

**Physiological Response of Heatwaves on a
California Native Species, *Heteromeles arbutifolia***

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ABSTRACT

Extreme weather events related to climate change, such as heatwaves, are predicted to increase in California. The impact of an increase in frequency and duration of heatwaves on Californian native shrubs has not been evaluated. Our research question investigates the physiological effects of a heatwave, and the differences in response between four populations of *Heteromeles arbutifolia*. We hypothesized that seedlings from southern sites would better withstand heatwaves because their parent sites experience a drier and warmer climate. We hypothesized seedlings from inland sites would better withstand heatwaves because their parent sites experience higher temperature extremes since there is no coastal fog buffering the temperature. We used two growth chambers to expose half of the plants to one artificial heatwave of 5°C above average spring temperatures seen in Berkeley, CA. The second chamber was the control. Each heatwave lasted 5 days with a 5-day recovery period. We measured stomatal conductance and chlorophyll fluorescence before, during, and after the heatwave. At the end of the experiment, we measured the total leaf area and xylem area of each plant to calculate the Huber value. Regardless of location, all plants responded to the heatwave treatment. Southern plants had significantly lower stomatal conductance than northern plants, $p= 3.25e^{-5}$. In order to self-regulate their energy balance, southern plants closed their stomata during the heatwave to prevent excess water loss from the leaf surface. There was no significant difference in stomatal conductance between inland and coastal plants. Huber value was significantly higher in plants from southern sites compared to northern sites, $p=0.027$. *H. arbutifolia* exhibited decreased stomatal conductance in response to an artificial heatwave, suggesting that an increase in the frequency and duration of heatwaves will have important impacts on this California native shrub.

KEYWORDS

Stomatal conductance, chlorophyll fluorescence, population differences, extreme temperature events, huber value

INTRODUCTION

Extreme temperature events related to climate change, such as heatwaves, are predicted to increase in California (Hayhoe et al. 2004). Global greenhouse gas (GHG) emissions due to human activity have grown 70% between 1970 and 2004 (Bernstein et al. 2007). By the end of the century, annual surface temperatures are expected to increase by 1.5 to 5°C under low GHG scenarios, and 3.5 to 9°C under high GHG scenarios (Hayhoe et al. 2004). Global warming has led to earlier timing of spring events, and upward shifts in plant niches (Bernstein et al. 2007). Warmer and drier conditions may threaten certain species and cause an overall loss in biodiversity. Mediterranean biomes, such as the one in California, is projected to experience the largest proportional loss of biodiversity of all terrestrial biomes due to multiple biodiversity threats and complex biotic and abiotic interactions between plants and the environment (Klausmeyer and Shaw 2009). There is high confidence that Mediterranean ecosystems will have less rainfall and exacerbated drought conditions due to the onset of global warming (Mooney and Dunn 1970).

The effects of climate change on California's native plant communities are varying in degree, and detrimental to certain life stages and species of plants (Padilla and Pugnaire 2007). Germinating seeds and young plants will be at greater risk to desiccation, due to their small root systems that are unable to tap into necessary water sources below ground. Seedlings are in the most vulnerable stage of life, and suffer from high mortality due to changes in temperature, competition, and pathogens (Padilla and Pugnaire 2007). Additionally, photosynthesis is sensitive to moderate heat stress due to temperature sensitive enzymes that do not function under high temperatures (Berry and Bjorkman 1980). A recent study found that heat stress of 5°C deactivates the protein Rubisco, which is responsible for driving photosynthesis (Salvucci et al. 2001). Under changing climate conditions, plant survival is unpredictable, and the long-term effects on plant communities are unknown (Padilla and Pugnaire 2007).

Woody shrubs living in California's Mediterranean ecosystems, such as *Heteromeles arbutifolia*, experience extremely hot and dry conditions during the spring and summer seasons (Padilla and Pugnaire 2007). Past studies have found that the high leaf temperatures due to summer heat stresses characteristic of Mediterranean climates are a limiting factor in *H. arbutifolia* seedling survival (Vallardes and Percy 2008). *H. arbutifolia* a California native

perennial shrubs found most commonly the chaparral communities. As mature adults, they grow to be several meters high, produce bright red fruit, and have strong sclerophyllous tissues. However, as seedlings they have pre-mature bark tissues that are structurally delicate (Balsamo et al. 2003). There is a need to better understand climate change affects a plant species differently depending on its geographical location. Populations of *H. arbutifolia* are widespread throughout California, and are great plants to study population differences on.

Plants handle changes to the environment either by adapting at the rate of climate change, or dying out with the next generation (Loarie et al. 2009). There are various ways that plants regulate their biological processes in response to the environment. Plants regulate the opening and closing of their leaf stomata as a primary method for controlling their water loss and maintaining plant energy balance (Shulze 1994). Stomatal conductance is measured as a rate of carbon dioxide and oxygen gas exchange between the inside chamber of a leaf stomata pore and the ambient air. Stomata are highly sensitive to their microclimate in order to prevent drought-induced cavitation and desiccation (Ogasa et al 2013). Stomata are affected by many factors including temperature, humidity, light, wind, and soil water availability (Davies 2006).

In a plant leaf, increases in stomatal conductance are coupled to simultaneous increases in the net rate of photosynthesis (Jarvis and Davies 1997). The stomata pore uptake of CO₂ from the environment is a key molecule in the photosynthesis reaction, and so an increase in CO₂ concentrations in the leaf also increases the rates of photosynthesis (Davies et al). A standard in plant physiology is to measure chlorophyll fluorescence in order to assess the plants photosynthesis levels (Kooten and Snel 1990). Chlorophyll fluorescence measures the activity of photosystem II (Berry and Bjorkman 1980). Photosystem II captures photons from light, which can either be absorbed for photosynthesis, reflected as light, or dissipated as heat (Berry and Bjorkman 1980). A successful plant will be able to keep up with the pace of climate change and regulate its photosynthesis and stomatal conductance amidst heat and water stressed conditions (Loarie et al. 2009).

Another strategy that plants undertake to cope with their changing environments is adjusting the allocation of resources into building structural xylem tissues or leave tissues during development, based on the demands of their microclimate (Carter and White 2009). The standard term in plant physiology to measure xylem-to-shoot ratio is the Huber value (Davidson 1968). Previous studies report a decrease in root-to-shoot ratios as temperatures increased. Xylem

tissues are used in the storage and transport of water and nutrients (Davidson 1968). Water leaving the plant is under negative tension traveling through the soil, roots, xylem, leaf and finally exits in the air (Tyree and Sperry 1988). The xylem goes under more hydraulic tension as the water availability in the soil decreases or as transpiration rates increase (Tyree and Sperry 1988). Under climate change scenarios, long-term adaptations to water stress include reducing overall plant growth rates to accommodate for reduced rates of net photosynthesis and stomatal conductance (Davidson 1968).

The increase in surface temperatures and a decrease of water availability in the soil, have important physiological effects on plants with regards to stomatal conductance, photosynthesis, leaf energy balance, and transport of water through the soil-plant-atmosphere pathway (Tezet et al. 2003). My study investigates the physiological effects of a heatwave, and the differences in response between four populations of *Heteromeles arbutifolia* seedlings throughout California. Before, during, and after the heatwave I measured stomatal conductance and chlorophyll fluorescence. After the experiment, I measured xylem-to-shoot area for each plant, to calculate the Huber value.

My first hypothesis is that seedlings from southern sites will better withstand the heatwave by closing their stomata in order to prevent losing excess water vapor from their leaves. Since plants from southern sites are originally from a drier and warmer climate, I think they are better adapted to regulate their biological processes during heatwaves than northern plants. My second hypothesis is that seedlings from inland sites will better withstand the heatwave by closing their stomata in order to prevent losing excess water vapor from their leaves. Since plants from inland sites experience higher temperature extremes in nature due to a lack of coastal fog buffering, I think they are better adapted to heatwaves than coastal plants. My third hypothesis is that southern plants will have a higher xylem to total leaf area ratio (i.e. Huber value) than northern plants. In terms of resource allocation, this means that southern plants will allocate more resources to their xylem tissues that store nutrients and transport water, rather than to allocating resources to its leaves that will provide higher rates of net photosynthesis. Understanding how *H. arbutifolia* cope with extreme temperatures will provide better insights on how other native species tolerate heatwaves and are affected by climate change.

METHODS

Study site

To determine the differences of physiological responses between populations of Toyon located in different regions of California, seeds were collected from Northern Coastal (Mendocino County), Northern Inland (Butte County), Southern Coastal (San Diego County), and Southern Inland (San Diego County) sites in the winter of 2009. I was not involved in the seed collection process. Michal Shuldman, a graduate student in the lab, collected the seeds and provided me with the seed collection and storage methods, and pictures of the sites.

The northern inland site was located at Big Chico Creek Ecological Reserve. The Toyon plants were located on a hilly terrain with a lot of vegetation cover including Mixed Oak, Bay Laurel, Grey Pine, grasslands, and shrubs. The northern coastal site was located in Laytonville.

Table 1. Summary of latitude, longitude, maximum/minimum average monthly temperature, and average annual precipitation. Data was collected from the Western Regional Climate Center.

	Latitude	Longitude	Max. average monthly temp. °C	Mix. average monthly temp. °C	Average annual precip. (inch)
Northern Inland (Chico)	39°51'13"N	121°42'36"W	17.1	15.2	23.72
Northern Coastal (Laytonville)	39°41'87"N	123°22'41.32"W	22.2	4.3	41.66
Southern Inland (Wrightsville)	32°50'29.81"N	116°45'48.32"W	24.7	10.2	16.15
Southern Coastal (San Diego)	32°50'29.81"N	125°23'41.44"W	23.8	11.3	12.93

The southern inland site was located in Wright's field managed by Backcountry Land Trust. The southern coastal site was located at Elliot Chaparral Reserve in CA. The southern sites have less annual precipitation than northern sites (Table 1). Southern sites experience a desert-like climate with cold, dry winters and hot, dry summers. The average annual precipitation is less in the

southern sites than the northern sites. Temperature data was collected from the Western Regional Climate Center. Cool sites experience a fog buffer with lower temperatures.

Seed collection and extractions

Toyon fruit was collected from the southern sites on December 14, 2009 and from the northern sites on December 28, 2009. The fruit was clipped from the plant, placed in a ziplock bag, and kept in a cooler until it could be refrigerated. The seeds were stored in ziplock bags in a refrigerator at 4°C for approximately two to three weeks until seed extractions. At the University of California, Berkeley the seed was extracted from the fruit using a blender with the blades covered to break up the fruit. Then the seeds were picked by hand from the slurry and placed in a container of clean water. The seeds were left in the water for five to seven days. Then they were washed with a dilute bleach solution (1:10), rinsed with distilled water, and immediately planted in germination flats. The soil used was a custom blend of six parts loamy sand (American Stone and Soil, Richmond, CA), four parts peat moss (Sunshine Peat Moss), superphosphate (Green All, E.B. Stone, Suisun, CA), and calcium carbonate (Fisher Scientific). Plants were grown in a greenhouse at the University of California, Berkeley. Planting began on January 6, 2009 and ended on January 16, 2009. All of the planting occurred over a 10-day period, there was seven different planting events during this period, for a total of 200 plants. The staggering of the planting episodes did not matter to the study because the time frame that all the plants were planted in was small.

Greenhouse growing conditions

To prepare the toyon for the growth chamber experiment, plants were allowed to mature into seedlings for two years in a natural light greenhouse. During the two years, the plants were monitored for pests and mold, and well-watered in the greenhouse. There were no problems with pests or mold during the two years that the plants grew to seedlings. Greenhouse conditions were that of a mediterranean climate since plants received the same temperature and lighting conditions. The average annual maximum temperature was 18.3°C, and the average minimum temperature was 9.6°C. The temperature data is from the Western Regional Climate Center. The

plants received natural sunlight from the cycle of the day and seasons in Berkeley, CA. The greenhouse is located on the 6th floor of the University of California, Berkeley's Valley Life Science Building. The temperature and humidity levels for the two years that the seedlings grew in are shown in a Figure.

Growth chamber preparation

I used two growth chambers, one as a control and the other as a treatment, to control for the temperature and light environment. The growth chambers I used were VWR Signature™ Diurnal Growth Chamber, Model 2015 and they measured 7 feet by 5 feet by 10 feet. I randomly selected 32 from the 100 seedlings for the experiment. Each growth chamber had 16 plants total, 4 from each site (e.g. 4 from Chico, 4 from Laytonville...ect.).

In this experiment, we simulated a spring heatwave, so the plants were well watered. We kept water, light, and ambient air constant in both chambers. I used a hydrosense to measure the volumetric water content of the soil (percentage units), in order to ensure all plants were uniformly watered.

The light cycle was 12 hours on and 12 hours off using nine 60-watt light bulbs. The lights turned off at 17:00 and turned at 7:00. Temperatures in the control chamber ranged from 19°C to 28°C, which was the same temperature range the seedlings experienced growing up in the greenhouse during spring (Table 2). Temperatures in the treatment chamber ranged from 24°C to 33°C, which is 5°C greater than the control settings to mimic a spring heatwave.

Table 2. Temperature settings for control and treatment growth chambers.

Time of day	Control Chamber	Heatwave Chamber
7:30	19°C	24°C
10:30	22°C	27°C
13:30	24°C	29°C
16:30	26°C	31°C
19:30	28°C	33°C

Experiment

I moved the plants into the two growth chambers in July 7th 2011, and allowed them to acclimate to the new environment for 19 days before the experiment began. The plants acclimated in the control chamber setting of 19°C to 28°C, with a 12-hour light cycle (Table 2).

The plants experienced one heatwave lasting 5 days with a 5-day recovery period. During the recovery period, the settings defaulted to control temperatures.

Measurements and Calculations

Michal Shuldman, a doctorate student at UC Berkeley, and I used a standardized data collection sheet to take measurements. The instruments were calibrated to the ambient air and humidity levels in the chambers before taking measurements.

Stomatal conductance

Measurements were taken before, twice during, and twice after the heatwave. Stomatal conductance measurements were taken using a SC-1 leaf porometer. Conductance measurements were taken five times a day at 7:30, 10:30, 13:30, 15:30, and 18:30 (Table 3).

Table 3. Experiment time line with measurements

Experiment day	Time point relative to heatwave	Measurements taken
Day 1	Pre-heatwave	Conductance & Fluorescence
	Heatwave start at 22:30	
Day 2	Heatwave	Conductance
Day 4	Heatwave	Conductance & Fluorescence
	Heatwave end at 22:30	
Day 8	Post-heatwave	Conductance & Fluorescence

Chlorophyll fluorescence

Chlorophyll fluorescence measurements were taken using a waltz mini-pam, photosynthesis yield analyzer and leaf clip 2030-B. Measurements were taken before, twice during, and twice after the heatwave. Chlorophyll fluorescence measurements were taken twice a day at pre-dawn and midday, 05:30 and 13:30 respectively.

Huber value, secondary traits

At the end of the experiment, I measured the xylem and total leaf area of each plant to calculate the Huber value. I used a sharp razor blade to cut the stems off of the plant, and thinly slice cross-sections, consistently cutting near the petiole, or base of the leaf. I placed three to seven slices of the cross sections of the stem into small glass vials with 95% ethanol solution. I capped the vials and labeled them for imaging under an electron microscope at 2.5X to 5X magnification (Figure 1).

Within 9 days, a graduate student in the lab imaged the cross-sectional slides of the main stem and saved the images to an external hard drive. I analyzed the images using ImageJ (Apple Inc. Cupertino, CA) to calculate the xylem area.

To calculate the total leaf area of each plant, I used a sharp razor to cut all the leaves off. Then, I placed the leaves onto a clear projector sheet and scanned them onto the computer. The leaves were analyzed using ImageJ (Apple Inc. Cupertino, CA) to calculate the total leaf area for each plant.

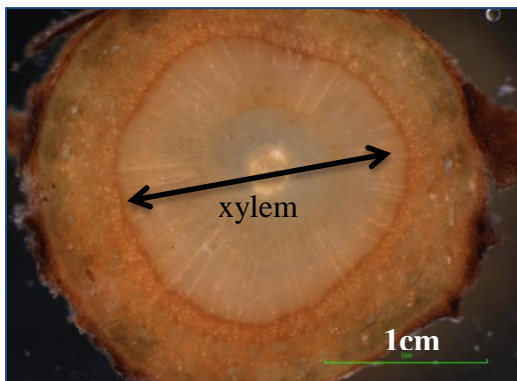


Figure 1. Cross-section of Toyon main stem under an electron microscope at 2.5X to 5X magnification.

Huber value calculations

Shown below is the calculation of the Huber value using total leaf area and xylem area.

$$\text{Total leaf area (mm}^2\text{)} \div \text{xylem area (cm}^2\text{)} = \text{Huber value (mm}^2\text{cm}^{-2}\text{)}$$

Data analysis

Analysis of variance (ANOVA) was used to test for differences between populations. To test for significant differences between experiment time points, I ran a pair-wise ANOVA for plants in the control and treatment group separately. Within the treatment group, I ran a two-sided independent sample t-test between coastal and inland populations, and again for northern and southern populations. Looking at the Huber values, I ran a pairwise ANOVA to compare the Huber values between the four regions. I also ran an independent sample t-test to compare between northern and southern populations, as well as between inland and coastal populations.

RESULTS

I found that the control plants were not behaving like controls relative to the treatment plants, most likely because of the small sample size. I threw out all of the control plant data, and used the pre-heat measurements as the “controls” for the treatment plants.

Effects of heatwave

I ran a pair-wise ANOVA to determine the differences in stomatal conductance between the experiment days (preheat1, heat1day2, heat1day4, postheat1day1, postheat1day8). There are highly significant differences between the first day after the heatwave compared to the second day of the heatwave (p-value= 0.001), and also the fourth day of the heatwave (p-value= 0.001). There are significant differences before the heatwave compared to the second day of the heatwave (p-value=0.001), the first day after the heatwave (p-value= 0.025), and the eighth day after the heatwave (p-value=0.001) (Figure 1). Looking at the boxplot of stomatal conductance

plotted throughout the duration of the heatwave, there are noticeable trends of high and low stomatal conductance. Stomatal conductance before the heatwave begins is high at 735.18, and then drops to 422.12 during the second day of the heatwave. Stomatal conductance remains low on the fourth day at 545.78. Immediately after the heatwave is over, the plants return to high stomatal conductance values at 904.89, higher than before their baseline values before the heatwave. On the eighth day after the heatwave, stomatal conductance values drop again to 552.35 which are similar to the preheat values (Figure 1).

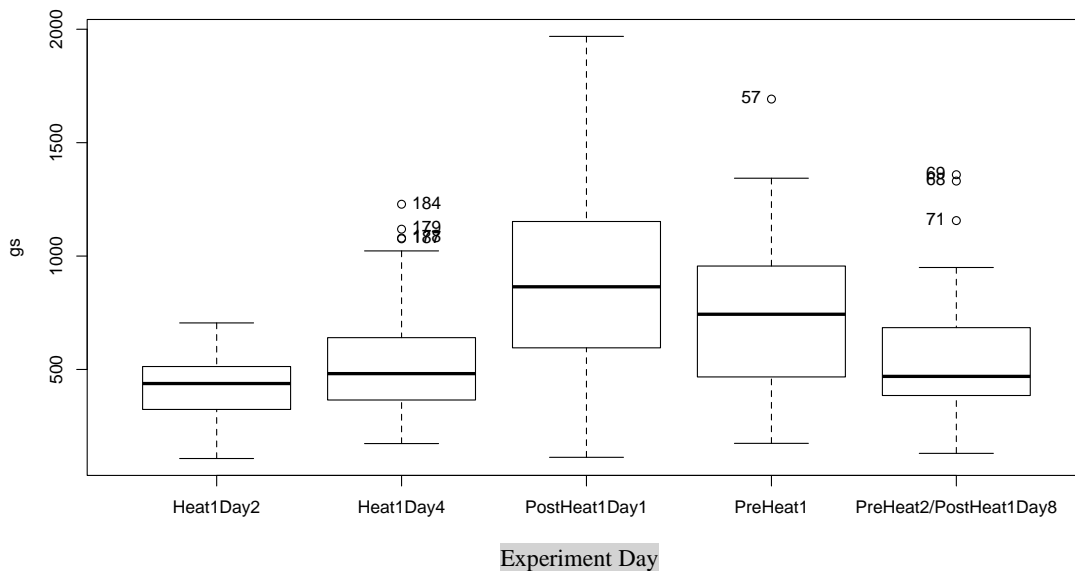


Figure 1. Stomatal conductance (gs) versus experiment day for plants in the treatment group.

Population differences

There was a significant difference ($t= 4.24$, $p= 3.25e^{-5}$) in stomatal conductance between the northern ($M= 724.65$ $SD= 344.75$) and southern sites ($M= 539.40$ $SD= 329.75$), but not between inland and coastal populations. Southern plants had significantly lower stomatal conductance than northern plants during all time points of the heatwave (Figure 2). Only the second day Figure is shown because they all look similar.

Northern and southern differences throughout heatwave

Second day. I found a significant difference (p-value= 0.01, t= 2.69) between northern (M= 25.31 SD=97.71) and southern (M= 371.35, SD=156.58) sites.

Fourth day. I found a significant difference (p-value= 0.03, t= 2.26) between northern (M= 627.71, SD=267.95) and southern (M= 463.86, SD=233.93) sites.

Post-heatwave, first day. I found a significant difference (t= 2.09, p-value= 0.042) between northern (M= 1034.73, SD= 423.35) and southern sites (M= 775.06, SD= 436.35).

Post-heatwave, eighth day. I found a significant difference (p-value= 0.002, t= 3.32) between northern (M= 671.93 SD=301.14) and southern (M= 432.79, SD=183.84) sites.

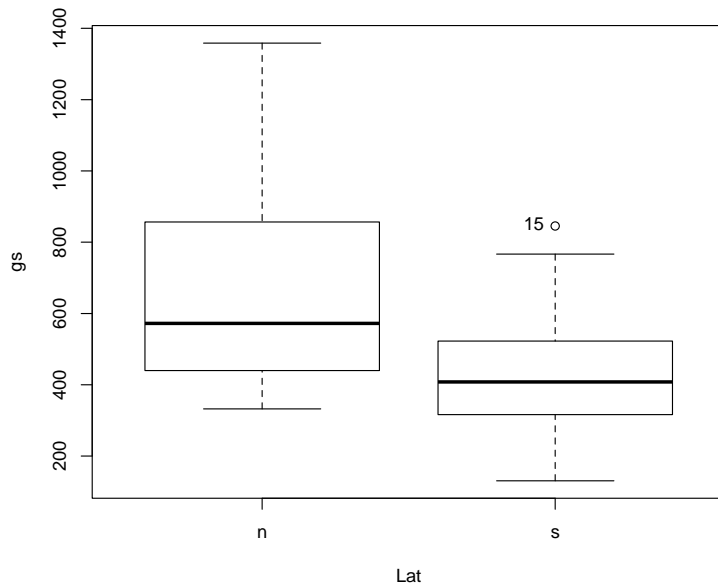
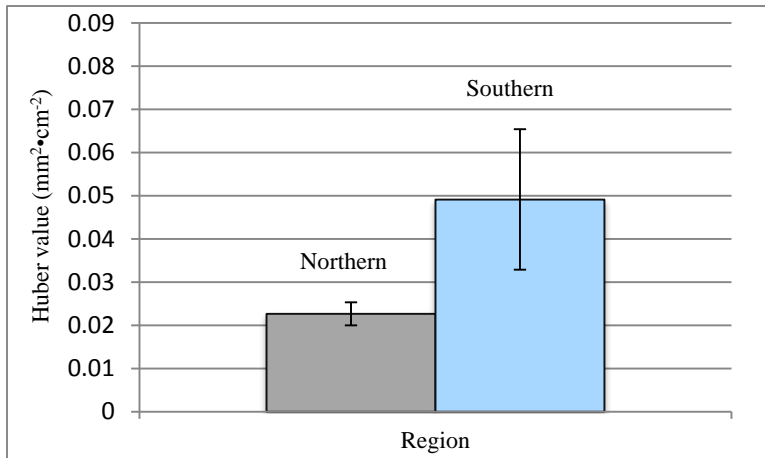


Figure 2. Stomatal conductance versus population for plants in the treatment group.

Huber value

Plants from southern sites had significantly lower ($0.011 \pm 0.003 \text{ m}^2$) total projected leaf area compared to northern sites ($0.015 \pm 0.001 \text{ m}^2$) ($p=0.048$). Southern plants had a significantly higher Huber value than northern plants ($p\text{-value}= 0.027$) (Figure 3).

Figure 3. Huber value versus region for all plants.



Chlorophyll fluorescence

Regardless of location, all plants responded to the heatwave treatment and had a stressed yield value less than 0.8. Northern and southern sites differed significantly in their responses, but coastal and inland sites did not. Northern populations had significantly higher chlorophyll fluorescence yield than plants from southern populations ($p\text{-value}= 0.016$).

DISCUSSION

Introduction

The data from the heatwave experiment confirms the first hypothesis that there are significant differences in stomatal conductance between northern and southern populations. Throughout the all experiment days of the heatwave, the southern plants had significantly lower stomatal conductance than northern populations. Contrary to the second hypothesis, I found no

significant differences between coastal and inland populations. Chlorophyll fluorescence yield data indicated that plants from northern regions were stressed, compared to plants from southern regions. The Huber value was significantly higher in southern compared to northern populations.

Stomatal Conductance

Southern populations had lower stomatal conductance throughout the heatwave, and this shows that they are better able to regulate their stomata during the heatwave. The plant strategy is to close their stomata to preserve water and prevent water stress. Parent plants from southern populations experiences dryer and warmer conditions in nature, experiencing low annual precipitation and high annual temperatures (Table 1). Plants southern sites have genetically selected for physiological traits and gene expression, which provide the tools for southern plants to better withstand extreme heat conditions (Strauss 2001). Past research suggest stomata are regulated with chemical signaling between the leaves and roots (Davies 2006). The plant hormone ABA, a stress hormone, is released when soil water availability is low and can effect the gene expression of plants during development to adapt to dry soil conditions (Downtown et al. 1988).

On the contrary, parent plants from northern populations experience a cooler and more temperate, which make them less adapted for heat stress conditions. During the heatwave, northern plants have significantly higher stomatal conductance than southern sites, which means that they are opening their stomata during the heatwave and losing more water vapor in the form of transpiration. Northern populations experienced more water stress during the heatwave because their stomata remained open, and the plants continued to transpire.

Huber value

Toyon plants have certain morphological characteristics that make them suited to withstand a heatwave (Valladares and Percy 2008). Their leaves have a medium-thick waxy layer on the top of the leaf, protecting it from heat, UV light and water damage. Mature plants have a thick, tough, and sturdy structural bark. However, the plants in the experiment were seedlings, so they did not have a mature bark or branch structure.

Southern populations had significantly higher Huber value than northern plants, which means that southern plants allocate more resources into building its xylem tissues to transport and store nutrients and water, as apposed to allocating more resources into its total leaf mass. Since southern populations come from warmer and drier environments than northern populations, it makes sense that southern plants build their structures to be more heat stress tolerant. Southern plants allocate more resources to building xylem which stores and transports water and nutrients, as a tradeoff for not conducting as much photosynthesis due to smaller leaf area.

Plants that live in water limited environments allocate more resources to building sturdy xylem that are more drought tolerant, because they can withstand more negative pressures before the water column in the xylem breaks (Sperry and Tyree 1988). Plants that allocate more resources to producing leaves have greater plant productivity, since the plant is producing higher net photosynthesis rates (Carter and White 2009). There is a tradeoff of whether it is favorable for plants to allocate resources to build the xylem tissues or to shoot tissues (Carter and White 2009).

Chlorophyll fluorescence

Northern populations had significantly higher chlorophyll fluorescence yield than plants from southern populations. This is most likely because northern plants had higher rates of stomatal conductance throughout the experiment, which increased the concentration of CO₂ and increased their net photosynthesis rates. Chlorophyll fluorescence data for all plants in the experiment showed that the plants were stressed before the experiment started. They had yield values below 0.83, which is the physiological threshold to measure stress in plants (Kooten and Snel 1990). This stress could be due to the low light conditions experienced by all plants in the growth chamber during the experiment. In the greenhouse, the plants were not experiencing low light conditions. They were receiving full sunlight exposure. Due to limited funds, the bulbs used in the growth chamber were 60-watt incandescent light bulbs and not adequate light for the plants to be not stressed. Even though the plants were allowed to acclimate to their chamber environment, they were not able to adapt to the low light conditions and started the experiment out being stressed.

Past research of drought stressed grapevines shows that photosynthesis was more strongly correlated with stomatal conductance than soil water availability (Medrano et al. 2002). However, there is still an unsettled debate about whether drought-stressed plants regulate photosynthesis by stomatal conductance, or metabolic impairment (Medrano et al. 2002). In a study of three woody tree species, water stress resulted in an uneven distribution of stomatal conductance over the surface of a leaf (Downtown et al. 1988).

Limitations

A limitation of the study is the small sample size, which made it difficult to detect significant differences in the data. In addition, the difference we found could be false-positives, which are due to individual differences and not necessarily traits from the sampled populations.

Future Research

In order to have a comprehensive understanding of how the plants responded during the heatwave, I could calculate the transpiration values as a better measure of plant physiology. Stomatal conductance is a key parameter in calculating transpiration, which is the total water loss from a plant leaf (Jarvis and Davies 1997). The results of the experiment highlighted the importance of considering heat waves in combination with other plant and environment interactions, such as atmospheric CO₂, nitrogen assimilation, and C¹³ intake.

Future research could look at genetic testing to determine if there is a genetic basis to explain the differences between southern and northern populations. This will answer the question of to what extent plants are adapting to their environment, and to what extent plants have genetically evolved to better perform in their natural climate. Future research could also look at the different ways in which plants respond to heat stress depending on what life-stage they are in when the stress is induced (germinating, seedling, fruiting-adult).

Toyon have C₃ photosynthesis, which means that they conduct photosynthesis during the day when their stomata are open (Jarvis and Davies 1997). Most plants have C₃ photosynthesis, and they thrive in areas with moderate levels of sunlight, moderate temperatures, and abundant ground water (Medrano et al. 2002). Future research questions could look at the extent to which

increasing surface temperatures due to climate change drives C₃ plants to start behaving like CAM plants photosynthetically (Nimmo 2000). CAM plants are able to adapt to warm temperatures by closing their stomata during the day, and opening their stomata for gas exchange at night (Nimmo 2000). Opening stomata at night when temperatures are cooler prevents the leaf from losing a lot of water due to high transpiration rates (Downtown 1988).

Future research could look at the consequences for Toyon if they are unable to physiologically adapt and better regulate their stomata to warmer conditions. Such consequences would be plant migration to higher altitudes up the slope where temperatures are cooler. The ecological niche where Toyon exists may begin to shrink as the climate heats up, especially in Mediterranean ecosystems (Klausmeyer and Shaw 2009). Future research could also document plant mortality rates through yearly surveys in California's Mediterranean ecosystem, keeping a close eye on native plant species.

Broader Implications and Conclusions

The increase in frequency and duration of heatwaves in California due to climate change have unknown effects on native plant species (Padilla and Pugnaire 2007). *Heteromeles arbutifolia* are found in abundance throughout California and respond to heat waves differently depending on their ability to regulate the opening of their stomata, which influences transpiration rates (Jarvis and Davies 1997). Global climate change has been increasing surface temperatures steadily, and this has unknown and potentially detrimental consequences for plants (Bernstein et al. 2007). Plants can live in a temperature range, but have to either evolutionarily adapt to the warmer surface temperatures, or become extinct (Loarie et al. 2009). I have aimed to answer some questions about how Toyon, a California native perennial woody shrub, will perform under heat stress depending on their parent site within a Mediterranean climate. With the changing climate, it is likely that Toyon will select for traits that are better suited for warmer temperatures. I found that plants originating from dry and warm climates will be better able to adapt to increased surface temperatures brought on by climate change due the better water use efficiency through regulating the closing of their stomata to prevent excess water loss that can dry plant tissues out and cause desiccation. With the onset of climate change, the most successful plants are the ones that are able to adapt and regulate their stomata by behaving more like CAM

photosynthesis, which is when plants open their stomata to photosynthesize during the night, and close their stomata during the day to prevent water loss due to high temperatures and little precipitation. Additionally, plants may allocate their resources differently depending on which growth stage the plant is in when the heat stress occurs. Plants who are originally from a temperate, cool, and moist climate that experiences an increase in surface temperatures, periods of drought, and high temperatures will respond differently to the onset of stressful events than a plant originally from an extreme climate such as in the desert.

In our changing climate, there is a need to better understand the strategies plants take to regulate their stomata to prevent excess water loss, and maintain energy balance (Ogasa et al 2013). Plants interact in complex ways with their environments, and there are many factors and perspectives to be considered when looking at the bigger picture (DeLucia et al. 2001). Climate change is happening, and this is driving up surface temperatures, which leads to an increase in the duration and frequency of drought periods and heat waves. Looking forward, it is important to consider the complex and interacting abiotic factors that work together to affect the plant physiology.

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