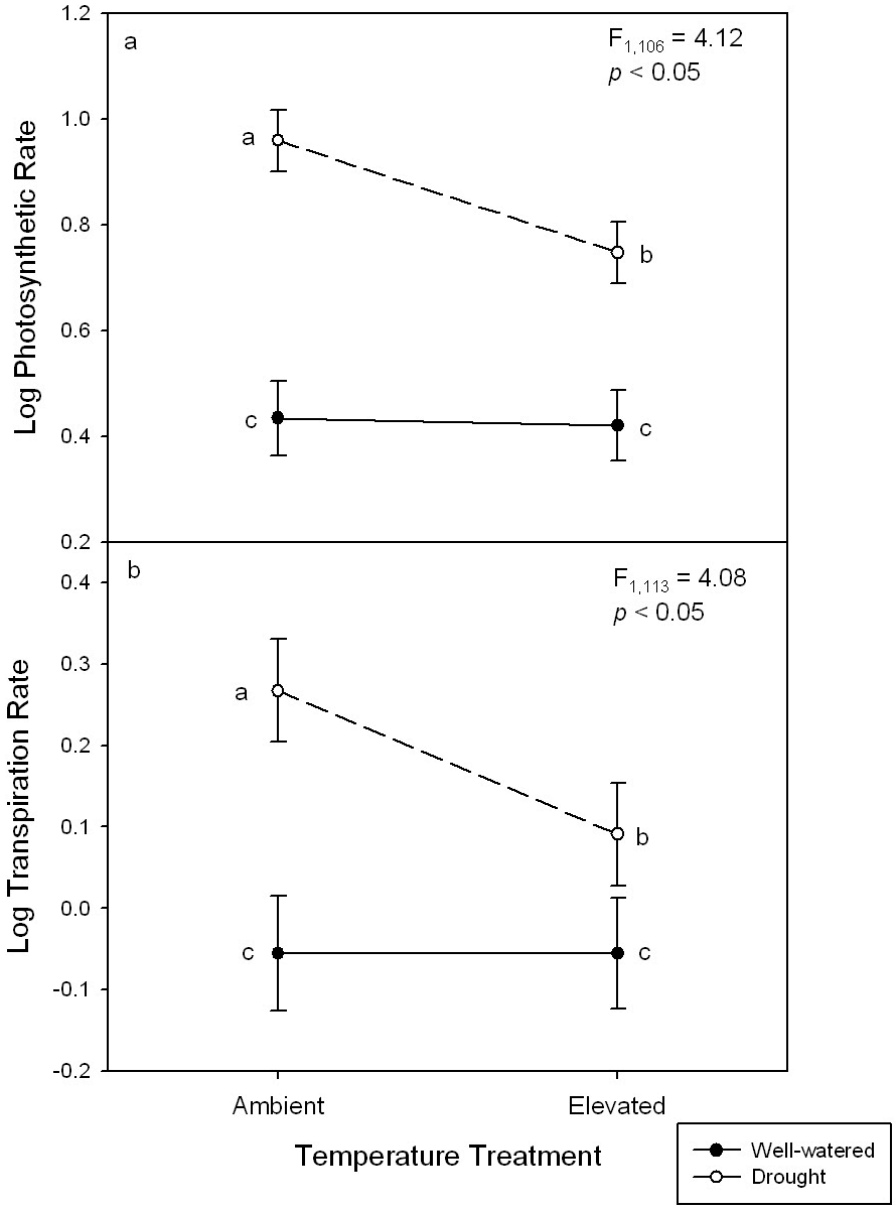
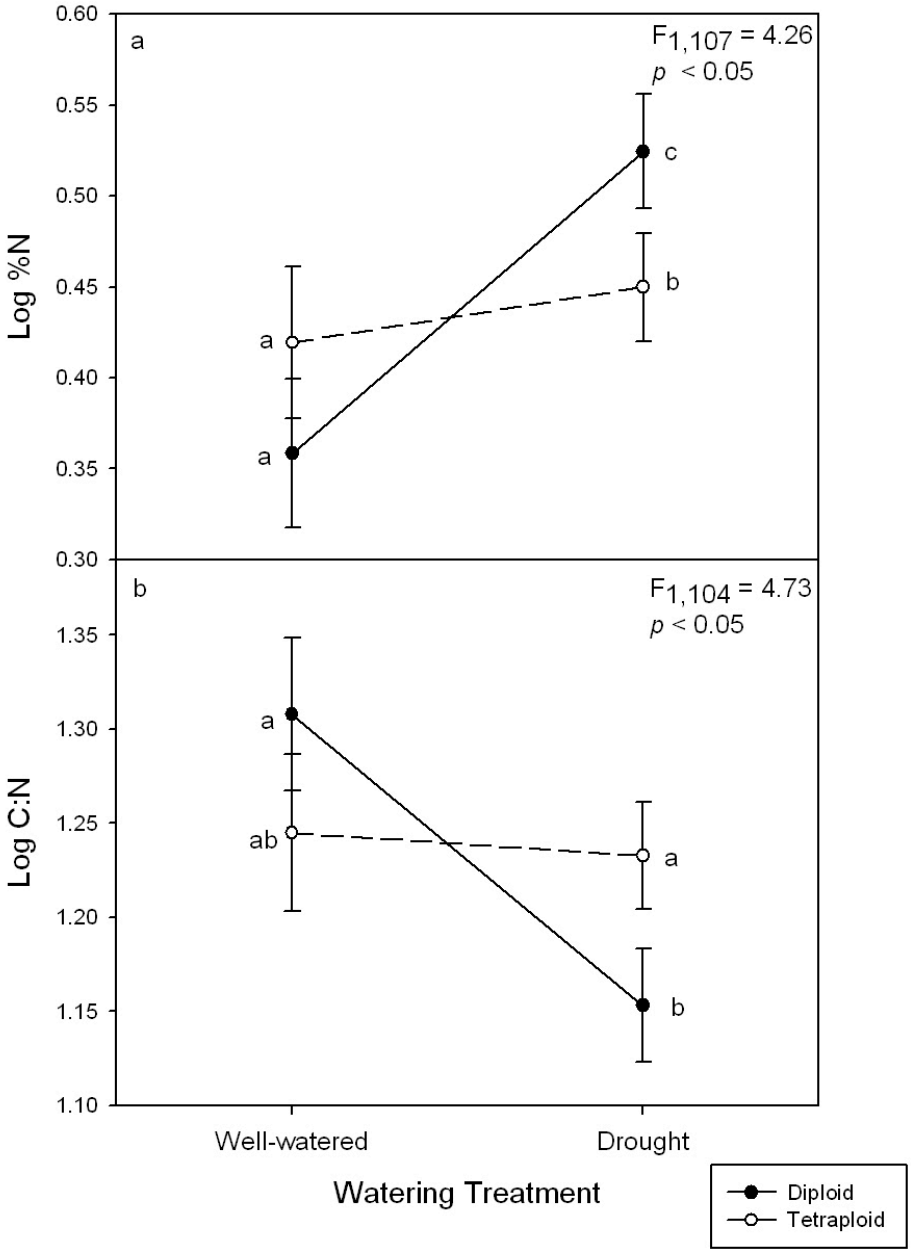

a
$F_{1,106}$ = 4.12
p < 0.05
Log Photosynthetic Rate
1.2
1.0
0.8
0.6
0.4
0.2
a
b
c
c
b
$F_{1,113}$ = 4.08
p < 0.05
Log Transpiration Rate
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
a
b
c
c
Ambient
Elevated
Temperature Treatment
Well-watered
Drought

Figure 2.3


a
$F_{1,107} = 4.26$
$p < 0.05$
c
b
a
a
0.60
0.55
0.50
0.45
0.40
0.35
0.30
Log %N
b
$F_{1,104} = 4.73$
$p < 0.05$
a
ab
a
b
1.35
1.30
1.25
1.20
1.15
1.10
Log C:N
Well-watered
Drought
Watering Treatment
Diploid
Tetraploid

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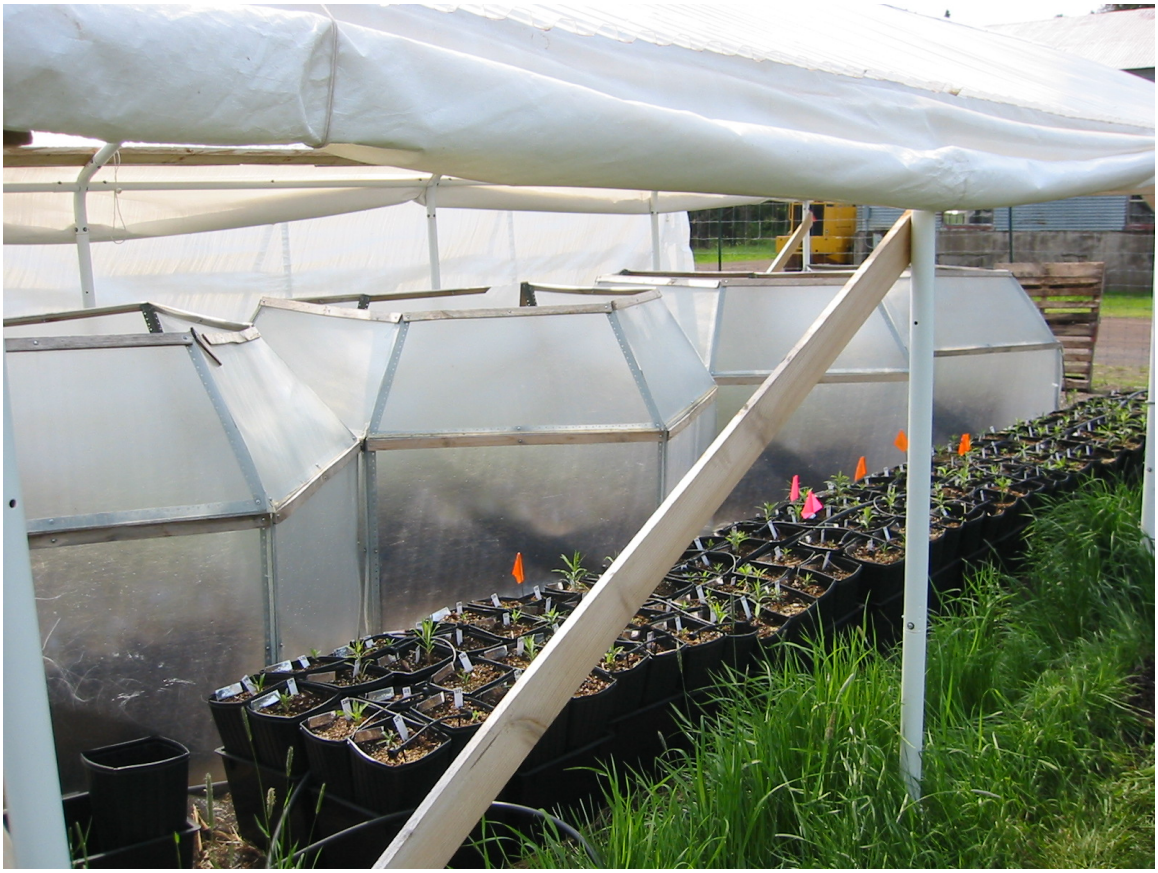
Appendix 1. Layout of elevated temperature chambers and ambient temperature treatment under 3.7 m x 7.9 m rainout shelters.



Appendix 2a. ANOVA test statistics (*F*) for morphological, physiological, and life history measurements taken on the date of first flower (or end of season for plants that did not flower) of diploid and tetraploid *Solidago altissima* subjected to drought or well-watered treatment and ambient or elevated temperature.

	Fixed Effect in Model												**Covariate**			
	Ploidy		Water		Temp		Ploidy x Water		Ploidy x Temp		Temp x Water		Log CloneWT		Log CloneWT * Water	
Factor	*df*	*F*	*df*	*F*	*df*	*F*	*df*	*F*	*df*	*F*	*df*	*F*	*df*	*F*	*df*	*F*
Julian Days to First Flower	1,52.4	**47.04*** **	1,118	**16.30*** **	1,96.1	**5.25***	1,51.2	**4.18***	1,36.2	0.89	1,149	0.94	1,181	**20.42*** **	1,165	**10.03***
Height	1,72.8	0.49	1,157	**492.41*** **	1,384	**22.70*** **	1,72.9	**5.46***	1,368	0.02	1,384	**5.11***	1,431	**86.27*** **	1,392	**78.61*** **
Stem Diameter	1,44.4	**5.50***	1,529	**937.73*** **	1,517	0.72	1,524	0.34	1,497	0.91	1,516	0.23	1,557	**98.46*** **	1,534	**51.92*** **
Leaf Number	1,41.9	**18.98*** **	1,93.3	**724.49*** **	1,513	0.25	1,41.2	0.46	1,525	2.11	1,514	0.01	1,553	**12.38** **	1,454	**13.09** **
Stem Branch Number	1,171	0.13	1,280	**153.94*** **	1,146	1.38	1,177	0.06	1,62.6	2.54	1,352	0.04	1,400	**7.14***	1,381	0.06
Basal Branch Number	1,364	0.49	1,364	**9.18***	1,368	2.41	1,364	0.06	1,370	0.37	1,368	1.02	1,366	0.98	1,365	0.01
Reproductive Branch Number	1,99.4	**16.48*** **	1,182	**530.10*** **	1,72.8	**7.24***	1,89.3	**3.82†**	1,46.7	0.71	1,413	1.80	1,497	**21.55*** **	1,467	**33.95*** **
Stomatal Density -Abaxial	1,39.4	**41.68*** **	1,82.2	**16.68** **	1,440	0.65	1,38.3	**5.70***	1,427	1.92	1,440	0.19	1,495	**3.14†**	1,404	**10.47***
Stomatal Density Adaxial	1,40.2	**18.07** **	1,540	**57.07*** **	1,37.4	0.38	1,521	0.34	1,37.1	**3.61†**	1,519	2.33	1,592	1.68	1,553	0.03
Vegetative Biomass	1,43.6	0.78	1,109	**1071.34*** **	1,528	**6.55***	1,46.8	0.67	1,518	0.07	1,528	**7.49***	1,479	**72.80*** **	1,392	**29.40*** **
Reproductive Biomass	1,64.8	**13.10** **	1,441	**632.48*** **	1,61.9	0.04	1,428	**3.84†**	1,36.9	1.50	1,431	2.47	1,491	2.50	1,454	**2.89†**
Total Biomass	1,43.6	0.16	1,578	**1138.92*** **	1,561	**3.49†**	1,568	0.02	1,558	0.01	1,561	**6.06***	1,484	**62.55*** **	1,586	**31.06*** **
SLA - Apical Leaf	1,63.1	1.48	1,103	0.03	1,52.4	0.10	1,67.8	**4.34***	1,42	0.03	1,130	1.78	1,142	0.16	1,141	0.80
SLA - Mid Leaf	1,48	**30.04*** **	1,102	0.00	1,415	**8.51***	1,47	0.91	1,447	0.00	1,414	0.21	1,462	0.34	1,434	**10.07***
Leaf Area -Apical Leaf	1,59	2.07	1,108	**14.43** **	1,72	0.94	1,59.1	**4.02***	1,37.5	0.32	1,132	**5.86***	1,143	**3.42†**	1,140	2.50
Leaf Area - Mid Leaf	1,84.8	1.47	1,174	**150.08*** **	1,509	**10.75***	1,84.8	**17.67*** **	1,505	0.73	1,508	2.50	1,520	0.05	1,519	**7.18***
Rosette Number	1,40.8	**27.53*** **	1,97.2	0.17	1,541	**5.73***	1,44.6	**11.35***	1,546	**5.71***	1,541	**6.50***	1,619	**2.84†**	1,358	0.24
Growth Rate	1,66.9	2.13	1,156	**439.55*** **	1,78.7	**16.70** **	1,71.4	**6.26***	1,37.9	0.61	1,354	2.10	1,439	**9.29***	1,375	**61.26*** **

† $p < 0.10$; * $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$

Appendix 2b. Chi square (χ^2) test statistics for random effects (df = 1). χ^2 values were calculated as the difference between -2 log likelihood values of the full model and a reduced model with each random effect removed with replacement.

	Random Effect in Model			
	Genotype(Ploidy)	Water x Genotype(Ploidy)	Temp x Genotype(Ploidy)	Block
Factor	χ^2	χ^2	χ^2	χ^2
Julian Days to First Flower	**10.96****	**3.43**†	0.00	**11.35****
Height	0.00	0.00	0.00	0.00
Stem Diameter	0.00	0.00	0.00	0.00
Leaf Number	2.13	1.38	0.00	0.00
Stem Branch Number	0.00	0.01	**3.67**†	0.00
Basal Branch Number	0.00	0.00	0.00	0.02
Reproductive Branch Number	0.00	**3.32**†	1.22	0.45
Stomatal Density-Abaxial	**9.14***	**3.59**†	0.00	0.23
Stomatal Density-Adaxial	**17.82*****	0.00	0.52	0.09
Vegetative Biomass	1.40	0.00	0.00	**5.85***
Reproductive Biomass	1.90	0.00	0.38	0.52
Total Biomass	1.71	0.00	0.00	2.64
SLA - Apical Leaf	0.00	2.39	0.19	0.20
SLA - Mid Leaf	0.21	1.61	0.00	0.00
Leaf Area - Apical Leaf	0.03	1.54	0.00	0.00
Leaf Area - Mid Leaf	0.00	2.57	0.00	0.01
Rosette Number	**16.73*****	0.00	0.00	0.90
Growth Rate	1.35	0.00	1.05	2.01

† $p < 0.10$; * $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$