Leaf Thermal Tolerance in *Populus fremontii*: Local Adaptation and Plasticity Across its

Range in the Southwestern United States

by

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A Thesis Presented in Partial Fulfillment
of the Requirements for the Degree
Master of Science

Approved May 2022 by the
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ARIZONA STATE UNIVERSITY

August 2022

ABSTRACT

The southwestern US will experience more frequent heat waves, prolonged droughts, and declining water supply. Riparian ecosystems are particularly at-risk under climate change predictions, but little is known about the thermal tolerance of plant species inhabiting these ecosystems. *Populus fremontii*, a pioneer and foundation tree species in riparian ecosystems throughout the southwest, is of concern given its importance in driving community structure and influencing ecosystem processes. This study compared leaf thermal tolerance across populations of *P. fremontii* to determine if local adaptation affects leaf thermal tolerance. I hypothesized that warm-adapted (low-elevation) populations would have larger leaf thermal tolerance thresholds, thermal safety margins, and thermal time constants than cool-adapted (high-elevation) populations. I expected warm-adapted populations to maintain lower maximum leaf temperatures due to local adaptation affecting leaf thermal regulation. Using a common garden at the warm edge of this species' range, I measured leaf thermal tolerance metrics in eight populations spanning a 1,200 m elevational gradient. Data collection occurred in May, during mild air temperatures, and in August, during high air temperatures. The first two metrics were leaf thermal tolerance thresholds. The critical temperature (T_{crit}) is the temperature at which the electron transport capacity of PSII is disrupted. T_{50} is the temperature at which the electron transport capacity decreases to 50%. The next metric was thermal safety margins (TSMs), which reflect a leaf's vulnerability to reaching thermal tolerance thresholds. TSMs are the difference between either T_{crit} or T_{50} and an experienced environmental variable such as leaf or air temperature. The last metric was the thermal time constant (τ), which is a trait that represents how quickly leaf temperatures respond to changes in air

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temperatures. T_{crit}, T_{50}, and τ were not correlated with elevation regardless of season, suggesting that acclimation or phenotypic plasticity is affecting these metrics. Conversely, TSMs using maximum leaf temperature were negatively correlated with elevation in August because warm-adapted populations maintained lower maximum leaf temperatures. These findings suggest that warm-adapted populations are locally adapted to maintain cooler leaf temperatures, which is critical for their future survival since they do not maintain higher leaf thermal tolerance thresholds than cool-adapted populations.

DEDICATION

To my parents, sisters, and grandparents who cheered me on throughout this entire process; without you I would not be where I am now. A special thanks to Casey Smith, not just your constant support, but for teaching me how to teach myself when I needed it the most. And finally, to everyone throughout my life who has fostered my passion for the natural world and all it holds, I am grateful and appreciative of you all.

ACKNOWLEDGMENTS

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INTRODUCTION

Background

NOAA National Centers for Environmental Information (2022) determined that, globally, 2021 was the sixth warmest year on record, and that North America had an annual temperature that was 1.40 °C above the 1910 to 2010 average. June 2021 was also North America's warmest month on record being 2.01 °C above average (NOAA, 2022). The southwestern United States is particularly affected by these increasing temperatures, and is expected to continue experiencing increases in drought, wildfires, and heat-wave frequency (Seager *et al.*, 2007; Garfin *et al.*, 2014; Gonzalez *et al.* 2018). Because of the threat that climate change poses to ecosystems that already experience climate extremes, it is important to investigate how organisms living in these ecosystems currently handle environmental stressors, and how they might be able to acclimate or adapt to future conditions.

Although the impacts of climate change are widely recognized, a better understanding of how foundation plant species might adapt and survive the threats posed by increasing global temperatures is urgently needed. The responses that foundation species have to climate change will directly affect future ecosystem dynamics, therefore having a comprehensive understanding of these responses can lead to better land management and conservation decisions. Plant biological processes such as growth, reproduction, and photosynthesis are fundamentally dependent on temperature (Geange *et al.*, 2021). Temperature is also considered one of the most important determinants of plant species adaptation and distribution globally (Nievola *et al.*, 2017), so thermal tolerance needs to be included in conservation decisions in order to better predict how

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plant populations and their surrounding communities might change as temperatures continue to rise. In a recent review, Geange *et al.* (2021) found that while most studies that investigated the heat tolerance of photosynthetic tissues occurred in warmer biomes, there were still more studies in these regions studying cold tolerance than heat tolerance. This pattern was even more prominent in cooler biomes where extreme heat events are also predicted to occur under current climate projections (Walsh *et al.*, 2014; Hayhoe *et al.*, 2018). By investigating the heat tolerance of plants, and strategies that they may use to cope with intensifying environmental conditions, we can better predict how population dynamics will change over time and how communities and ecosystems will be affected.

Maintaining and regulating leaf temperature is an important strategy that plants use to cope with thermal stress. The air temperature thermal limit for tissues in vascular plants is believed to generally be around 60 °C, but it is sometimes higher in species that routinely experience extreme heat in the summer (Osmond *et al.*, 1987; Hatfield & Prueger, 2015; Nievola *et al.* 2017). The air temperature at which irreversible damage to photosystem II (PSII) occurs—a leaf's physiological thermal tolerance for conducting photosynthesis—generally falls between 40 and 60 °C (Feeley *et al.*, 2020). There is often a difference between leaf temperature and air temperature, the pattern of which is not consistent across all species, which can impact a plant's ability to cope with excessive heat or drought (O'Sullivan *et al.*, 2017, Perez & Feeley, 2020). Typically, leaf temperatures above 50 °C can cause irreversible damage to PSII (e.g., Curtis *et al.*, 2014, 2016; Knight & Ackerly, 2002; Krause *et al.*, 2010; O'Sullivan *et al.*, 2017; Perez & Feeley, 2020; 2021; Zhu *et al.*, 2018), so plants that repeatedly experience extreme air

temperatures close to or above 50 °C must reliably cool their leaves below ambient temperature to avoid damage to PSII.

'Thermal tolerance' itself is a term that can be applied to many different types of data that are not always fully compatible for comparison across studies (Geange *et al.*, 2021). Here, thermal tolerance is defined as the air temperature beyond which PSII experiences substantial or lasting damage. Past research has shown that photosynthetic thermal tolerances vary among species, and that they are generally correlated with latitudinal gradients in temperature (e.g., O'Sullivan *et al.*, 2017). Methods of studying thermal tolerance since the 1960s have ranged from techniques such as quantifying tissue damage visually, measuring photosynthetic gas exchange and tracking the recovery of photosynthetic capacity after stress exposure, monitoring heat shock proteins, and more (Geange *et al.*, 2021). This study uses chlorophyll fluorescence measurements, which is the amount of light energy reflected by PSII and therefore not absorbed for photosynthetic purposes (Maxwell & Johnson, 2000), to quantify leaf thermal tolerance thresholds. Measuring how chlorophyll fluorescence changes across a temperature gradient can pinpoint the temperature at which the electron transport capacity of PSII is initially disrupted (critical temperature or T_{crit}; Knight & Ackerly, 2002; O'Sullivan *et al.*, 2017) and the temperature at which there is a 50% decrease electron transport capacity of PSII (T_{50}; Knight & Ackerly, 2002; Sastry *et al.*, 2018). Knowing these thermal thresholds will help inform land-management decisions, species distribution modeling, conservation action plans, and other efforts to protect foundation plant species.

Light energy can either be absorbed and used by PSII or re-emitted as fluorescence, but not both. Therefore, chlorophyll fluorescence can be used to measure

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photosynthetic capacity and efficiency (Smillie & Nott, 1979; Maxwell & Johnson, 2000; Baker, 2008). Higher levels of fluorescence indicate that less light energy is being absorbed, and therefore used, by PSII. A recognized strength of measuring fluorescence is its ability to yield quantitative insight on how plants tolerate environmental stress and the extent to which that stress damages photosynthetic processes (Maxwell & Johnson, 2000; Baker, 2008). Focusing on the thermal tolerance of photosynthetic tissues in foundation plant species allows us to determine a temperature at which these species have an increased risk of losing photosynthetic capacity, which will in turn reduce plant productivity and negatively impact other organisms within the larger community.

To quantify thermal tolerance in this study, I measured minimum chlorophyll fluorescence (F_0)—the fluorescence level of a dark-adapted leaf when all PSII reaction centers are open (Baker, 2008)—along a steady and linear temperature gradient (see Table 1 for abbreviations and definitions). When F_0 is measured along a thermal gradient, the resulting temperature-dependent fluorescence response (T-F_0) can be used to calculate T_{crit} and T_{50} (Knight & Ackerly, 2002). There is a slow and fast rise portion of the T-F_0 curve, and T_{crit} is the temperature at which the inflection point between slow and fast rise phase of T-F_0 occurs (Fig. 1). Functionally, T_{crit} is the temperature threshold at which electron transport capacity of PSII is disrupted (Knight & Ackerly, 2002; O'Sullivan *et al.*, 2017), and this drop in photosynthetic capacity is what sparks the rapid increase of fluorescence. Because T_{50} is the temperature threshold at which there is a 50% decrease in the electron transport capacity of PSII, T_{50} is the point in the T-F_0 curve where F_0 reaches 50% of its maximum value (Knight & Ackerly, 2002; Sastry *et al.*, 2018). Past research has shown that these two heat tolerance (H_{tol}) thresholds are highly plastic

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(Knight & Ackerly, 2003; Zhu *et al.*, 2018), generally correlated with large-scale

latitudinal (O'Sullivan *et al.*, 2017; Zhu *et al.*, 2018) and smaller-scale elevational

gradients (Feeley *et al.*, 2020; Slot *et al.,* 2021), increase with leaf age (Marias *et al.*,

2017), and vary seasonally as plants acclimate to changes in environmental conditions

(Zhu *et al.*, 2018).

 Leaf H_{tol} thresholds can also be used to calculate a thermal safety margin (TSM),

which is a temperature range that is used to determine an organism's vulnerability to heat

stress (O'Sullivan *et al.*, 2017). TSMs for leaves are calculated by finding the difference

between a physiological tolerance (T_{crit} or T_{50} in this case) and an environmental

temperature metric (Cook *et al.*, 2021). Examples of environmental temperature metrics

in recent works have included extreme leaf temperatures (Perez & Feeley; 2020; Cook *et

al.*, 2021), regional or local air temperatures (Curtis *et al.*, 2016; Sastry & Barua, 2017,

Cook *et al.*, 2021), air temperatures during extreme heatwave events (O'Sullivan *et al.*,

2017), and mean annual temperatures (Slot *et al.,* 2021). Regardless of which

environmental metric is used, the smaller the TSM is, the closer an organism is to

meeting its thermal threshold, and the higher its vulnerability.

 A plant's ability to regulate and stabilize its leaf temperatures can significantly

impact the vulnerability of photosynthetic tissues to heat damage. The thermal time

constant (τ) is a composite leaf trait that quantifies the thermal stability of a leaf by

calculating the ratio of a leaf's ability to store heat versus exchange heat with the

environment (Michaletz *et al.*, 2015). A shorter τ, measured in seconds, corresponds to

leaf temperature changing in response to air temperature more quickly, and larger τ

corresponds to a slower response time (Michaletz *et al.*, 2015, 2016; Slot *et al.*, 2021).

Either a short or long τ is not inherently better than the other; a leaf with a shorter τ might reach high temperatures quickly, but can cool down faster if air temperatures drop. A leaf with a larger τ will be able to maintain lower body temperatures than air temperatures for a longer period, but if the leaf overheats it will also take longer to cool down. When considering τ in the context of leaf H_{tol} and climate change, it follows that short τ species are expected to experience higher maximum and lower minimum temperatures more readily than large τ species living under the same temperature conditions (Michaletz *et al.*, 2016; Fauset *et al.*, 2018). It is unknown the extent to which τ is affected by local adaptation and plasticity, but it is an important leaf trait that can be used to more comprehensively understand how leaf thermal stability will change with regards to increasing temperatures.

Study Overview

In this study, I investigated the heat thermal tolerance of Fremont cottonwood (*Populus fremontii*) leaves, comparing populations across its range in Arizona. *Populus fremontii* is a dominant, phreatophytic tree species that is common in riparian ecosystems in arid and semi-arid regions throughout the southwest United States and northern Mexico (Taylor, 2000; Hultine *et al.*, 2020, Blasini *et al.*, 2022). It is recognized as a foundation species that drives community structure and influences ecosystem processes throughout its range (Whitham *et al.*, 2006; Ikeda *et al.*, 2017). This species also experiences high landscape genetic diversity which means that there is a certain degree of local adaptation and phenotypic plasticity occurring across populations that is potentially influencing leaf thermal tolerance (Cushman *et al.*, 2014; Cooper *et al.*, 2019; Blasini *et*

al., 2021; Sankey *et al.*, 2021). The degree to which the range and plasticity of leaf

thermal tolerance depends on factors such as seasonality or location within a single

species is currently unknown. It is important that leaf thermal tolerance within foundation

species like *P. fremontii* be studied because the structure and genetic diversity of their

surrounding communities will be affected by the foundation species' response to rising

temperatures (Whitham *et al.*, 2006). This is particularly urgent in the southwestern US,

which is already the hottest and driest region in the US, and is expected to get even hotter

and drier going forward (Seager *et al.*, 2007; Garfin *et al.*, 2014). I explored patterns of

local adaptation in leaf thermal tolerance across the broad elevational distribution in

which *P. fremontii* occurs using an experimental common garden located in Yuma, AZ,

which is the warm edge of this species' geographical distribution. I also compared my

findings in May and August to better understand how H_{tol} varies across different

populations seasonally, as well as between ideal and extreme temperature conditions.

Eight populations and 10 genotypes per population were chosen to be included in this

study. Previous work has shown that there is significant local adaptation and plasticity

within *P. fremontii* as a species and at the population-level (Cushman *et al.*, 2014;

Cooper *et al.*, 2019; Blasini *et al.*, 2021, 2022), this work aims to add information to that

growing body of knowledge in hopes of better informing conservation and land-

management decisions.

The leaf H_{tol} thresholds, T_{crit} and T_{50}, were used to calculate mean thermal safety

margins in conjunction with the following environmental temperature metrics at the

population level: (1) highest recorded leaf temperature, (2) maximum air temperature at

the common garden site during sampling days, and (3) the maximum air temperature at

the source population locations during the sampling months. Thermal time constants were calculated for each population in May and August as a separate indicator of potential response to thermal stress, as well as to see how τ changes seasonally on the same plants. The populations were categorized as either warm-adapted or cool-adapted. Warm-adapted here is defined as populations whose source locations experienced mean maximum summer temperatures above 40 °C for the last 30 years, while cool-adapted are those where mean maximum summer temperatures were below 40 °C (Blasini *et al.*, 2022; Fig. 2). I hypothesized that in a common garden setting, warm-adapted populations would have larger mean T_{crit} and T_{50} values, thermal safety margins, and thermal time constants than cool-adapted populations. Significant differences in thermal tolerance metrics across an elevational gradient would support my prediction that local adaptation plays a role in population level thermal tolerances. I predict that mean T_{crit}, T_{50}, and τ across all of the populations regardless of elevation will increase from spring to summer, indicative of seasonal acclimation of leaf thermal traits in *P. fremontii* as a species. For seasonal increases in TSMs to appear, T_{crit} and T_{50} would have to increase in August by a larger number of degrees than the maximum leaf temperature and air temperature will. By comparing the H_{tol} thresholds, TSMs, and τ over time across all populations, we can better predict whether *P. fremontii* will be able to acclimate to increasing temperatures as climate change progresses and to what extent local adaptation affects leaf thermal tolerance.

MATERIALS AND METHODS

Study Site

The scope of this study was to analyze the differences in leaf H_{tol} thresholds within one tree species across its elevational range in Arizona. My study site was an experimental common garden located at Mittry Lake near Yuma, Arizona (32°50'59.28" N, -115°30'25.92" W; 49 m elevation), and is maintained by the Bureau of Land Management (Cooper *et al.*, 2019). The common garden was originally established in October of 2014 and contained 4,096 propagated cuttings collected from 16 populations of *P. fremontii*. These populations represented the climatic and elevational range of the Sonoran Desert and Utah High Plateau ecotypes of the species (Ikeda *et al.*, 2017; Cooper *et al.*, 2019; Hultine *et al.*, 2020). Out of the original 16 populations in the common garden, eight populations from the Sonoran Desert ecotype and 10 genotypes from each population were selected to be included in this study. These populations represent a 1,158 m elevation gradient across *P. fremontii*'s range in Arizona. Yuma experiences a mean annual temperature of 22.8 °C (Cooper *et al.*, 2019), and will often experience summer maximum temperatures upwards of 40.0 °C (Fig. 2). Data were collected in May, when temperatures are generally mild, and in August, during the warmest time of the growing season. The common garden is well-watered through monthly flood irrigation, but the higher elevation populations would not naturally experience temperatures as high as they do in this common garden. The source locations of these populations have experienced maximum summer temperatures over the last 30 years ranging from 38.0 °C to 44.9 °C, with the four highest elevation populations—the cool-adapted populations—never experiencing temperatures above 40.0 °C (data obtained through PRISM,

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; Fig. 2). Exposing the cool-adapted populations to these extreme temperatures in a common garden setting allows for the investigation of the effects local adaptation have on leaf thermal tolerance within a single species.

Previous common garden studies have used transfer function analysis to estimate the changes in a trait as a result of population differences in environmental response relative to the garden environment (Grady *et al.*, 2011). Transfer distance is the difference in mean annual temperature (MAT), between the common garden site and the original source location (Grady *et al.*, 2011; Cooper *et al.*, 2019; Blasini *et al.*, 2022). The transfer distances for MAT increased fairly linearly across source populations (Table 2). Therefore, the results of the air temperature TSMs should reflect patterns of populations with smaller transfer distances/lower elevations having increased thermal tolerance in the common garden site compared to those with larger transfer distances/higher elevations.

Chlorophyll Fluorescence Measurements

In order to study temporal plasticity in leaf thermal tolerances, 3-5 fully expanded sun leaves were collected from each of the 80 individuals in May and August of 2021. During both collection periods, the leaves were immediately put in Ziplock bags and placed in a dark cooler with ice packs to be shipped overnight to Phoenix, Arizona, so that they could be processed at the Desert Botanical Garden (DBG). Care was taken to maintain the leaves under cool, but not frozen conditions until they were unpacked and placed in a refrigerator in the lab. Leaf samples were sent to the DBG two populations at a time in both May and August were processed within a two week period.

All chlorophyll fluorescence measurements were taken using a Closed FC-800-C FluorCam coupled to a TR2000 thermoregulator (Photon System Instruments, Drasov, Czech Republic). When the leaves initially arrived in Phoenix, one leaf from each of the 10 genotypes was randomly pulled out of its bag to be tested for the maximum quantum yield of photosystem II (F_v/F_m) in order to gauge their photosynthetic health. A healthy, dark-adapted leaf should have a F_v/F_m value of 0.83 (Maxwell & Johnson, 2000). Once the F_v/F_m of the leaves had been assessed, three 5.5 mm disks were punched out of every leaf and each disk was placed adaxial-side up in one of the wells in the thermoregulator's heating block. The wells were filled with 0.9 ml of distilled water prior to the leaf disks being placed. The heating block was placed inside the FluorCam with the lid closed for 30 minutes so that the leaves could become dark adapted before measuring for F_0. The thermoregulator was set to linearly increase from 30 to 60 °C over the course of an hour, while the FluorCam simultaneously was set to take F_0 measurements every 30 seconds. Five F_0 measurements were taken in bursts (one measuring flash every 40 ms for a total of 160 ms) every 30 seconds with low-level measuring flashes. These flashes measure ambient levels of fluorescence without closing PSII centers and pulling the leaf samples out of their dark-adapted state.

Calculating T_{crit} and T_{50}

All analyses were conducted using R version 4.1.2 and closely followed procedures from Arnold *et al*. (2021). Using the F_0 data, T_{crit} was calculated by conducting breakpoint regression and finding the temperature at which the inflection point between the slow- and fast-rise portions of the T-F_0 curve was located. In order to

do breakpoint regression calculations for T_{crit}, the T-F_0 curve had to be modified so that one F_0 value could be paired with one temperature value. Due to the measuring flashes in the FluorCam occurring milliseconds apart (5 flashes over 160 ms, repeated every 30 seconds), there was not always a unique temperature value for each F_0 measurement. To account for this, every cluster of five F_0 values were averaged, allowing for the averaged F_0 values to have a unique temperature value. The adjusted T-F_0 curves were then normalized to fall between 0 and 1, or between 0 and 100% of the maximum recorded F_0 value, following the procedure of Knight and Ackerly (2002). Breakpoint regression analysis was used to extrapolate the inflection point between the slow- and fast-rise phase regression lines, identifying T_{crit}. T_{50} was measured by identifying the temperature at which the normalized F_0 value most closely matched 50% of the maximum recorded F_0.

Thermal Safety Margins

For this study, TSMs were calculated using the difference between T_{crit} or T_{50} and (1) highest recorded leaf temperature during both May and August, (2) mean maximum air temperatures for the source populations during May and August of the sampling year, and (3) maximum air temperatures during sampling days in Yuma. Leaf temperature was recorded using thermal imaging. Three sun-exposed canopy leaves from each genotype were photographed using a FLIR ONE Pro (Third Generation, for iOS; Teledyne FLIR). These photographs were repeated three times a day in order to track daily leaf temperature patterns. Thermal images were taken during the morning (8:00 - 10:00), midday (12:00 - 14:00), and afternoon (15:00 - 17:00) in both May and August. The

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thermal images were analyzed using FLIR Thermal Studio Pro (Teledyne FLIR), and maximum recorded leaf temperature was compiled.

Monthly maximum regional air temperatures for the source population locations during May and August of 2021 were gathered from PRISM (http://prism.oregonstate.edu) to be used for the TSM with regional air temperature as the environmental metric. A weather station located at the common garden collected continuous air temperature data from May to August every 15 minutes at 2.0 m above the ground surface. This weather data was used to find the local maximum air temperature during the sampling days that would then be used to calculate TSMs.

Thermal Time Constants

Thermal time constants were calculated using the following equation from Michaletz *et al.* (2016):

$$\tau = \varphi LMA \left[\frac{c_{p,w}}{LDMC \cdot h} + \frac{c_{p,d} - c_{p,w}}{h} \right] \qquad (1)$$

Where φ is the ratio of projected-to-total leaf area (dimensionless), which for flat leaves is 0.5. LMA (kg m^{-2}) is the dry leaf mass per area (fresh), LDMC (kg kg^{-1}) is the leaf dry matter content, $c_{p,w}$ is the specific heat capacity of water (4,183 J kg^{-1} °C^{-1}), $c_{p,d}$ is the specific heat capacity of dry leaf matter (J kg^{-1} °C^{-1}), $c_{p,w}$ is the specific heat capacity of water (J kg^{-1} °C^{-1}), and h is an overall heat transfer coefficient (W m^{-2} °C^{-1}). For this study, h was calculated as $h = \rho_a c_{p,a} g_h$ (Slot *et al.*, 2021), where ρ_a is air density (kg m^{-3}), $c_{p,a}$ is the specific heat capacity of air (J kg^{-1} °C^{-1}), and g_h is heat conductance (mm s^{-1}). g_h was calculated as g_h = 1.5 x 6.62 x U/w where 1.5 is a factor to account for outdoor turbulence (Jones *et al.*, 2014; Slot *et al.*, 2021), U is the wind velocity (1.5 m s^{-1} here),

and w is leaf width (m). This version of h excludes the effects of radiation and transpiration, which allows us to highlight how morphological traits alone cause variation in thermal time constants (Slot *et al.*, 2021). A paired t-test was conducted to determine if there was a significant difference between the mean τ values in May and August. Given that there is known plasticity in leaf functional traits at the population level for *P. fremontii* (Blasini *et al.* 2021), τ was expected to differ across populations along an elevational gradient.

Statistical Analyses

All of the thermal tolerance metrics (T_{crit}, T_{50}, TSMs, and τ) were calculated at a genotypic level, and the means and standard errors were then collected to represent each population. Thermal tolerance metrics were also assessed for normality using a Shapiro-Wilk test. Paired t-tests and linear regression were conducted to compare the difference in mean T_{crit} and T_{50} values between May and August, and to see if there was a significant relationship between the tolerance thresholds over time. Each thermal tolerance metric was compared to source population elevation using linear regression to explore the relationship of local adaptation to leaf thermal tolerance. One-way ANOVA was conducted separately for each H_{tol} threshold in May and August (produced four ANOVAs total), where elevation was the factor, treated as a categorical variable, and H_{tol} thresholds were the response variables. These tests were used to determine if there were significant differences in the mean T_{crit} and T_{50} across elevations in the spring and summer. Post hoc Tukey HSD tests were used when the ANOVA results showed that there were significantly different mean H_{tol} thresholds across elevations.

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RESULTS

T_{crit} and T_{50} Analysis

The differences in mean leaf H_{tol} thresholds between spring and summer across all populations between August and May was 1.21 °C (t_{79} = 8.737, p < 0.0001) and 1.45 °C for T_{50} (t_{79} = 10.317, p < 0.0001; Fig. 3). No linear relationship was present when leaf H_{tol} thresholds were plotted against elevation, however the range in thermal tolerance values across all elevations was smaller in August when ambient air temperature at the common garden site was higher than most populations would experience in their source location (Fig. 4).

There was a difference in means across elevations for both of the May H_{tol} thresholds (T_{crit}: $F_{7,72}$ = 9.416, p < 0.0001; T_{50}: $F_{7,72}$ = 11.27, p < 0.0001) and for August T_{50} ($F_{7,72}$ = 3.747, p = 0.0016), but not for the August T_{crit} means ($F_{7,72}$ = 0.843, p = 0.556). The post hoc test for May H_{tol} thresholds and August T_{50} showed that there are differences in thresholds at the population level, but that these differences are not directly correlated to elevation (Fig. 5). The variation in mean T_{crit} and T_{50} is higher among populations than variation within each population, and in August the range in mean T_{crit} and T_{50} across populations is much smaller than in May.

The final linear regression conducted with T_{crit} and T_{50} was comparing them to the difference in maximum recorded leaf temperature and Yuma air temperature (ΔT). The Yuma air temperature was chosen based on which timestamp from the weather station data most closely matched the time of the highest recorded leaf temperature. The difference between maximum recorded leaf temperature and ambient air temperature also

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had no correlation with T_{crit} and T_{50} values (Fig. 6), however it was significantly different from May to August.

Thermal Safety Margins

While ΔT was not correlated to T_{crit} or T_{50}, it was significantly correlated with elevation in August ($F_{1,6} = 29.62$, $p = 0.002$; Fig. 7). The coefficient of determination was also high in August ($R^2 = 0.832$). There was a surprising positive correlation with elevation for ΔT, especially when considering the results of the TSMs. TSMs based on leaf temperature were calculated using T_{crit} or T_{50} and highest recorded leaf temperature (Fig. 8), with the T_{crit} and T_{50} TSMs in August both having significant negative correlations with elevation (T_{crit}: $F_{1,6} = 41.790$, $p < 0.0001$; T_{50}: $F_{1,6} = 41.22$, $p < 0.0001$). The coefficient of determination was also high in this regression model (T_{crit} $R^2 = 0.874$; T_{50} $R^2 = 0.873$). No specific pattern arose in the May data for these TSMs at the population level. Maximum recorded leaf temperature per genotype decreased by about 1.49 °C from May to August ($t_{79} = -3.278$, $p = 0.002$), which likely contributed to the pattern of correlation that was detected in the August leaf temperature TSMs. Two of the mean leaf temperature TSMs for cool-adapted populations had negative values for their May T_{crit} LT_{max} TSM (986 m = -1.368 °C; 988 m = -0.556 °C), but otherwise all other mean TSM values in this group were positive. The full range of these LT_{max} TSM values went from -1.37 °C to 8.57 °C, which was the most conservative range out of the three different TSMs that were calculated.

The second TSMs were calculated based on the maximum air temperature of the source population during the sampling months. Using recorded leaf temperatures to

calculate TSMs generally will yield more meaningful vulnerability assessments (Cook *et al.*, 2021), however most studies that do this used continuous leaf temperature measurements which was not possible for this study. Since leaf temperatures were taken three times a day rather than on a continuous schedule, TSM using leaf H_{tol} thresholds and the maximum air temperature for the source population location in May and August of 2021 (ie., regional AT_{max}) as the environmental metric were also calculated to be used as a comparison. Lastly, TSMs using the same leaf H_{tol} thresholds, but with the highest recorded air temperature in Yuma on the sampling days in May and August of 2021 (Yuma AT_{max}) as the environmental metric, were also calculated.

For the mean regional AT_{max} TSMs, there was a significant positive linear correlation with elevation found in August for TSMs with both leaf H_{tol} thresholds (T_{crit}: $F_{1,6} = 77.37$, p <0.0001; T_{50}: $F_{1,6} = 67.11$, p < 0.0001), but not in May (Fig. 9). The coefficient of determination was also very strong in August (T_{crit}: $R^2 = 0.928$; T_{50}: $R^2 = 0.918$), indicating that regional AT_{max} could very strongly predict the mean TSMs. The range of these mean TSMs was 12.28 °C, with the lowest mean TSM occurring in the August T_{crit} category with 8.93 °C (161 m) and the highest mean TSM occurring in the May T_{50} category (988 m).

The final mean TSM calculations involved leaf H_{tol} thresholds and maximum local air temperature on the day each population was sampled in May and August (Yuma AT_{max}).. The range in Yuma AT_{max} in May across the sampling days was 2.55 °C, and was 1.00 °C for the sampling days in August. They reflected the results of the mean leaf H_{tol} threshold regression model and elevation in that the mean Yuma AT_{max} TSMs also did not have a significant relationship with elevation (Fig. 10).

Thermal Time Constants

There was not a significant difference in mean τ between May and August, and population mean τ values did not have any significant relationship with elevation (Fig. 11). Across May and August, no single population consistently had the highest or lowest τ value either, which is indicative of a lack of determination of τ from May to August. The τ value in May will not accurately predict the τ for the same population in August. Similarly, there was no significant relationship between τ and H_{tol} thresholds, regardless of season, at a population level, which was not expected. The top two factors that contribute the most to changes in τ are leaf area and dry leaf mass (Michaletz *et al.*, 2016). As a follow up to these non-significant results, paired t-tests were run for both of these leaf traits to determine if there was a significant difference in them between May and August. The difference in means for both traits between May and August were significant (leaf area: $t_7 = 3.286$, $p = 0.013$; dry leaf mass: $t_7 = 2.411$, $p = 0.046$), suggesting that seasonal changes to these morphological traits were likely not responsible for the lack of correlation between τ and elevation.

DISCUSSION

T_{crit} and T_{50}

As expected, the leaf H_{tol} thresholds for all populations, regardless of their source location elevation, acclimated to seasonal temperature changes in the common garden. These results confirm that *P. fremontii* is able to acclimate to increased temperatures to some degree given that every population increased their leaf H_{tol} thresholds in August relative to May when it was cooler (Zhu *et al.*, 2018). Past research on this common

garden, and the two other gardens established at the same time in mid- and high-elevation

environments, found variation in the expression of traits relating to phenology, leaf

economic spectrum, whole-tree architecture, and wood economic spectrum according to

transfer distances (Cooper *et al.*, 2019; Blasini *et al.*, 2021). Other studies have shown

that warm-adapted species are able to maintain higher thermal tolerance thresholds if they

are adapted to warmer climates (O'Sullivan *et al.*, 2017, Zhu *et al.,* 2018; Lancaster &

Humphreys, 2020), but that pattern does not always carry over as clearly in common

garden settings (Knight & Ackerly, 2002; 2003). H_{tol} thresholds, both T_{crit} and T_{50}, have

also previously been found to have a significant negative correlation with elevation

(Feeley *et al.*, 2020; Slot *et al.*, 2021). However, while there was a significant increase in

these thresholds between May and August, there was not a significant relationship

between population mean threshold values and elevation in this study. All of the

populations converged around similar T_{crit} and T_{50} values in the summer. This lack of

relationship did not support my hypothesis, but resembles results from Knight & Ackerly

(2002) who did not find a significant relationship between any T-F_0 parameters and

regional maximum temperature in July, a trend that roughly correlates to elevation in my

data. The lack of difference in means for these thresholds across elevations might suggest

that phenotypic plasticity, specifically physiological changes within the plant in response

to increased ambient temperatures (Cooper *et al.*, 2019), is affecting leaf H_{tol} more so

than any local adaptations that might differ across the populations (Goa *et al.*, 2018).

Alternatively, the pattern of warm-adapted species typically having higher H_{tol} thresholds

has been supported by past studies (O'Sullivan *et al.*, 2017; Zhu *et al.*, 2018), however

these were conducted on an interspecies level. There is a chance that this pattern is not

reciprocated on a population level, but further testing should be done at the source locations of *P. fremontii* in conjunction with ongoing common garden studies since the two different sites sometimes yield different results with comparable experimentation (Gao *et al.*, 2018; Kitudom *et al.*, 2022).

The range of the mean T_{crit} and T_{50} values decreased from May to August, indicative of lower variation across populations in the summer when extreme heat is continuous (Fig 4). There is likely a true T_{crit} and T_{50} value for the Sonoran Desert ecotype of *P. fremontii*, but more research would need to be done to determine if phenotypic plasticity does truly impact H_{tol} thresholds as these data suggest. Zhu *et al.* (2018) suggested that 50 - 55 °C might be the upper limit by which acclimation or adaptation can increase T_{crit}, and both T_{crit} (maximum of 51.6 °C) and T_{50} (maximum of 53.4 °C) calculated in this study followed that pattern across all populations and both seasons.

The results of my ANOVA on T_{crit} and T_{50} respectively in relation to elevation showed that the values among populations was significantly different in all cases except the August T_{crit} ANOVA. The significant differences in mean thermal thresholds first indicates that thresholds are likely affected by their local adaptation, but that the pattern does not occur linearly across elevations (Fig. 5). The non-significant ANOVA results of August T_{crit} suggest that as temperatures rise and as cool-adapted populations are exposed and acclimating to these hotter temperatures, they are very capable of meeting the tolerance thresholds of those in the warm-adapted groups. The lack of difference in mean T_{crit} in August implies that all of the populations will experience the initial disruption of PSII around the same air temperature limit. The result of the August T_{50} ANOVA

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determined that there was a difference in means across populations, however the post hoc test found that only the 570 m population was the most distinct (Fig. 5). While there were other populations whose mean T_{50} values were similar to the 570 m population, all other seven populations had means that were similar to each other as well. This suggests that, for the most part, the populations will all experience the 50% decrease in the electron transport capacity of PSII around the same temperature as well, with the 570 m population experiencing this at a slightly lower temperature than the rest. Downton *et al.* (1984) showed that growing plants at a higher temperature than they would generally experience in their provenance can lead to higher thermal tolerance, which is likely what has happened in this study. This convergence on August T_{crit} and T_{50} thresholds across elevations indicates that young plants transferred to warmer locations might stand a better chance at tolerating extreme heat than their mature counterparts.

The results of the regression model comparing May and August H_{tol} thresholds suggested that H_{tol} thresholds measured during the summer are not highly predictable based on those measured in the spring. Further analysis of all of the metrics analyzed in this study could benefit from being repeated at a genotype level. Any significant trends for genotypes can help inform comprehensive conservation efforts, particularly for agencies or organizations that are responsible for the land in which one or more of these populations inhabit.

The last linear regression that was conducted was on leaf H_{tol} thresholds and ΔT, i.e., the difference between LT_{max} and the Yuma air temperature that was recorded as close to the LT_{max} timestamp as possible. Mean ΔT was used as an indicator of how effectively each population was able to regulate their leaf temperature. Previous work has

shown that ΔT in the Sonoran Desert ecoregion, which occupies the warmest and wettest region of *P. fremontii*'s range (Ikeda *et al.*, 2017), was significantly higher than ΔT in the High Plains ecoregion which experiences much colder annual temperatures (Hultine *et al.,* 2020b). This pattern was found again across populations defined warm- and cool adapted based on the 40 °C maximum summer temperatures threshold (Blasini *et al.*, 2022), confirming that there is variation in ΔT at the population level. An unexpected trend emerged in my data. ΔT was actually found to be lower in the warm-adapted trees than in the cool-adapted trees in August, contrary to previous findings (Fig. 7). However, the mean maximum leaf temperature per population did follow the findings of previous work, where warm-adapted populations had cooler maximum leaf temperatures on average than the cool-adapted populations (Fig. 8). This indicates that while ΔT was lower in the warm-adapted populations, they were still able to maintain lower mean maximum leaf temperatures overall. Because what I found here contradicts much previous work, this ΔT pattern should be verified in future research. My data would be improved by including continuous measurements of leaf temperature, rather than periodically throughout the day.

Leaf thermoregulation is affected by leaf functional traits such as leaf width and stomatal conductance (Fauset *et al.*, 2018), traits that have also been found to differ among warm- and cool-adapted populations of *P. fremontii* (Blasini *et al.,* 2022). If the effects of local adaptation with regards to leaf H_{tol} thresholds are in fact specific to warm- and cool-adapted populations, there would have been a positive correlation between H_{tol} thresholds and ΔT. However, no relationship emerged between the two (Fig. 6). This suggests that phenotypic plasticity might be coming into play more than anticipated,

particularly with the high-elevation populations at this common garden site. Since there was no relationship between these two variables, there was no evidence that maintaining cooler leaves is correlated to increased leaf H_{tol} thresholds. Similar to previous suggestions, future research should be done on leaf H_{tol} thresholds and ΔT specifically with trees at each source location to see if this trend persists outside of the common garden setting and to better understand the roles of phenotypic plasticity and local adaptation in these processes. Stomatal conductance data that was collected during this same field season will be compared to these H_{tol} metrics in the near future as well to see how stomatal conductance relates to changes in ΔT and H_{tol} thresholds.

Thermal Safety Margins

Much of previous research involving thermal safety margins uses air temperature as the environmental metric to subtract from H_{tol} thresholds (Curtis *et al.*, 2016; O'Sullivan *et al.*, 2017; Sastry & Barua, 2017; Slot *et al.*, 2021), however it has since been found that using leaf temperature is a better indication of thermal vulnerability since that is the true body temperature of the leaf (Perez & Feeley *et al.*, 2020; Cook *et al.*, 2021; Kitudom *et al.*, 2022). Leaf temperature data for this study was not continuous (Cook *et al.*, 2021), so true maximum leaf temperature is not necessarily known, but thermal images were taken during the hottest times of the day ensure that our highest recorded leaf temperature is close to the true maximum. The mean LT_{max} TSMs had a negative relationship with elevation in August for both T_{crit} and T_{50}, but not in the May H_{tol} thresholds. Since all of the populations converge around the same T_{crit} and T_{50} values in August, the differences in these TSMs is heavily dependent on variation in leaf

temperature across populations. This partially supports my hypothesis that the warm-adapted, lower elevation populations are better able to regulate their leaf temperature, so they have lower LT_{max} values which translates to larger TSMs, but only in August. These results also support previous findings that TSMs based on LT_{max} will increase along with increases in habitat air temperature (Perez & Feeley, 2020; Blasini *et al.*, 2022; Kitudom *et al.*, 2022). Lower elevation populations had cooler maximum leaf temperatures during August than their higher elevation counterparts, and because of that they were able to keep their leaf temperatures below thermal tolerance thresholds. Elevation of the source population predicts most of the variation in these summer thermal safety margins (87.44% and 87.29% of the variation in TSMs using T_{crit} and T_{50} respectively are predicted by elevation).

The 72 m elevation population consistently had the largest LT_{max} TSMs for both seasons and for both thermal tolerance thresholds. This is likely because it is the source population that experiences climate that is the most similar to the common garden (Table 2). There were two populations with T_{crit} mean TSMs that fell below zero, the 986 m and 988 m populations, which indicated that they were likely already dealing with seasonal temperatures that were warmer than what they were adapted to cope with (Sastry & Barua, 2017). LT_{max} surpassing the T_{crit} threshold does not necessarily mean that there will be permanent damage to the chlorophyll in these populations, but it is important to note that even in the spring the leaf temperatures for some of the cottonwoods were already close to their H_{tol} thresholds. Compared to the other two TSMs that were calculated, the one using LT_{max} was by far the most conservative, and is also the one that

is most biologically meaningful because it incorporates the actual experiences of these plants (Cook *et al.*, 2021).

The mean TSMs found using regional AT_{max} had the opposite relationship to elevation as those for LT_{max}. While this does not support my hypothesis, it does make sense when considering what the temperature gradient was in these source locations for May and August of 2021. In May, there was a 5.8 °C range with the 988 m site being the lowest (29.7 °C) and the 161 m site being the highest (35.5 °C). However, in August that range of regional temperatures almost doubled to 10.0 °C, with the lowest temperature occurring in the 1230 m site (31.6 °C) and the highest still occurring at the 161 m site (41.6 °C), highlighting the significance of the intense heat that is experienced in the common garden setting. Elevation of the source population also predicted most of the variation in these summer TSMs (92.80% and 91.80% of the variation in TSMs using T_{crit} and T_{50} respectively). As with all TSMs, the smaller the environmental metric is (either LT_{max} or AT_{max} for this study), the larger the TSM will end up being. Given that there is such a strong regional AT_{max} gradient in August between the low- and high-elevation, and no distinct pattern in mean H_{tol} thresholds across populations, these findings make sense. However, because the higher elevation populations had lower mean TSMs for LT_{max}, these results should be interpreted cautiously. Similar to what was found in Cook *et al*. (2021), the regional AT_{max} TSMs were much wider than those of the LT_{max} TSMs. The LT_{max} TSMs are reflective of physiological mechanisms, therefore they should be considered a more rigorous assessment of leaf vulnerability. These TSMs were also likely affected by acclimation or plasticity since the trees from high-elevation sources have had years to grow in this really hot environment. Comparing AT_{max} TSMs from this common

garden to those in the field will likely show that the higher elevation population has smaller heat TSMs (O'Sullivan *et al.*, 2017; Zhu *et al.*, 2018).

The mean Yuma AT_{max} TSMs were expected to follow a similar pattern to the leaf H_{tol} threshold TSMs when compared to elevation. Since my hypothesis suggested that lower elevation populations would have higher leaf thermal tolerance thresholds, it would follow that calculating TSMs using local weather station data (AT_{max} of the sampling day) would just further highlight that pattern. However, there was not a significant relationship between T_{crit}/T_{50} and elevation, and these mean Yuma AT_{max} TSMs reflect that lack of correlation. Even though the AT_{max} in Yuma may have differed depending on the sampling day, the AT_{max} range for May and August was 2.55 °C and 1.00 °C, respectively. If there had been a more significant relationship between the thermal tolerance thresholds and elevation, this TSM would have likely also mirrored that pattern. Due to the lack of a correlation between the local AT_{max} TSMs and elevation, these TSMs are considered to be the least effective out of the three that were found in this study.

Thermal Time Constants

Between May and August there was not a significant difference in mean τ values across all populations. The range for population mean τ in May was 1.61 s (8.40 to 10.02 s) and the range in August was 1.05 s (8.25 to 9.29 s). Since τ is a composite leaf trait, variation in it can mostly be attributed to total leaf area and dry leaf mass (Michaletz *et al.,* 2016), therefore it would make sense to not see seasonal changes in τ if leaf traits are also not changing seasonally. However, there was a significant difference in means between May and August for both of these leaf traits in these populations. These data,

coupled with the fact that Equation 1 excluded radiation and transpiration as part of the heat transfer coefficient (h), suggest that seasonal variation in leaf functional traits across these populations does not correlate with changes in τ (Slot *et al.*, 2021).

I expected there to be a negative linear relationship between τ and elevation, where τ is higher in warm-adapted populations and lower in cool-adapted populations. This is because a high τ means that a leaf is slow to respond to external air temperature fluctuations, and I predicted that warm-adapted populations would be better at internally regulating leaf temperatures. However, such a relationship was not found at the population level. Thermal time constants do affect leaf temperature (Leigh *et al.*, 2016; Halbritter *et al.*, 2022), so τ was expected to follow a similar pattern when compared to elevation as leaf temperatures did, however there might be too many similarities in leaf morphological traits across populations at this time. The trees in this common garden are fairly young, so the thermal time constant might be even across all populations given because these specific individuals have leaves that are much smaller than their mature counterparts in their native habitats. Mature cottonwoods have leaves that are larger than the ones at our common garden site, and much of the preliminary experimentation for this study was conducted on well-watered, mature cottonwoods located at DBG. The leaves of these few individuals had higher temperatures at which maximum F_0 was reached (T_{max}) than the young populations at the common garden site. T_{max} directly affects T_{50}, so it is likely that as the trees in the common garden mature, they will have an increased T_{50} threshold.

Leaf width in particular has been found to have a positive relationship with τ, so the thermal time constants might continue to increase as the trees in the common garden

mature (Leigh *et al.*, 2016). Collecting data on mature trees in the provenances of those in this common garden might also yield different results that more closely match my original hypothesis. These studies can also be repeated on the common garden trees over time to track changes in these traits and H_{tol} thresholds as they continue to grow and mature.

CONCLUSIONS

This study aimed to better understand intraspecies variation in thermal tolerance thresholds, TSMs, and τ. Most studies done on leaf thermal tolerance have remained at the species level, and not enough work has been done at the population level to account for local adaptation and/or phenotypic plasticity. My hypothesis was that in a common garden setting, warm-adapted populations would have higher T_{crit} and T_{50} values, larger thermal safety margins, and larger thermal time constants than cool-adapted populations. I also predicted that T_{crit}, T_{50}, and τ across all of the populations regardless of elevation will increase from spring to summer, indicative of seasonal acclimation of leaf thermal traits in *P. fremontii*. Some of the mean H_{tol} thresholds were significantly unique from each other, but there was not a significant relationship between H_{tol} thresholds and elevation. The exception to this finding was with T_{crit} in August, when all of the populations did not have significantly unique thresholds, which suggests that T_{crit} in extreme temperatures might have a true value across the whole species. Regardless, all populations converged on similar T_{crit} and T_{50} thresholds in August when air temperatures were at their highest, suggesting that local adaptation does not strongly affect H_{tol} thresholds. This is the opposite of what was found with LT_{max}, where there was an

important trend of warm-adapted populations having lower maximum leaf temperatures than their cool-adapted counterparts.

The leaf H_{tol} thresholds did increase from May to August, while simultaneously all of the LT_{max} values either decreased or stayed fairly even as air temperatures rose. These data together show that LT_{max} TSMs increased from May to August across all elevations, indicative of phenotypic plasticity particularly in the high elevation groups who would not normally experience maximum summer temperatures as high as they are in Yuma. Both of the AT_{max} TSMs were likely not biologically relevant, and this study mirrors results from previous work that found leaf temperatures are a better indicator of vulnerability for TSMs. Thermal time constants were not significant across elevations either, however this metric should continue to be measured as the trees mature to better understand if there is an age-related confounding factor or if it is actually a metric that is not as privy to local adaptation as other traits are.

This research contributes to the growing body of knowledge about *P. fremontii*, dominant riparian trees, and plants of the southwest which are all predicted to suffer from the ongoing consequences of climate change. Knowing that local adaptation does not strongly impact *P. fremontii*'s H_{tol} thresholds, specifically on these younger trees, suggests that trees across the southwestern US have a chance to acclimate to their increasingly hot environment. All of these populations demonstrated disruption and damage to PSII between 47.9 and 52.4°C (T_{crit} and T_{50} combined across seasons), the lower limit of that range currently already being experienced in the warmer parts of Arizona during the summer. However, the ability of the high elevation populations to acclimate their thermal thresholds is encouraging, and hopefully the low elevation

populations will also be able to adapt as temperatures continue to increase. The ability of *P. fremontii* to survive climate change is likely going to rest on leaf thermal regulation rather than H_{tol} thresholds. As droughts become increasingly frequent in this region, this riparian species will likely need to take significant risks in its water-use strategies in order to successfully maintain acceptable leaf temperatures.

Future work with this species should include common garden and provenance comparisons of thermal tolerance metrics to better identify the effects of local adaptation and plasticity on leaf thermoregulation. The inclusion of stomatal conductance and other functional traits that affect leaf thermal regulation should be analyzed in conjunction with chlorophyll fluorescence to better understand the role of thermal regulation in maintaining the integrity of PSII's electron transport chain. Negative changes in this species' ability to regulate its leaf temperatures would likely result in decreases in its H_{tol} thresholds, so understanding how leaf thermal regulation capabilities can be maintained through climate change will be of the utmost importance.

Table 1. List of Abbreviations and Symbols

Abbreviation	Definition	Units
F_0	Minimum chlorophyll fluorescence; the fluorescence level of a dark-adapted leaf when all PSII reaction centers are open	Dimensionless*
T_{crit}	Critical temperature; the temperature at which PSII's electron transport chain is initially disrupted. Calculated as the inflection point between the slow and fast rise portions of the T-F_0 curve	°C
T_{50}	The temperature at which there is a 50% decrease in PSII's electron transport capacity. Calculated as the temperature where F_0 reaches 50% of its maximum recorded value	°C
T-F_0	Temperature dependent F_0 response (see Fig. 1)	%
H_{tol}	Heat tolerance; H_{tol} thresholds include T_{crit} and T_{50}	No units
TSM	Thermal safety margin; difference between either T_{crit} or T_{50} and an environmental metric	°C
τ	Thermal time constant; a composite functional leaf trait that reflects how quickly leaf temperature reacts to changes in air temperature	s
LT_{max}	Maximum recorded leaf temperature	°C
ΔT	Difference between LT_{max} and Yuma AT_{max}	°C
AT_{max}	Maximum air temperature	°C

* Chlorophyll fluorescence units are relative to the instrument that is used. All metrics calculated with F_0 values are done so after the T-F_0 curve has been normalized to fall between 0 and 1 (ie., 0-100% of the maximum F_0)

Table 2. Sampled Populations. All variables except for transfer distance gathered from Cooper *et al.*, 2019. MAT is mean annual temperature.

Symbol	Elevation (m)	Latitude	Longitude	MAT (°C)	Transfer Distance (°C)
MYN-MLY*	49	32.8498	-114.4928	22.8	0
CCR-COL	72	33.36077	-114.69856	22.6	0.2
LBW-BIL	161	34.27595	-114.05856	22.3	0.5
WHY-HAS	570	33.8901	-112.66784	19.6	3.2
NRV-NEW	666	33.954	-112.13526	19.9	2.9
TSE-TUM	986	31.56538	-111.04478	17.5	5.3
CAF-AUG	988	34.25771	-112.06456	17.2	5.6
TSZ-SAN	1219	31.60676	-110.17009	16.9	5.9
PSA-SON	1230	31.53646	-110.7626	15.7	7.1

* MYN-MLY is the symbol for the common garden at Mittry Lake, Yuma, AZ. There are no populations associated with this location.

Example of a T-F$_0$ Response from a Leaf Sample in August of 2021

Tmax = 57.43 °C
T50 = 52.35 °C
Tcrit = 50.52 °C

% of maximum recorded F$_0$
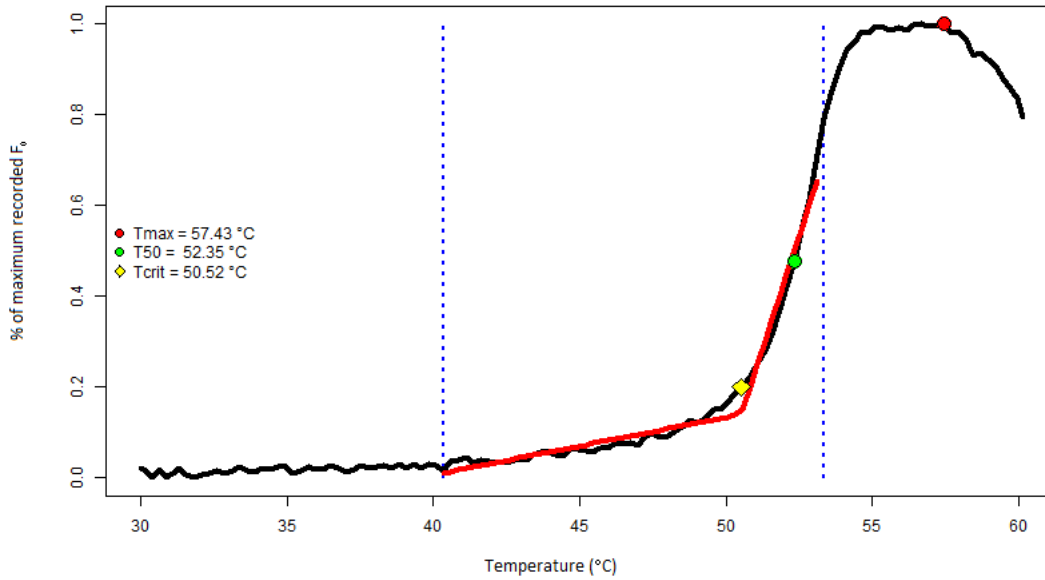
Temperature (°C)

Figure 1. This was the T-F$_0$ response for one leaf sample in this project. T$_{max}$ is the temperature at which the maximum recorded F$_0$ occurs, T$_{50}$ is the temperature at which 50% of the maximum F$_0$ occurs, and T$_{crit}$ is the inflection point between the slow and fast rise regression lines in the T-F$_0$ response (slow and fast rise phases highlighted by the red line). The blue dashed lines are the region specified for the regression to occur. This graphic was created by following the methods of Arnold *et al*. (2021).

30 Year (1991-2020) Maximum Summer Temperatures for Source Locations of *P. fremontii*
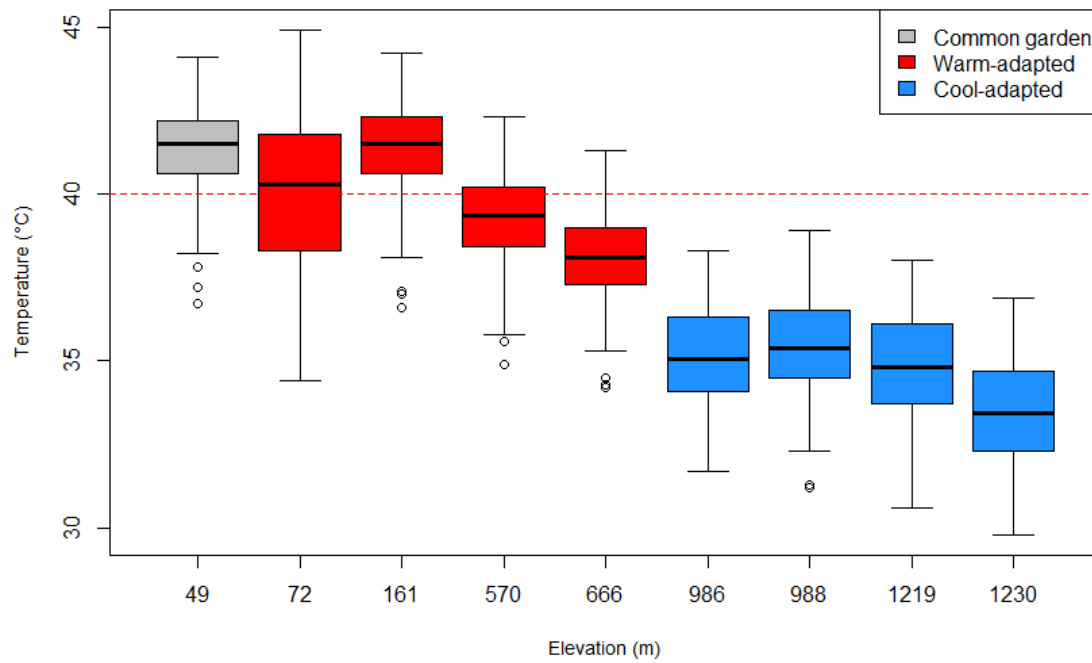
Figure 2. The distinction between warm-adapted and cool-adapted populations follows Blasini *et al.* (2022) where any population with a source location that experienced maximum summer temperatures greater than 40 °C over the last 30 years were designated as warm-adapted populations, and those that did not were designated coo-adapted populations. The four populations with the lowest elevations met that summer maximum temperature threshold, and the four populations with the highest elevations did not. Data were collected from http://prism.oregonstate.edu.

Seasonal Differences in Leaf Thermal Tolerance Thresholds
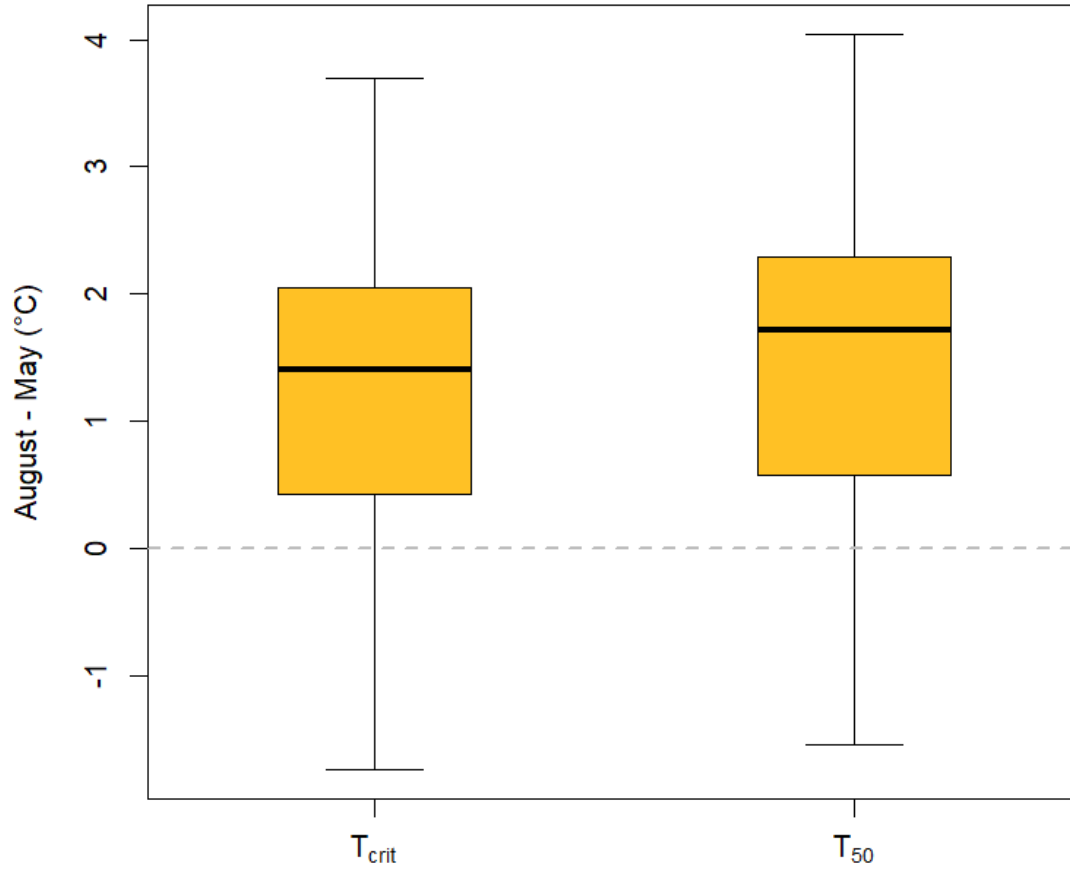
Figure 3. Boxplot showing the distribution of differences in T_{crit} and T_{50} across all genotypes. Differences were calculated by subtracting May thermal tolerance values from those in August, as seen in the y-axis label.
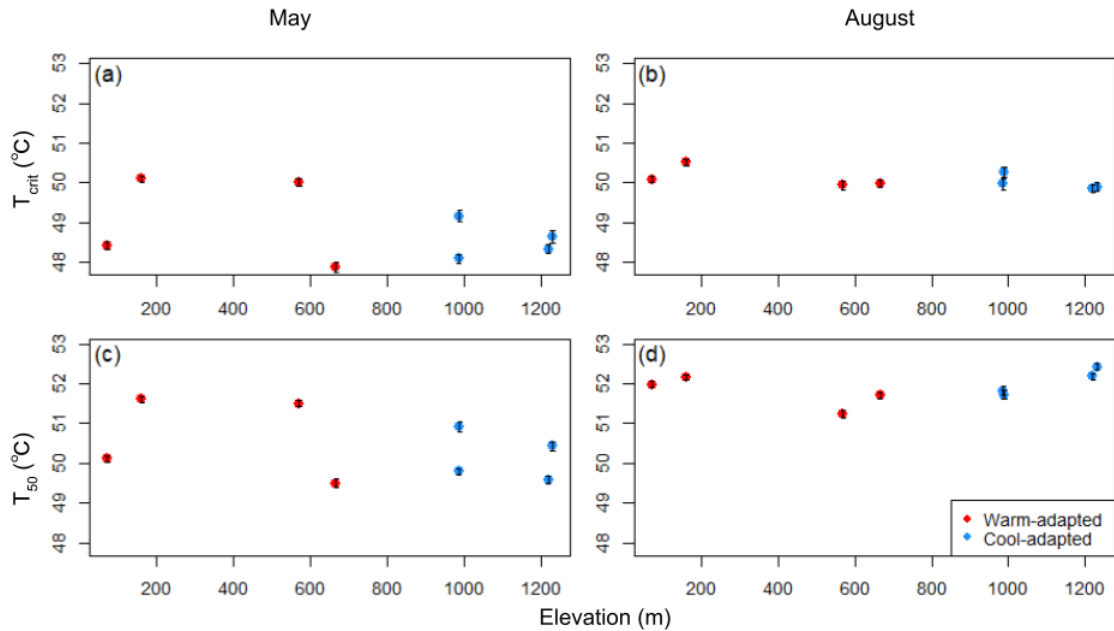
Figure 4. Comparing H_{tol} thresholds to elevation. (a) is May T_{crit} values, (b) is August T_{crit} values, (c) is May T_{50} values, and (d) is August T_{50} values. The red points are warm-adapted populations (>40 °C maximum summer temperatures) and the blue points are cool-adapted populations (<40 °C maximum summer temperatures)
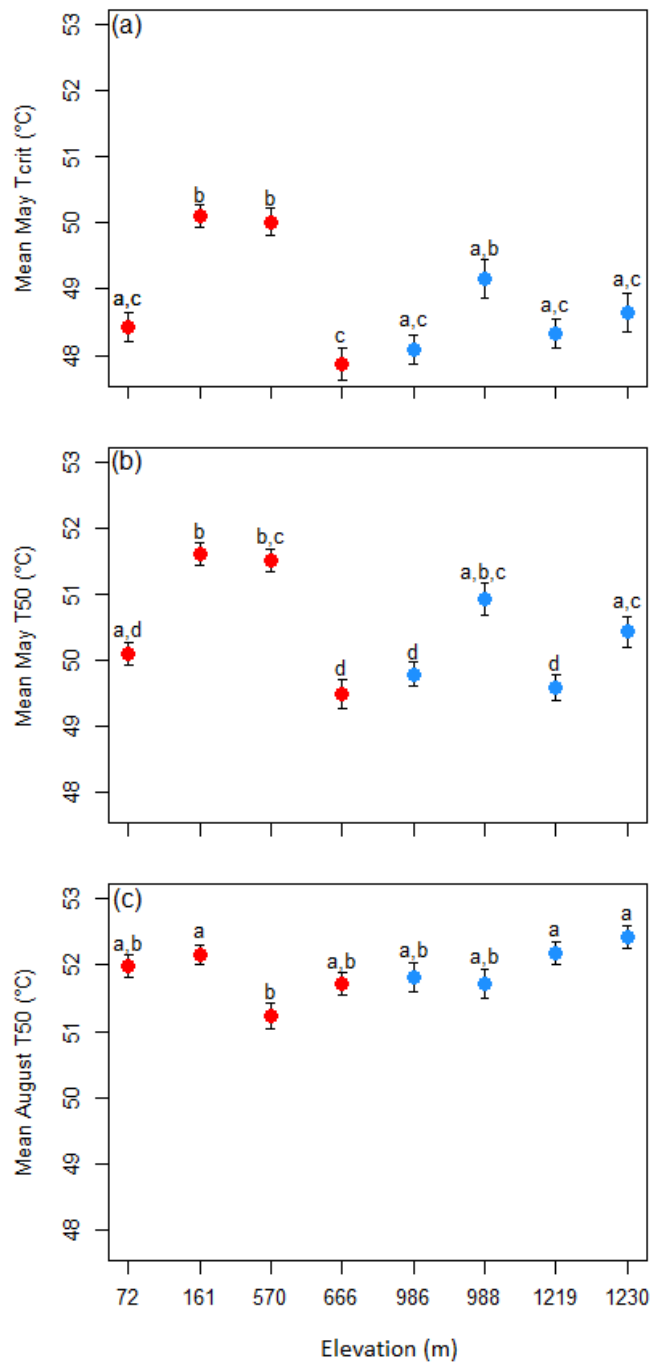
Figure 5. Results of the post hoc Tukey HSD tests for the significant ANOVA results. Matching letters mean that those populations did not have significantly different means, differing letters means they did. Letters are unique to each individual plot, and do not correlate to each other. Plot (a) is the results of the May T_{crit} Tukey test, (b) is the results for May T_{50}, and (c) is the results for August T_{50}. August T_{crit} did not have a significant p-value in its ANOVA test.
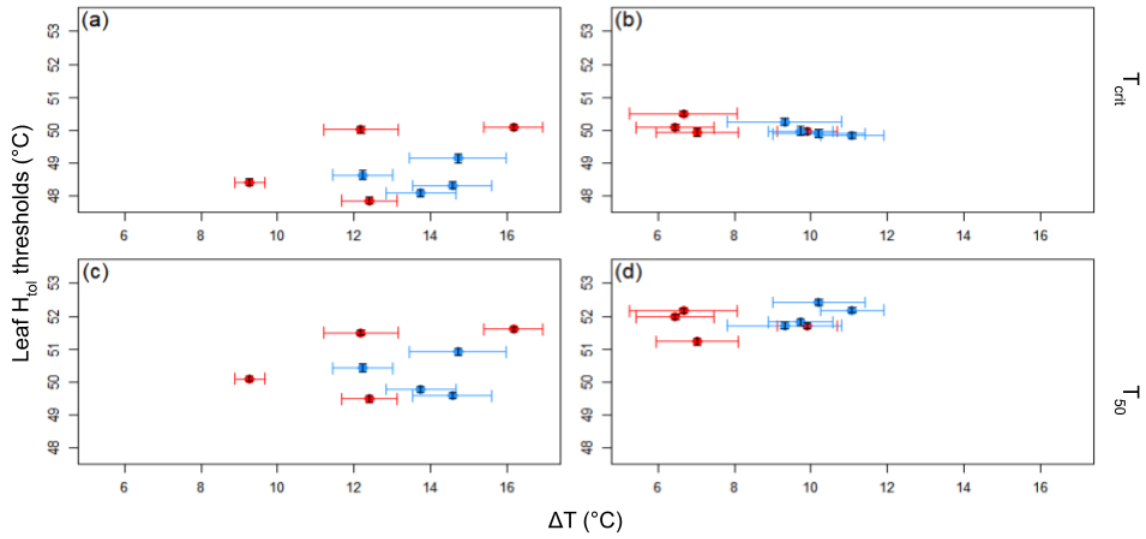
Figure 6. Comparison of leaf H_{tol} thresholds (T_{crit} and T_{50}) and the difference between highest recorded leaf temperature and the corresponding air temperature (ΔT) in Yuma as close to the time of LT_{max} as possible. (a) H_{tol} metric is May T_{crit}, (b) is August T_{crit}, (c) is May T_{50}, and (d) is August T_{50}.
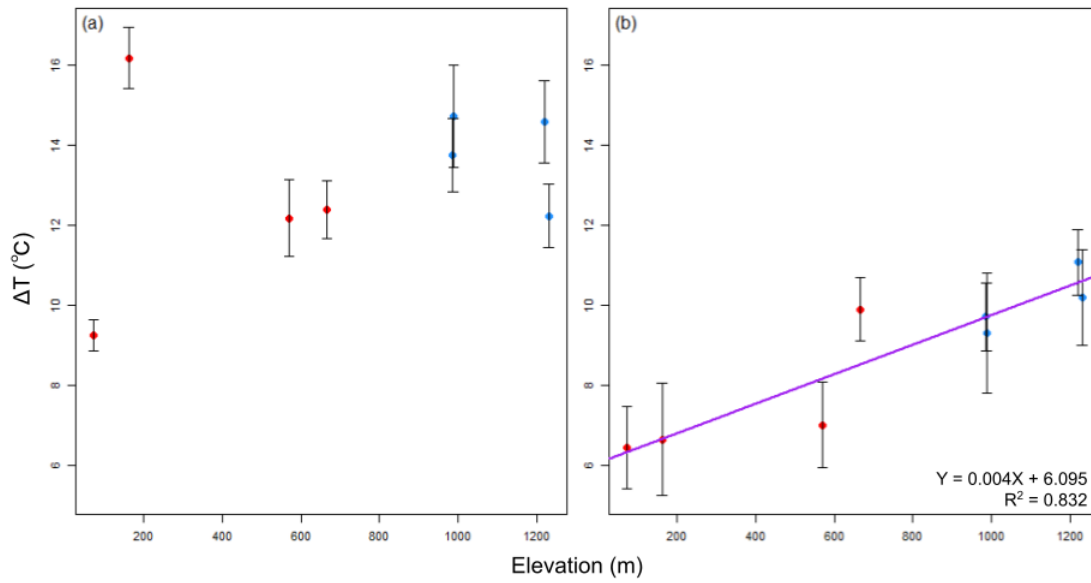
Figure 7. The difference in highest recorded leaf temperature and ambient air temperature in Yuma at as close a time to the LT_{max} as possible for (a) May and (b) August. The purple, solid line represents the significant linear regression model in August; May did not have a significant regression model.

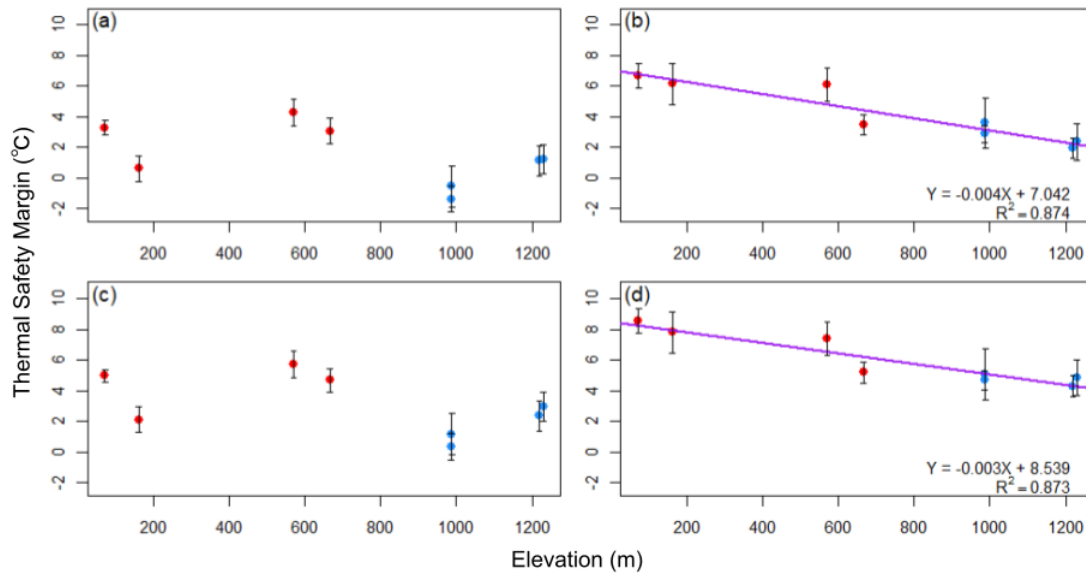## Thermal Safety Margins Calculated Using LT_{max}

Figure 8. Thermal safety margins were calculated at a genotype level, and the mean and standard error were taken from those values to produce these population level mean TSMs. Each panel is one H_{tol} threshold (T_{crit} or T_{50}) minus LT_{max} for May or August. (a) is the May T_{crit} TSM, (b) is the August T_{crit} TSM, (c) is the May T_{50} TSM, and (d) is the August T_{50} TSM. The purple, solid line represents the significant linear regression models in August; May did not have significant regression models.
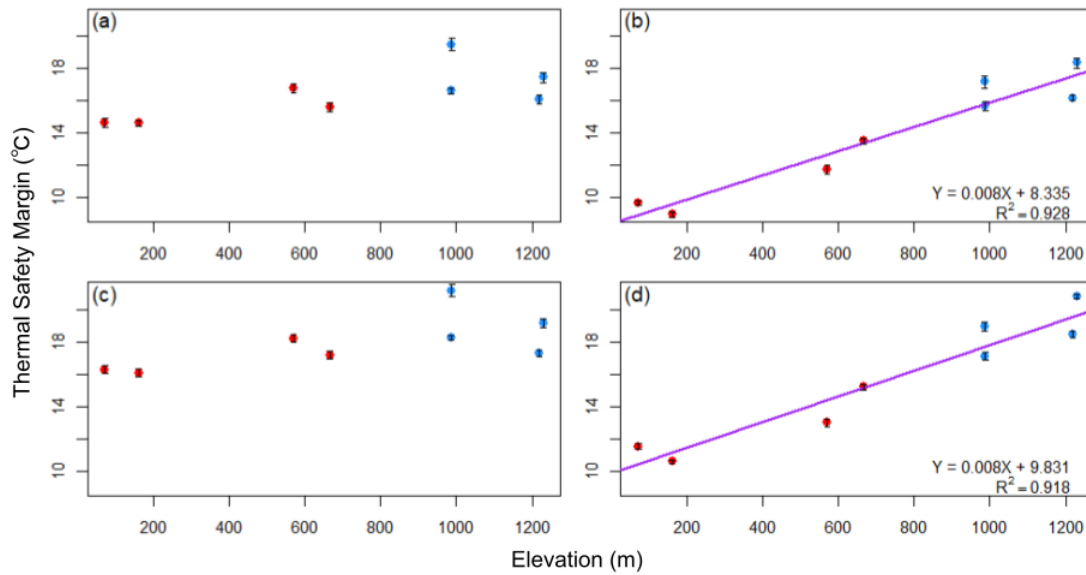
Figure 9. TSMs were calculated at a genotype level, and the mean and standard error were taken from those values to produce these population level mean TSMs. Each panel is one H_{tol} threshold (T_{crit} or T_{50}) minus regional AT_{max} for May or August. Regional AT_{max} is the maximum air temperature at each population source location for the months of May and August, respectively, in 2021. (a) is the May T_{crit} TSM, (b) is the August T_{crit} TSM, (c) is the May T_{50} TSM, and (d) is the August T_{50} TSM. The purple, solid line represents the significant linear regression models in August; May did not have significant regression models.

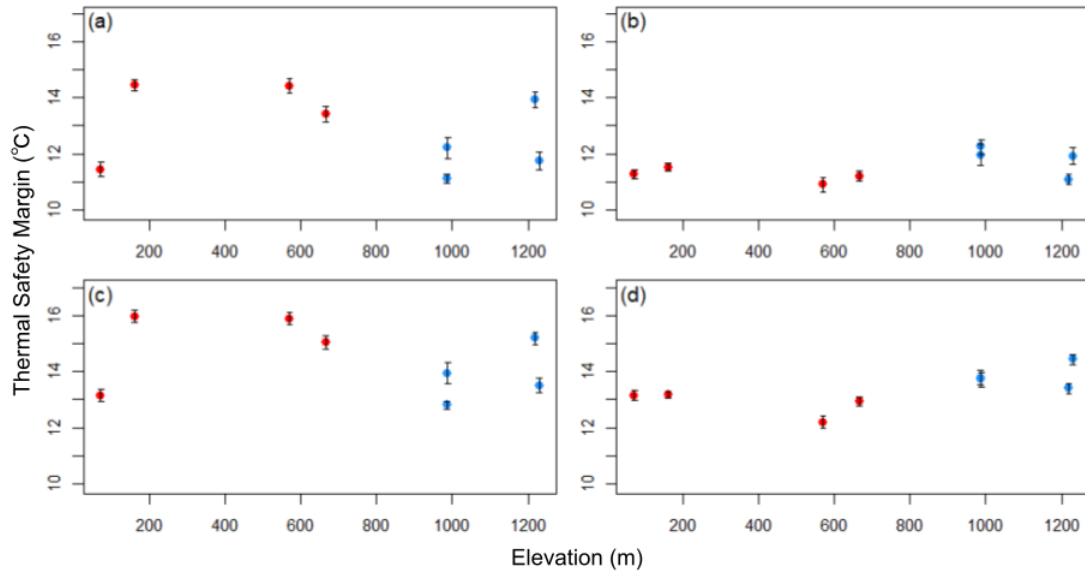Thermal Safety Margins Calculated Using Yuma AT_{max} During Sampling Days

Figure 10. Each panel is one H_{tol} threshold (T_{crit} or T_{50}) minus Yuma AT_{max}. Yuma AT_{max} is the maximum recorded air temperature at the Yuma common garden site during the sampling days in May and August. (a) is the May T_{crit} TSM, (b) is the August T_{crit} TSM, (c) is the May T_{50} TSM, and (d) is the August T_{50} TSM. There were no significant linear models for these TSMs.
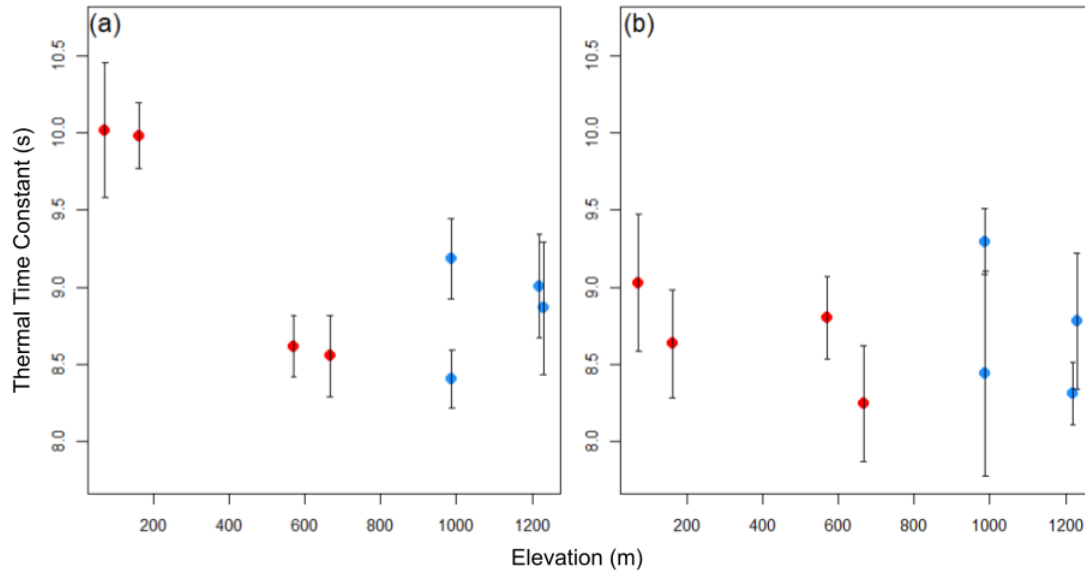
Figure 11. Thermal time constants were originally calculated at the genotype level, then the mean and standard error of each population was calculated to get the values for (a) May and (b) August. No significant linear relationship was found.

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