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The Coordination of Drought and Heat Tolerance of Woody Species Across Ecosystems

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The Coordination of Drought and Heat Tolerance of Woody Species Across Ecosystems

A Thesis submitted in partial satisfaction
of the requirements for the degree of

Master of Science

in

Plant Biology

by

Denise Eileen Mitchell

June 2021

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The Thesis of Denise Eileen Mitchell is approved:

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Committee Chairperson

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Acknowledgements

I would like to acknowledge Dr. Louis S Santiago as my advisor, and the American Association of University Women (AAUW), Riverside East Rotary Club and the UC Riverside Graduate Student Association for funding this research.

Dedication

I would like to dedicate my thesis to my father Roger Yangues Merino, who continues to be my inspiration for lifelong learning, and my mother, Ann Elizabeth Merino, who introduced me to nature by making me pull weeds.

“May we all continue to grapple with the conundrums and disentangle the dilemmas in an effort to reveal the patterns that will unlock the mysteries of nature… because that is indeed what we have been commissioned to do.”

DMM

Table of Contents

List of Tables

List of Figures

Introduction

When one studies drought tolerance, it is helpful to examine heat tolerance as well, because high heat accelerates drought. Across ecosystems woody plant mortality has been observed as warmer temperatures amplify the effects of arid conditions. Of particular concern is the potential for warmer temperatures to compound the effects of increasingly severe droughts by triggering widespread vegetation shifts because of woody plant mortality (Adams HD, et al 2009).   But the "die out" concern is not restricted to natural systems. In crops, drought and heat combinations have a synergistic impact (Awasthi et al. 2014; Qaseem et al. 2019). Drought and heat stress in combination significantly impacts crop yield by decreasing harvest index, shortening life cycles, and altering seed number, size, and composition (Cohen I, et al 2021). These impacts are more severe when the stress combination occurs during the reproductive stage of the plants (Cohen I, et al 2021). Combined heat and drought stresses were found to negatively impact the yield of major crop plants including legumes such as soybean, chickpea, and lentil, as well as cereals such as wheat, maize and rice, which are the mainstay of global food and feed supply in terms of both calorie and protein intake (Cohen I, et al 2021; Awasthi R, et al 2014; Nelimor, Badu-Apraku, et al 2019; Obata T, Witt S, et al 2015; Sehgal A, et al 2017). Although plant heat and drought tolerance are frequently studied, the combination of these two stresses has received little attention, but is essential, because we cannot extrapolate responses to stress combinations simply by the addition of responses to the two single stresses given the nature of potential interactions, both in climate and physiological regulation (Cohen I, et al 2021).

Accordingly, the objective of this original research is to characterize the coordination of drought tolerance and heat tolerance in woody species from three distinct ecosystems, that differ vastly in both mean annual precipitation (MAP) and mean annual temperature (MAT; Table 3), to understand the coordination of the physiological responses of leaf cell turgor loss, due to drought, and photosynthesis decline, due to heat.

While both high heat and aridity are environmental extremes that can negatively affect the productivity of plants, many species have traits that mediate their heat and drought risk (Table 1 & Table 2). One well documented avoidance strategy is the drought deciduous growth habit, shedding leaves during the dry period and growing new leaves when conditions become more favorable (Pivovaroff AL, et al 2016). Leaves of deciduous species undergo greater leaf hydraulic dysfunction to maximize carbon uptake, yet they are shed and replaced annually, whereas evergreen species hold on to their leaves, so there is a greater necessity to preserve the vasculature (Kikuta SB 1992). However, for woody species that do not avoid these stresses by losing their leaves, how can they endure combined heat and drought? Surviving the stress does not come without a cost. The plant may need to display effective osmotic control within leaf cells coupled with efficiently regulating stomata to minimize water loss, while still evaporatively cooling the leaf and gaining carbon. Plants often adjust their leaf turgor loss point (π_{tlp}), the leaf water potential at which the leaf mesophyll cells lose turgor, or wilt, during drought periods through the accumulation of cell solutes, or osmotic adjustment. Such an adjustment results in a lowering of π_{tlp} and can contribute to drought tolerance of vegetation worldwide (Maréchaux I, et al 2015). Species may also put down deep roots to have access to the water table, as seen with evergreen needleleaf tree root tissue which has a greater density relative to other taxa (Mackay DS, et al 2020).

Table 1. Physiological and biochemical drought tolerance strategies associated with the ecosystem where it is mostly observed shown.

	Desert	Shrubland	Forest
Deep Roots/ Ectomycorrhizae			X (*Quercus, Conifers*)
↑ Capacitance			X (Quercus, *Conifers*)
Water Storage Organs	*(In Cactaceae, not woody)*		X (Quercus, Conifers)
↑ Root: Shoot	X		
Drought Deciduous Growth Habit	X	X	
Photosynthetic Stems	X	X	
Effective Osmotic Control/ can withstand low Ψ (*McDowell et al 2008*) (*Bartlett et al 2016*)	X	X	
Pubescent Leaves (*Moles, AT et al 2020*)	X		

Plants also exhibit unique thermoregulatory traits, both physiological and morphological, that influence leaf temperatures and decouple them from ambient air temperatures (Perez TM, Feeley KJ 2020). For example, leaves with a low leaf area and narrow leaves, are generally associated with a thin boundary layer, and can cool down more quickly with evaporative cooling, relative to leaves with a greater leaf width and a larger leaf boundary layer (Meinzer FC, et al 1994). Leaf width is the most important leaf morphological driver of species differences in the leaf-to-air temperature differential, as is overall leaf size with wide and large leaves warming up more than narrow and small ones under similar conditions (Leigh A, et al 2017). In addition, leaves displaying epidermal modifications, such as hairs and cuticular wax, can moderate the incoming radiation, and light- colored leaves can reduce absorptivity. Leaves that roll, have a vertical angle, or maintain a parallel orientation to incoming radiation can also moderate temperature.

Table 2. Morphological and physiological strategies for heat tolerance associated with the ecosystem where it is mostly observed shown.

	Desert	Shrubland	Forest
↓ Leaf Area/ Pubescent Leaves *(Moles AT et al 2020)*	X		X (*Needles/Conifers*)
Narrow leaves/ Thin Boundary Layer (↑ *Evap. Cooling*) (*Michaletz et al. 2016*)	X		X (*Needles/Conifers*)
Leaf Epidermal Modifications *(Hairs/ waxes)*	X *(Hairs)*	X(*Sclerophylls*)	X (*Thick cuticles & sunken stomata/Conifers*)
↓ Absorptivity *(Light- colored leaves)*	X	X	
↑ Emissivity (*Absorb & Radiate*)	X		
Leaf Wilting		X	
Leaf Rolling		X	
Leaf Angle *(Vertical)*	X	X	
Paraheliotropism/ *Leaf Parallel to Incoming Radiation (Ehleringer et al 1980*)		X	

We were interested in identifying quantifiable patterns within single ecosystems involving drought and heat tolerance that can be observed across species? Further, we question if suites of traits for tolerating these two stresses are conserved across ecosystems that have distinctly different precipitation and temperature regimes, possibly due to pleiotropic effects, the upstream regulatory processes that lead to coordinated stress tolerance (Chapin FS, et al 1993). We posit that if the same suite of traits consistently recurs in diverse evolutionary lineages, it seems likely that it evolved as a coherent unit selected for fitness, rather than by parallel selection of each individual trait (Chapin FS, et al 1993). We know that species which thrive in their distinct locale have acclimated efficiently to the temperature extremes and periods of drought by employing a combination of physiological, morphological, and biochemical traits, (Table 1 & Table 2) which if correlated statistically, suggests that suites of traits have been selected for as part of an evolutionary response to abiotic stresses (Chapin FS, et al 1993). Comparing relative heat tolerance and drought tolerance of woody species across three distinct ecosystems has implications for a deeper understanding of resource allocation by the plant to sustain viable water potentials, minimize heat damage, and ultimately gain enough carbon to maintain integrity of the plant body.

Materials and Methods

Study Sites

In Southern California there are numerous distinct ecosystems that occur within a one-hundred-kilometer radius. The Mediterranean-type shrubland, one of five such uniquely biodiverse regions across the globe, is characterized by warm and dry summers coupled with mild, wet winters. By bordering a cold ocean on the western side of the continent, foggy conditions can moderate the climate, but with a stable laminar onshore flow that prevents the development of unstable air and convective storms. This unique geographical phenomenon provides for a wealth of biodiversity and floristic complexity known as the coastal sage scrub and chaparral. This dense vegetation carries with it a beauty of elusiveness all its own. With *Salvia mellifera* nestled secretly amongst the surrounding *Adenostoma fasciculatum,* catching a glimpse of the elevated bloom of purple hue above the shrub horizon is a prized moment during the slow and calculated trek through nature. Going inland from the coast, we lose the moderating effects of the marine layer, the range of air temperature broadens significantly, and this gives rise to the serene Mojave Desert ecosystem. After just a few steps into this landscape one is struck by the vastness that unfolds, drawing you in, and transforming your consciousness to one of a simpler time in California's historical past. Ascending in elevation to 1,770 m, we witness another shift in the resident flora within the Idyllwild Mixed Evergreen Forest with temperate summers and cold winters. The majestic *Pinus lambertiana* towers overhead displaying its cones of record length both as hanging ornaments and as a decorative carpet protecting the soil at its base. These three field sites within these distinct ecosystems provide an ideal framework for relative heat and drought comparison studies. (Table 3) The Philip L. Boyd Deep Canyon Desert Research Center (33° 38’ N 116° 24’W) is a field station of the University of California, Riverside, and the Natural Reserve System (Ford, LD et al. 1988). The Santa Margarita Ecological Reserve (33.4744° N 117.1379° W, 970’) is a field station of the San Diego State University (see website Table 3). The James San Jacinto Mountains Reserve (33.8° N 116.79° W) is a field station of the University of California, Riverside, and the Natural Reserve System (Ford, LD et al. 1988). The James San Jacinto Mountains Reserve is located on an alluvial bench situated at the lower end of Hall Canyon, a steep, western flank of Black Mountain and is nestled between 1623 m and 1692 m in elevation. Habitats include mixed conifer and hardwood forest, montane chaparral, and montane riparian forest. The James Reserve flora is unique with elements from Baja California (South), Colorado Desert (East), Sierra Nevada (North), and the Pacific Ocean (West).

Table 3. Climatic gradients of mean annual precipitation and mean annual temperature, as well as elevation and soil type differences between the forest, shrubland and desert shown.

Biome	**MAP (mm)**	**MAT (°C)**	**Elevation (m)**	**Soil Type**
Forest (https://james.ucnrs.org/)	637	12.6	1770	Soils are entisols loamy-sand texture (83% sand, 10% silt, 7% clay), a bulk density of 1.2 g cm^{-3}, underlay weathered granitic bedrock.
Shrubland (https://fsp.sdsu.edu/)	360	16.4	150	Soils are alluvial and sandy and classified as typic torripsamments (Soil Survey Staff 2008).
Desert (https://deepcanyon.ucnrs.org/)	139	23.3	275	Soils are classified as a eutric fluvisol with loam texture, based on (Batjes 2012).

Study Species

Drought tolerance and heat tolerance were quantified on a wide range of taxa including 21 woody species spanning 14 families. (Table 4) Desert and shrub biomes included only Angiosperms, whereas the forest biome included both Angiosperms and Gymnosperms. Three leaf phenological habits, evergreen, drought deciduous and semi-deciduous were included in this study. Study species include both native shrubs and trees, encompassing an average height range of <2m to >70 m, respectively. A wide range of leaf shapes and sizes were collected in this study including ovate, oblong, and linear shapes with the smallest pubescent leaves collected from desert species *Senegalia greggi,* and the longest from forest species *Pinus coulteri.* Wet season measurements taken in February 2020 and 2021 served as the control data. Dry season measurements taken in July 2020 served as the experimental/stress data. A young, mature leaf was collected from three to five individuals of each woody plant species, stored in a Whirl-Pak bag to minimize moisture loss, and placed in a cooler for transport back to the laboratory. The drive from the field site to the laboratory was 1 hr. 15 minutes or less. Once in the laboratory the bags were placed in the refrigerator to prevent further evaporation and processed within three days.

Drought Tolerance

Leaf mass per area (LMA), leaf thickness (T), and leaf dry matter content (LDMC) all have previously been proposed as drought tolerance indicators. It has been recently demonstrated that neither LMA values nor degree of thickness/toughness of leaves plays a direct role in drought tolerance globally (Bartlett, et al 2016). Rather, across plant species, drought tolerance and distributions with respect to water availability are strongly correlated with two physiological traits, the leaf water potential at wilting, (π_{tlp}), and the cell solute potential at full hydration, osmotic potential (π_o) (Bartlett, et al 2016) When the water potential in the leaf cells cannot sustain turgor, it is termed the turgor loss point (π_{tlp}). This occurs when the hydrostatic pressure potential equals zero, thereby making the water potential of the leaf cells only dependent on the solute potential. A lower, more negative value for turgor loss point would indicate higher drought tolerance. Conversely, a higher, less negative turgor loss point would indicate a relatively higher vulnerability to drought. Plants have a large range of water potential values at turgor loss. Further, some species can withstand exceptionally low water potentials well past the turgor loss point, and still survive the environmental stress (Kikuta SB, Richter H 1992). In a synthesis study of 262 woody angiosperms and 48 gymnosperms, it was found that wilting (π_{tlp}) occurred at water potentials lower than the water potential at 50% decline in stomatal conductance ($g_s\,\Psi_{50}$) as predicted, but higher than the water potential at 95% decline in stomatal conductance ($g_s\,\Psi_{95}$) contrary to the expectation that plants would undergo stomatal closure at sufficiently high-water potentials to prevent wilting (Bartlett MK, et al (2016). Therefore, according to the current literature, at π_{tlp} stomates may remain open as during the decline of plant gas exchange.

Table 4. List of species studied with respective biome, family, leaf growth habit and code shown.

Species	**Family**	**Biome**	**Leaf Growth Habit**	**Code**
Abies concolor	Pinaceae	Forest	Evergreen	*Abco*
Arctostaphylos pringlei	Ericaceae	Forest	Evergreen	*Arpr*
Calocedrus decurrens	Cupressaceae	Forest	Evergreen	*Cade*
Pinus coulteri	Pinaceae	Forest	Evergreen	*Pico*
Pinus lambertiana	Pinaceae	Forest	Evergreen	*Pila*
Quercus chrysolepis	Fagaceae	Forest	Evergreen	*Quch*
Adenostoma fasciculatum	Rosaceae	Shrubland	Evergreen	*Ad fa*
Artemisia californica	Asteraceae	Shrubland	Drought Deciduous	*Arca*
Eriogonum fasciculatum	Polygonaceae	Shrubland	Evergreen	*Erfa*
Heteromeles arbutifolia	Rosaceae	Shrubland	Evergreen	*Hear*
Malosma laurina	Anacardiaceae	Shrubland	Evergreen	*Mala*
Rhamnus illicifolia	Rhamnaceae	Shrubland	Evergreen	*Rhil*
Salvia apiana	Lamiaceae	Shrubland	Semi Deciduous	*Saap*
Salvia mellifera	Lamiaceae	Shrubland	Semi Deciduous	*Same*
Condea emoryi	Lamiaceae	Desert	Semi Deciduous	*Coem*
Encelia farinosa	Asteraceae	Desert	Evergreen	*Enfa*
Fouquieria splendens	Fouquieriaceae	Desert	Drought Deciduous	*Fosp*
Justicia californica	Acanthaceae	Desert	Drought Deciduous	*Juca*
Larrea tridentata	Zygophyllaceae	Desert	Evergreen	*Latr*
Parkinsonia florida	Fabaceae	Desert	Semi Deciduous	*Pafl*
Senegalia greggi	Fabaceae	Desert	Semi Deciduous	*Segr*

The relative drought tolerance of 21 woody species across three distinct ecosystems was assessed using turgor loss point as a metric. A vapor pressure osmometer (Vapro; Wescor, Inc.) was used to determine the relative drought tolerance parameter π_{tlp} more rapidly and cell solute potential at full hydration (π_0), as compared to the standard pressure-volume (P-V) approach (Bartlett MK, et al 2012). Whereas it may take 2 days to generate a P-V curve for 4 – 6 leaves, we were able to average approximately 1 hour per leaf sample using the osmometer. The osmometer method enabled accurate measurements for both needles with fascicles and leaves. The osmometer was left turned on for a few days prior to processing leaves to ensure a ready state and temperature stability of the thermocouple. Following this prep period, the instrument was calibrated using the Opti-Mole 290, 1000 and 100 mmol/kg calibration standards, beginning with the 290-set point, and following with the 1000 and 100 mmol/kg. For each successive day that leaves were processed, the osmometer was left turned on, and a new calibration was performed. A leaf disc was made using the #2 Leaf Corer, centrally between the midrib and margin, avoiding first- and second-order veins. In the case of needles, the same #2 Leaf Corer was used, and the disc consisted of 3 – 5 needle pieces taken from the base of the fascicle. Two aluminum foil discs were made using the #5 Leaf Corer. The leaf disc was placed between the two aluminum foil discs, and the corners of aluminum were turned over to completely wrap leaf tissue to limit condensation or frost after freezing and evaporation prior to processing. The leaf disc wrapped in aluminum foil was then submerged in LN_2 for two minutes. The minimum time used, 2 min, was adequate to completely freeze leaf tissue and fracture the cell walls. Upon removal from LN_2, the leaf disc was punctured ten times with sharp-tipped forceps to facilitate evaporation through the cuticle and decrease equilibration time before sealing in the osmometer chamber, using the standard 10μL chamber well (Kikuta SB, et al 1992). The discs were exposed to air for < 40s for all steps between removing the leaf from the Whirl-Pak bag and sealing the disc in the osmometer. Leaf samples from which water may not readily evaporate require long periods to reach vapor equilibrium. Process Delay Mode allows the instrument to seal the sample chamber but delays the measurement until achieving vapor equilibrium. A wait period between ten minutes and one hour was implemented. The equilibrium solute concentration value c_0 (mmol/kg) was recorded from the osmometer as consecutive 2-min measurements. When the difference between consecutive 2-min measurements fell below 5 mmol/kg, the process was complete. This final reading for c_0 from the osmometer was converted to π_{osm} using the van't Hoff equation relating solute concentration to vapor pressure (eqn. 1) (Maréchaux, et al 2015). The value of π_{tlp} was estimated from π_{osm} using the regression equation relating π_{osm} to pressure-volume curve turgor loss point values. (eqn. 2) (Bartlett MK, et al 2012)

eqn. 1: van't Hoff equation $\pi_{osm} = 2.5/1000 \times c_0$

(where the numerator of the first term represents $R \times T = 2.5$ L MPa/mol at 25°C, with R, the ideal gas constant, and T, the temperature in Kelvin, c_0 is the equilibrium solute concentration value, π_{osm} is the vapor pressure.)

eqn. 2: regression equation $\pi_{tlp}= 0.832 \times \pi_{osm} - 0.631$

(where π_{osm} is the vapor pressure, and π_{tlp} is the turgor loss point)

Leaf Thermal Tolerance

At some point of extreme heat, the photochemistry becomes damaged causing a decrease in photosynthetic efficiency. Perhaps most surprising is that there is little or no permanent damage to photosystem II because of moderate heat stress even though moderate heat stress can reduce the photosynthetic rate to near zero. Further, rubisco normally deactivates at these moderately stressful temperatures. Therefore, the deactivation of Rubisco at moderately high temperature could be a parallel deleterious effect or a regulatory response to limit damage to thylakoid reactions (Sharkey TD 2005). Further, the literature suggests that in a range of diverse species, photochemical damage is reached only after full hydraulic disruption (Trueba, et al 2019). Earlier photochemistry research found that four abundant glycerolipids of thylakoid membranes in the chloroplasts of higher plants and in the cells of cyanobacteria play important roles in maintaining the photosynthetic electron-transport machinery, and further proposed that electron transport is the leading limiting step of photosynthesis at high temperature (Sharkey TD 2005). Therefore, after reviewing the literature describing the various heat effects on the photochemistry, we chose chlorophyll *a* fluorescence as a proxy for photosynthetic efficiency in this study.

Photosynthetic electron transport driven by Photosystem II (PSII) is one of the most heat-sensitive processes in green leaves (Krause GH, et al 2010). The oxygen- evolving activity in the PSII complex is more sensitive to heat than other photosynthetic activities, as demonstrated with the release of functional manganese ions from the PSII complex as a direct result of heat inactivation (Berry J, 1980; Nash D, et al 1985). In comparison, in Photosystem I (PS I), the chloroplast envelope and enzymes of the chloroplast stroma are less heat labile (Krause GH, et al 2010). Heat tolerance determined using fluorometry is referred to as photosynthetic heat tolerance (PHT) because it provides insight into the effect of high temperature on the function of Photosystem II (PSII), but does not directly measure carbon assimilation (Perez TM, et al 2020). Our metric for photosynthetic efficiency, F_v/F_m is the ratio of the variable fluorescence to the maximum fluorescence. F_v is the difference between F_m and the initial fluorescence (F_o). Heating the leaf causes

F_o to increase and approach F_m. The increase in F_o reflects the decrease in the number of open reaction centers of PSII. A major factor involved in the mechanism of the F_o rise is an inhibition of electron transport from the primary quinone electron acceptor, Q_A, to the secondary acceptor, Q_B, of PSII, relating to the damage of the oxygen evolving complex (Krause GH, et al 2010). In theory with extreme heat, both types of PSII alterations manifested by the F_o increase and the F_m decline, respectively, result in the decrease in F_v/F_m indicating a decline in potential PSII efficiency (Krause GH, et al 2010).

The relative heat tolerance of 21 woody species across three distinct ecosystems was assessed using chlorophyll *a* fluorescence as a metric for photosynthetic efficiency. Using the # 5 leaf coring tool, a leaf disc was made, centrally between the midrib and margin, avoiding first- and second-order veins. In the case of needles, the same # 5 Leaf Corer was used, and the disc consisted of 3 – 5 needle pieces taken from the base of the fascicle. The leaf disc was then placed in a small Ziplock sample bag, one disc per bag. The bag with leaf disc was immersed in a preheated water bath for fifteen minutes. Leaf discs reached water temperature within 2 min, as shown by measurements with a fine-wire thermocouple. (HH11B/Omega) Subsequent to heat exposure, the discs were dark-acclimated for ~15 min using a Walz leaf clip with a sliding cover that blocked all incoming irradiance (Baker NR 2008). Following dark acclimations, a saturating pulse of white light was applied to the leaf disc still in the small Ziplock sample bag using a MINI - PAM fluorometer (Walz, Effeltrich, Germany). Determining the maximum quantum yield of Photosystem II in this heat-induced system using this apparatus is based on two factors: (1) the rise of the initial fluorescence emission, F_o, and (2) the decrease in the ratio of the variable to the maximum fluorescence, F_v/F_m. Both variables serve as indicators of the heat effects on PSII. The overall quantum yield of photochemical energy conversion, with a dark- adapted sample, can be assessed by the expression:

$$\mathrm{YIELD} = Y/1000 = (M-F)/M = \Delta F/M = \Delta F/F_m' = F_v/F_m$$ (Genty et al. 1989)

(*where YIELD is the maximum yield of photochemical energy conversion, Y is the display output, F is the fluorescence yield measured briefly before the last saturating light pulse triggered by START,* ***M*** *is the maximum fluorescence yield measured during the last saturating light pulse triggered by START (M =* F_m *or* F_m*'),* F_v *is referred to as the variable fluorescence,* F_m *is referred to as the maximum fluorescence)*

The parameter for relative heat tolerance was assigned as the temperature at 50 % loss of maximum quantum yield of Photosystem II (T_{50}). Therefore, the higher the value for T_{50}, the higher the relative heat tolerance.

Statistical Analysis

The π_{tlp} and T_{50} means calculated for species in each of the three ecosystems and two leaf growth habits were compared using a one- way ANOVA /Tukey test and *t* test, respectively. Logistic nonlinear least square models with the 'nls' function in the R STATS package was used for extracting the T_{50} parameter. Scatter plots of π_{tlp} (MPa) vs T_{50} (°C) were created, and a regression model was run with one predictor. Bivariate relationships were assessed using standardized major axis estimation (model II regression) with software (Falster, Warton & Wright 2003). The program first tested for differences in slope between ecosystems. If no significant difference in slope was detected, tests for differences in intercepts, and whether ecosystems were separated along the standardized major axis with a common slope were performed using randomization routines.

Results

ANOVA comparison of the π_{tlp} means of the three ecosystems in the wet season revealed an *f-value* of .005 and *p-value* of 0.946, and comparison in the dry season revealed an *f-value* of 2.856 and *p-value* of 0.109. (Figure 1a) Comparison of the T_{50} means of the three ecosystems in the wet season revealed an *f-value* of .307 and *p-value* of 0.585 and comparison in the dry season revealed an *f-value* of 15.99 and *p-value* of 0.0016. (Figure 1b) Further using the Tukey test, the shrub/desert comparison revealed a *p-value* of 0.12, the forest/desert comparison revealed a *p-value* of 0.0061, the forest/shrub comparison revealed a *p-value* of 0.34. (Table 6)

t-test comparison of the π_{tlp} means of the two leaf growth habits in the wet season revealed a *t-value* of 1.83 and a *p-value* of 0.084 and comparison in the dry season revealed a *t-value* of 1.52 and a *p-value* of 0.15. Comparing T_{50} means of the two leaf growth habits in the wet season revealed a *t-value* of -0.67 and a *p-value* of 0.51 and comparison in the dry season revealed a *t-value* of 1.30 and a *p-value* of 0.203.

Table 5. Species trait measurements of π_{tlp} (MPa) and T_{50} (°C) for both the wet and dry seasons shown.

Species	π_{tlp} (MPa) wet	π_{tlp} (MPa) dry	T_{50} (°C) wet	T_{50} (°C) dry
Abies concolor	-4.23	-3.25	48.85	48.35
Arctostaphylos pringlei	-3.59	-3.10	50.75	50.23
Calocedrus decurrens	-4.26	-2.73	48.79	48.47
Pinus coulteri	-3.26	-3.05	48.21	48.70
Pinus lambertiana	-3.93	-2.97	47.19	47.50
Quercus chrysolepis	-4.83	-2.97	48.25	47.90
Adenostoma fasciculatum	-3.73	-3.87	49.25	51.10
Artemisia californica	-2.57	-2.44	45.16	47.74
Eriogonum fasciculatum	-2.65		48.78	
Heteromeles arbutifolia	-3.68	-3.79	49.39	51.15
Malosma laurina	-3.30	-2.85	51.15	50.97
Rhamnus illicifolia	-4.15		49.70	
Salvia apiana	-2.03	-2.12	47.14	49.58
Salvia mellifera	-2.17	-2.54	45.86	48.01
Condea emoryi	-3.02	-2.65	49.43	49.67
Encelia farinosa	-3.70	-4.65	47.56	52.47
Fouquieria splendens	-3.20	-2.17	46.15	49.45
Justicia californica	-3.59	-3.23	48.17	54.43
Larrea tridentata	-5.23	-5.33	51.27	52.54
Parkinsonia florida	-4.67	-4.20	50.18	50.61
Senegalia greggi	-4.54	-4.04	51.84	51.48

Table 6. Values for π_{tlp} and T_{50} means ± SD for the forest, shrubland and desert shown. Letters represent one way ANOVA results, where a change in letters between two ecosystems within a row indicates a statistically significant difference between the means.

	Forest	Shrubland	Desert
π_{tlp} mean (MPa) wet	-3.97 ^{a} ± 0.54	-3.03 ^{a} ± 0.74	-3.79 ^{a} ±0.62
π_{tlp} mean (MPa) dry	-3.03 ^{a} ± 0.17	-2.94 ^{a} ± 0.73	-3.75 ^{a} ±1.12
T_{50} mean (°C) wet	48.67 ^{a} ± 1.08	48.30 ^{a}± 1.92	49.23 ^{a} ± 1.90
T_{50} mean (°C) dry	47.81^{b} ± 2.00	49.76 ^{a} ± 1.57	51.52^{a} ± 1.78b

Figure 1a. The range of values of π_{tlp} (MPa) for forest, shrubland and desert during both the wet season and the dry season, where the lower end of the box represents the minimum, the upper end of the box represents the maximum, and the line represents the median.

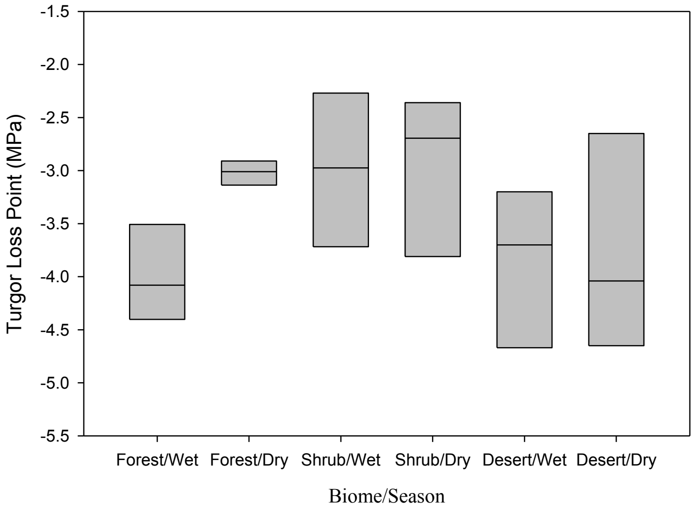


Figure 1b. The range of values of T_{50} (°C) for forest, shrubland and desert during both the wet season and the dry season, where the lower end represents the minimum, the upper end represents the maximum, and the line represents the median.

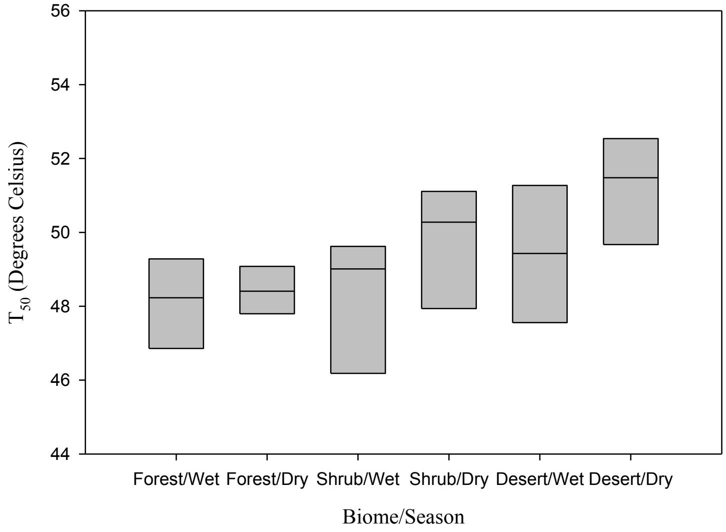


Table 7. Values for π_{tlp} and T_{50} means ± SD for the evergreen and deciduous leaf growth habit shown. Letters represent *t*- test results, where a change in letters between the leaf growth habits within a row indicates a statistically significant difference between the means.

	Evergreen	Deciduous
π_{tlp} mean (MPa) wet	$-3.89^{a} \pm 0.68$	$-3.22^{a} \pm 1.00$
π_{tlp} mean (MPa) dry	$-3.48^{a} \pm 0.80$	$-2.92^{a} \pm 0.81$
T_{50} mean (°C) wet	$48.88^{a} \pm 1.47$	$48.44^{a} \pm 2.25$
T_{50} mean (°C) dry	$49.42^{a} \pm 2.50$	$50.12^{a} \pm 2.13$

Figure 2a. Range of values of π_{tlp} (MPa) for both the deciduous and evergreen leaf growth habits during both the wet and dry seasons, where the lower end represents the minimum, the upper end represents the maximum, the line represents the median, and the whiskers indicate outliers.

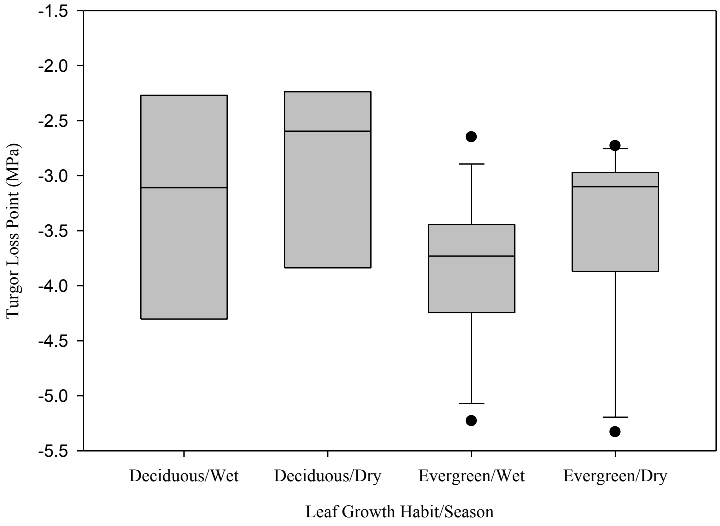


Figure 2b. Range of values of T_{50} (°C) for both the deciduous and evergreen leaf growth habits during both the wet and dry seasons, where the lower end represents the minimum, the upper end represents the maximum, the line represents the median, and the whiskers indicate outliers.

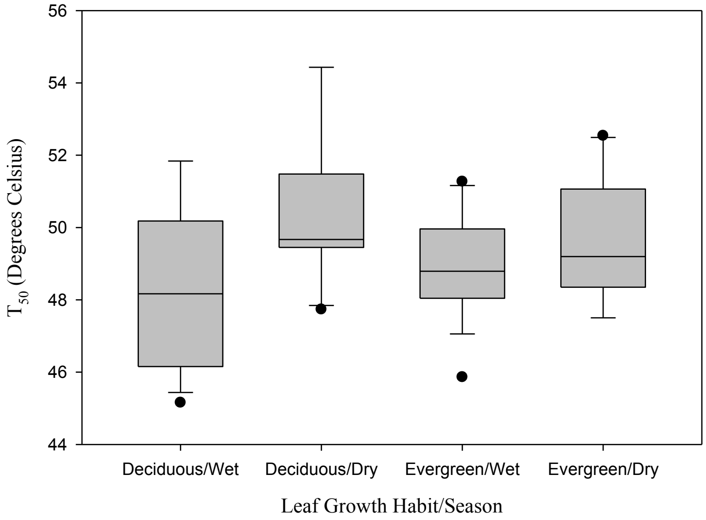
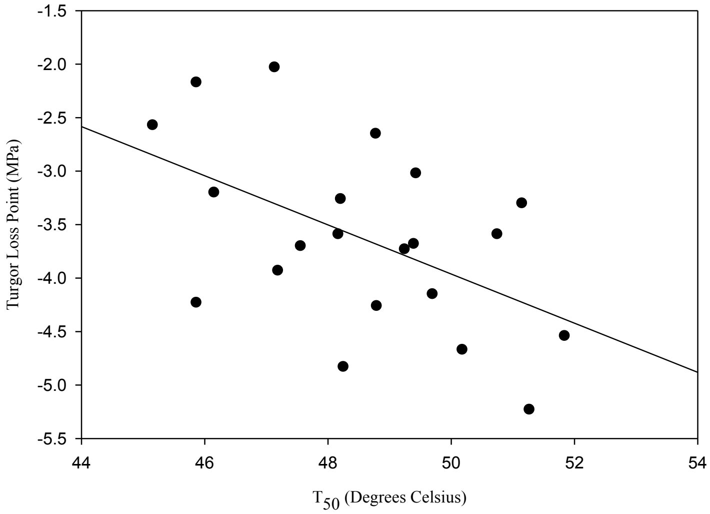


Table 8. Standard major axis regression analysis of the relationship between leaf photosynthetic heat tolerance (T_{50}) and leaf turgor loss point (π_{tlp}). Tests for heterogeneity of slope, shifts in intercept, and whether biomes are separated along a standardized major axis with a common slope and intercept for relationships among tolerance traits.

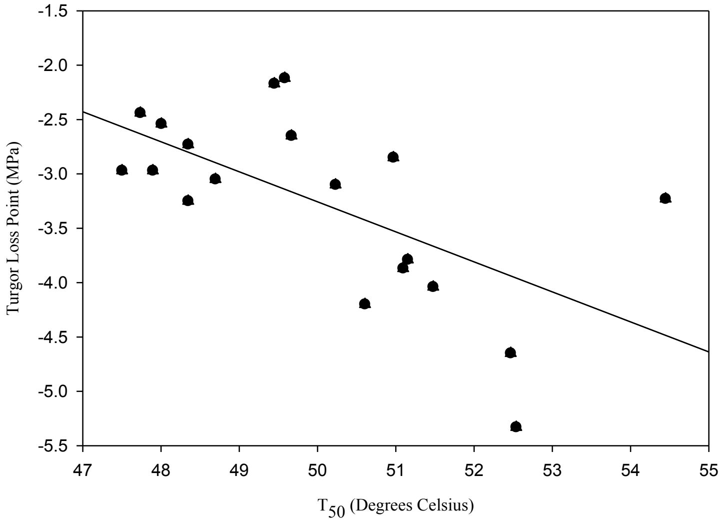
Season	Relationship	equation	*n*	r^2	*p*
Wet season	All data	**$T_{50} = -2.23 \cdot \pi_{tlp} + 40.5$**	**22**	**0.26**	**0.018**
	Desert	**$T_{50} = -2.48 \cdot \pi_{tlp} + 39.3$**	**7**	**0.58**	**0.047**
	Forest	$T_{50} = 2.95 \cdot \pi_{tlp} + 60.0$	6	0.09	0.57
	Shrubland	**$T_{50} = -2.61 \cdot \pi_{tlp} + 40.4$**	**8**	**0.52**	**0.041**
	Heterogeneity of slope				0.958
	Intercept heterogeneity				0.095
	Shift in common slope				0.261
Dry season	All data	**$T_{50} = -2.23 \cdot \pi_{tlp} + 42.7$**	**19**	**0.38**	**<0.005**
	Desert	$T_{50} = -1.58 \cdot \pi_{tlp} + 45.6$	7	0.24	0.267
	Forest	$T_{50} = -5.44 \cdot \pi_{tlp} + 32.1$	6	0.06	0.638
	Shrubland	$T_{50} = -2.41 \cdot \pi_{tlp} + 43.5$	6	0.53	0.102
	Heterogeneity of slope				0.195
	Intercept heterogeneity				1.000
	Shift in common slope				**0.020**

Figure 3a. Scatter plot of mean values for T_{50} (°C) and π_{tlp} (MPa) per species, during the wet season from the forest, shrubland and desert with regression line shown.



Multiple R-squared: 0.2623, p-value: 0.01762

Figure 3b. Figure 3a: Scatter plot of mean values for T_{50} (°C) and π_{tlp} (MPa) per species, during the dry season from the forest, shrubland and desert with regression line shown.



Multiple R-squared: 0.3835, p-value: 0.004695

Figure 4a. Relationship between mean annual precipitation and π_{tlp} (wet and dry season) where every point is the mean of a species. The vertical lines show the range in π_{tlp} values, and the non-vertical line is a regression line.

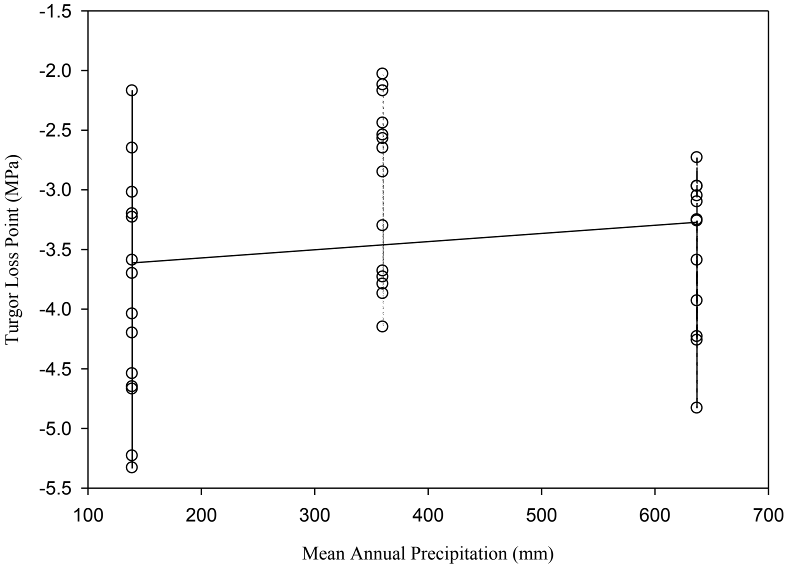


Figure 4b. Relationship between mean annual precipitation and T_{50} (wet and dry season) where every point is the mean of a species. The vertical lines show the range in T_{50} values, and the non-vertical line is a regression line.

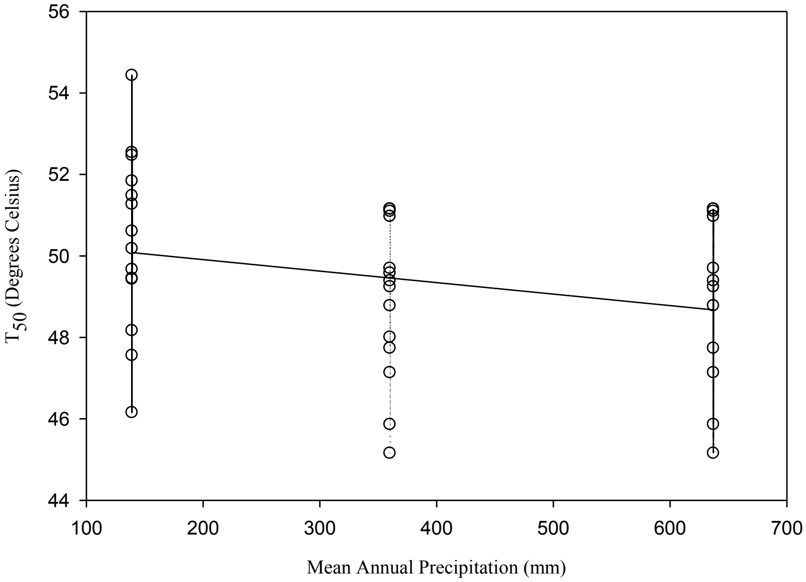


Figure 5a. Relationship between mean annual temperature and π_{tlp} (wet and dry season) where every point is the mean of a species. The vertical lines show the range in π_{tlp} values, and the non-vertical line is a regression line.

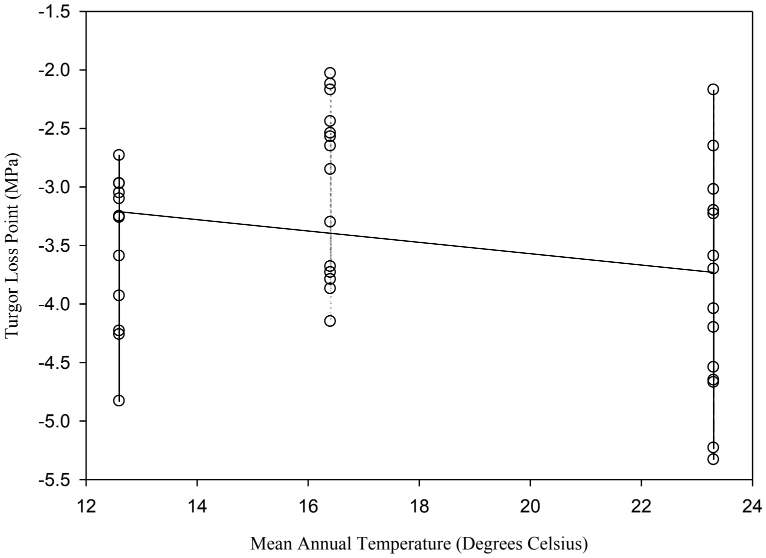


Figure 5b. Relationship between mean annual temperature and T_{50} (wet and dry season) where every point is the mean of a species. The vertical lines show the range in T_{50} values, and the non-vertical line is a regression line.

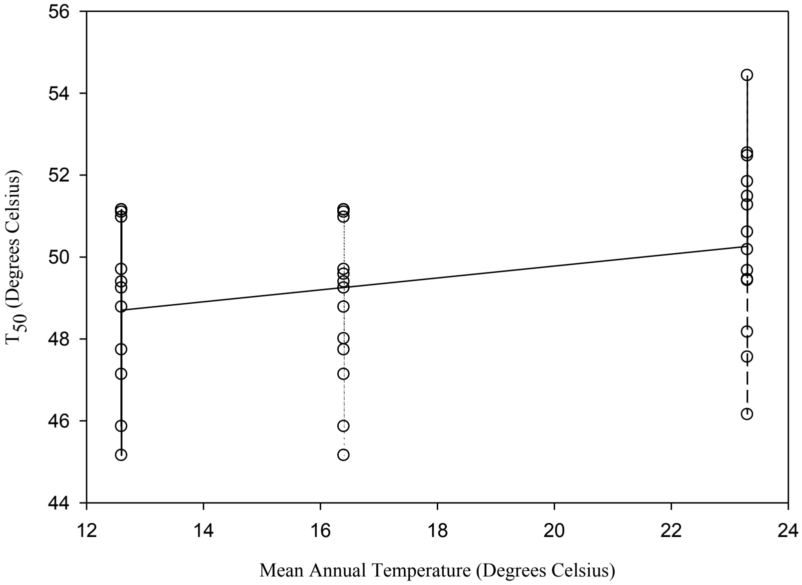


Figure 6a. Values for the change in π_{tlp} (MPa) for all species, where distance from zero represents the change from the wet season values to the dry season values. Forest (bottom), shrub(middle) and desert (top)

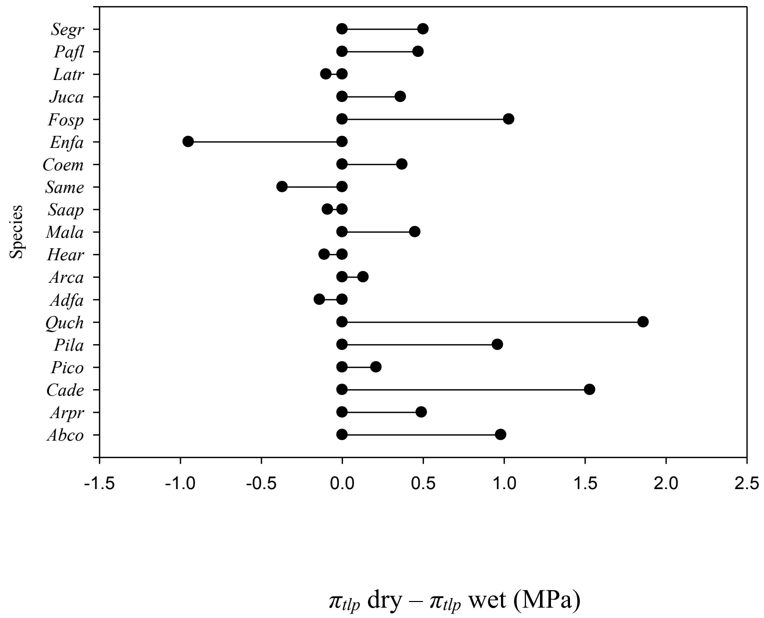
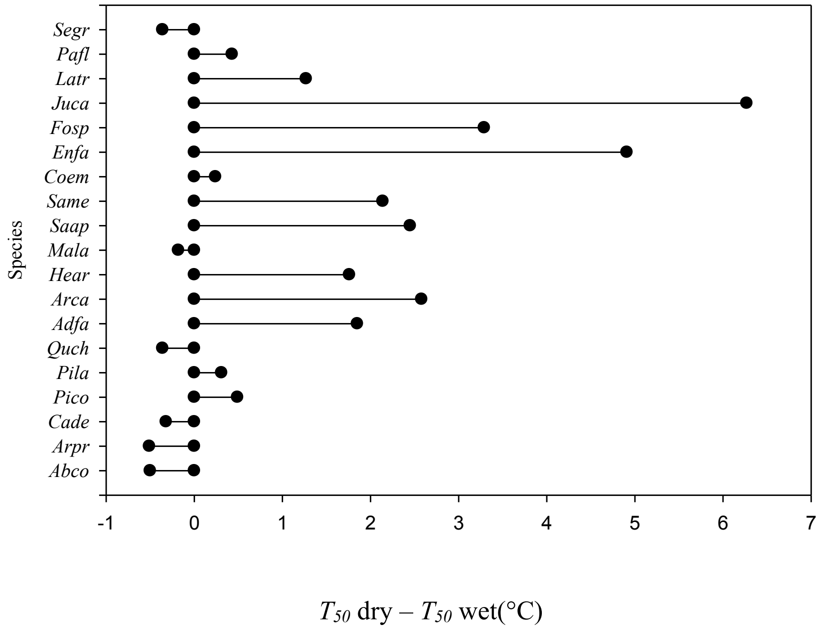


Figure 6b: Values for the change in T_{50} (°C) for all species, where distance from zero represents the change from the wet season to the dry season values. Forest (bottom), shrub (middle) and desert (top)



Discussion

Trait Measurements

In reviewing the species trait measurements, we found ample agreement with documented drought and heat tolerant functional trait research. The deciduous species *Salvia apiana*, *Salvia mellifera* and *Artemisia californica* in the shrubland displayed some of the highest values for turgor loss point of -2.03, -2.17 and -2.57 MPa, respectively. This agrees with a low relative drought tolerance playing into the drought avoidance strategy. Similarly, these species also show a parallel trend with heat tolerance displaying some of the lowest T_{50} values of 47.14 °C, 45.86 °C and 45.16 °C, respectively. But, in our study it was not only the deciduous species that displayed low drought tolerance. *Pinus lambertiana* and *Quercus chrysolepis* in the evergreen forest, known to put down deep roots, displayed weak osmotic control with both species having a dry season turgor loss point of -2.97 MPa supporting the "trade-off" theory of drought tolerant traits, and suggesting that the variation in rooting depth is a major determinant of operational water potential ranges therefore contributing to drought survival strategies (Pivovaroff AL, et al 2016).

Interestingly, *Justicia californica* a desert deciduous species displayed the highest T_{50} value of all the species, 54.43 °C. Upon collecting leaves from this specimen in the dry season, we noticed that the shrub was not completely void of leaves, rather there were leaves down low in the center of the plant being shielded by the upper canopy. These leaves showed extreme heat tolerance and seemed to hang around through the dry conditions. For this reason, I would challenge the literature that labels this species as deciduous and argue that it is more accurate to say it is semi-deciduous. In another research study involving woody savanna species in southern China, it was observed that species with long-lived leaves, generally associated with conservative resource use, had higher heat tolerance than species with short-lived leaves (Zhang S-B, et al 2012). Further in the tropics, among lowland species, T_{50} increased with leaf mass per area, so species with structurally more costly leaves reduce the risk of leaf loss during hot spells (Slot M, et al 2021). In agreement in our study, *Malosma laurina*, an evergreen shrub of the Anacardiaceae family has tough, leathery leaves that fold up like the shape of a taco allowing the leaf lamina to be parallel with the sun's rays, minimizing the incoming radiation, displaying a relatively high T_{50} value of 51.15 °C. Heat tolerance research tells us that the thermal time constant (τ) in seconds, quantifies the thermal stability of a leaf, i.e. how rapidly leaf temperature responds to temporal variation in the microclimate (Michaletz ST, et al 2016). The low τ of relatively small and thin leaves indicates that they heat up and cool down quickly. The photosynthetic capacity of species with low τ peaks at higher ambient temperatures than for species with high τ, suggesting that species with low τ are better acclimated to higher temperatures. Supporting this finding in our study, the evergreen desert shrub *Larrea tridentata,* with pubescent resinous leaves only measuring 7-18 millimeter in length, displayed a T_{50} in the dry season of 52.54 °C. Also in the desert, *Encelia farinosa*, strategized for heat tolerance with hairy light-colored leaves displayed a T_{50} in the dry season of 52.47 °C.

ANOVA/Ecosystem (π_{tlp} and T_{50})

We observed the widest range of turgor loss point values in the desert during the dry season -2.17 MPa to -5.33 MPa, and a substantially narrow range in the forest during the dry season -2.73 to -3.25 MPa. These results would suggest typical conservative isohydric behavior in the forest with the species closing their stomata early to prevent water loss and sustaining higher turgor loss point values, as compared to a more liberal anisohydric behavior in desert species sustaining much lower water potentials at turgor loss point. But ANOVA results revealed no statistically significant differences between the turgor loss point means as compared across the three ecosystems for both the wet season and the dry season. With T_{50} values, we observed the widest range in the shrubland during the wet season 45.16 °C – 51.15 °C, and a substantially narrow range in the forest during the dry season 47.50 °C – 50.23 °C. ANOVA results revealed one statistically significant difference in the T_{50} means between the shrubland and the forest during the dry season 49.76 ± 1.57 (°C) and 47.81 ± 2.00 (°C) (Table 6). It is worthy to note that the variation within ecosystems is greater than the variation between ecosystems making the use of heat tolerances to understand plant ecology complicated by the fact that tolerances can vary markedly among co-occurring species (Perez TM, et al 2020). Recent studies have shown that the interspecific variation of heat tolerances within communities often exceeds variation in community – mean heat tolerances across coarse climatic gradients. (Feeley KJ, et al 2020; O'Sullivan, et al 2017). It follows that heat tolerances should therefore correlate more with extreme leaf temperatures than with regional climates – especially since leaf and air temperatures can be decoupled

(Michaletz ST, et al 2016). This would suggest that leaf temperatures are determined by the physical properties of leaves and their interactions with the environment. Since plant species possess unique combinations of thermoregulatory traits including leaf size, stomatal conductance and thermal absorptivity, different species should experience different leaf temperatures even in identical environmental conditions (Leigh A, et al 2017; Lambers, et al 2008; Meinzer FC, et al 1994; Smith WK, et al 1977).

t-test/Leaf Growth Habit (π_{tlp} and T_{50})

We observed the widest range of turgor loss point values in the deciduous species during the wet season -2.03 to -4.67 MPa. This speaks to the range of deciduousness to include the intermediate leaf growth habit of semi-deciduous, where species may not lose all their leaves and will need to exercise some degree of osmotic control to sustain lower water potentials. We observed the narrower range in turgor loss point in the evergreen species during the wet season where most turgor loss points resided between -3.26 and -4.83 MPa. A *t*-test revealed no statistically significant differences between the turgor loss point means between the deciduous and evergreen leaf growth habit in the wet or dry seasons. However, in both the wet and dry seasons, deciduous species revealed higher, less negative, turgor loss points than evergreen species. But leaf habit does not appear to have as large as an effect on T_{50} as it does on π_{tlp}. A *t*-test revealed no statistically significant difference between the T_{50} means of deciduous and evergreen species.

Standardized Major Axis (SMA) Regression

We see a significant shift in the common slope for dry season data showing that the desert occupies a significantly different range in the relationship compared to the forest and shrubland. The desert values extend to the low π_{tlp} and high T_{50} portion of the relationship that is not occupied by the forest and shrubland. However, there were no differences in slope or intercept between ecosystems within a season, indicating a high degree of functional convergence to the physiological and biochemical constraints of the heat-drought tolerance relationship.

T_{50} vs π_{tlp} (wet and dry season)

We found a significant *p-value* (<0.05) in both the wet and dry seasons with the scatter plots of T_{50} vs π_{tlp} using data from all three ecosystems. The regression line reveals a strong negative correlation between these two parameters with data essentially void in the regions representing low turgor loss point values coupled with low T_{50} values, as well as high turgor loss point values coupled with high T_{50} values. It is worthy to note that the *p-value* generated from plotting all species on the T_{50} vs π_{tlp} scatter plot is more statistically significant than the *p-values* generated when stratifying the data by ecosystem because the *p-value* is driven by the higher sample size. But, if you look at the r squared values, the shrubland has the stronger relationship. These results suggest that drought and heat tolerance suites of traits could be conserved across all ecosystems possibly as an evolutionary response to stress and possibly controlled by the same upstream regulatory processes that lead to coordinated stress tolerance.

Climate regression

The data reveals an increase in turgor loss point and a decrease in T_{50} across an increasing MAP gradient. In addition, the data reveals a decrease in turgor loss point and an increase in T_{50} across an increasing MAT gradient.

Plasticity (π_{tlp})

The results show that all the species in the forest (bottom of the plot) increased their turgor loss point value from the wet season to the dry season, with *Quercus chrysolepis* increasing the most at nearly 2.0 MPa. This reflects an earlier observation that the forest species take on more conservative turgor loss points in the dry season displaying anisohydric behavior closing stomata early, and for *Pinus lambertiana* and *Pinus coulteri*, relying on deep roots as a trade- off for poor osmotic control. It is also worthy to note that as the leaf ages between the wet and dry seasons, there could be a loss of ability to tolerate the loss of turgor. We see the shrubs (middle of plot) displaying less plasticity compared to the forest. But in the desert species (top of plot), we see *Encelia farinosa,* an evergreen species, decreasing turgor loss point nearly 1.0 MPa from the wet season to the dry season, thereby becoming more drought tolerant. In contrast *Fouquieria splendens*, a deciduous species, displays an increase in turgor loss point nearly 1.0 MPa from the wet season to the dry season, thereby becoming less drought tolerant.

Plasticity(T_{50})

The results show the forest species (bottom of plot) displaying relatively low plasticity between the wet season and dry season. The shrubs (middle of plot) display relatively more plasticity between the wet and dry seasons increasing T_{50} by nearly 2 to 3 ˚C, with the exception of *Malosma laurina,* Lastly, the desert species (top of plot) display the most plasticity relative to the other ecosystems with all species displaying an increase in their T_{50} value in the dry season, with the exception of *Senegalia greggi* , and *Justicia californica* showing the greatest increase of more than 6˚C. Significant plasticity was seen in photosynthetic heat tolerance among tropical trees in India, with higher T_{50} values in the hot, dry season than in the cooler wet season (Slot M, et al 2019). In addition, Mediterranean trees that experience considerable seasonal temperature variation exhibited similar dynamic changes in heat tolerance (Froux F, et al 2004).

Conclusion

In our study, our first key revelation was that the interspecific variation, with respect to drought and heat tolerant functional traits within a community of co-occurring species was as great as the variation between different communities across a coarse climatic gradient. This finding would suggest that it is in fact not the ecological climate parameters that are driving drought and heat tolerance. Rather it is the physiology of the organisms with respect to functional traits for drought and heat tolerance that allow species to acclimate and not succumb to climate change type drought. Our second key revelation manifested with the emergence of a significant negative correlation between T_{50} and π_{tlp} using species data taken across all ecosystems. This correlation suggests that drought and heat tolerance may be co-selected in response to evolutionary stress, or that acclimation or adaptation for greater heat or drought tolerance causes a necessary physiological change of the other trait in the direction of greater tolerance. This has implications for predicting which species are likely to survive global change type drought, where arid conditions are compounded with increasingly errant heat waves. Our study supports the trait co-selection hypothesis based on the significant correlations of drought and heat tolerant measurements across ecosystems with a broad range of climatic conditions. Our study linked the implications of drought and heat tolerance trait correlations to the trait co-selection hypothesis, in agreement with other research studies finding drought tolerance trait correlations alone implying functional coordination, concerted convergence and shared ancestry (Bartlett MK, et al 2016). A further line of support for this hypothesis is in the literature pertaining to the *sequence* at which species respond to both drought, in succumbing to hydraulic failure, and heat, with a precipitous photosynthesis decline, being conserved amongst species. This research posits that across species, tissue dehydration thresholds were interconnected, suggesting trait co-selection. The stomatal and leaf hydraulic systems show early functional declines before cell integrity is lost. Substantial damage to the light harvesting function of the photochemical apparatus, as shown with F_v/F_m measurements, occurred at extreme dehydration, after complete stomatal closure, and seems to be irreversible. These findings reveal that photochemical damage is reached only after full hydraulic disruption in a range of diverse species (Trueba S, et al 2019). Our research findings represent initial steps towards illuminating the path in the pursuit of developing predictive tools to accurately identify the species that are likely able to survive, and continue to reproduce, amidst the forecasted combined heat and drought stress in our relatively near future. In tropical tree research it was posited that further studies to resolve the phylogenetic signal in π_{tlp} are needed to assess the evolutionary history of drought tolerance in tropical trees (Maréchaux I, et al 2015). We open the window to explore the evolutionary underpinnings that may lead to the phylogenetic signal for both drought and heat tolerance functional traits. Implications for understanding these evolutionary legacies coupled with genomic research may progress to the identification of pleiotropic genes and lead to improved predictive power.

Further Research

In our study, we focus our view of relative drought tolerance through the lens of effective osmotic control, using turgor loss point as a parameter and relative heat tolerance through the lens of the decline of Photosystem II efficiency, using F_v/F_m as a parameter. This research explores the intimate connection between drought and heat tolerance. A change in one may necessarily impart a change in the other, which might explain why species are so vulnerable to global change type drought. Our first area of possible further research pertains to heat acclimation in leaves, described in the research as a complex phenomenon, which, among many molecular processes, comprises changes in photosynthetic pigment composition such as increases lutein, α- and β-carotene and can alter interactions between xanthophyll-cycle pigments and thylakoid membranes (Volkova, et al 2010; Havaux M, et al 1996). The more recent literature suggests that when the stomata are closed, leaf cuticular conductance continues (Cochard H 2019). This would imply that at higher temperatures the leaf is still losing water through this mechanism, thereby decreasing the time to total hydraulic failure. The temperature at which stomatal closure occurs, but cuticular conductance continues, is termed the phase transition temperature, (T_p). The time to hydraulic failure appears clearly more determined by the water losses beyond the point of stomatal closure rather than the speed at which plants empty the soil water reserve when stomata are still open (Cochard H 2019). Of interest to us, in the context of heat acclimation, is the species *Rhazya stricta*, a typical woody hot desert plant, which possesses a high T_p value (> 50℃), and a greater proportion of triterpenoids in its cuticle (Schuster A-C, et al 2016). This may suggest that plants could achieve a higher cuticular thermostability by modifying their chemical composition of their cuticles, but the degree of genetic variability or plasticity of T_p is not yet known (Cochard H 2019).

A secondary area of future research involves exploring an additional parameter for assessing relative heat tolerance in leaves, the critical temperature (T_{crit}). T_{crit} is the highest temperature that does not yet cause PSII damage when F_v/F_m begins to decline, whereas T_{50} is the temperature at which PSII functionally is reduced to 50 % (Slot M, et al 2019) Both parameters are referred to as Photosynthetic Heat Tolerances (PHTs) in the literature. We propose that the differential (T_{50} - T_{crit}) in these PHTs may be a better informant in our research. I liken this to the daredevil who walks across hot coals. The ability of the daredevil to “tolerate” the extreme heat imparted on the soles of his feet for a given amount of time, does not necessarily translate to the thermal energy not imparting damage to the epithelial tissue during his trek across the glowing embers. Similarly, leaf tissue exposed to extreme heat may have a relatively high T_{50} value, but just how much tissue damage has occurred is not clear. Pulling out the T_{crit} value from the data enables us to track when the tissue damage to Photosystem II begins. The differential value (T_{50} - T_{crit}) will allow us to assess relative heat tolerance within the context of heat damage effects, run a non- linear regression against turgor loss point, and compare its correlation strength to the reported data in this manuscript. In other words, we wish to investigate the claim that a species with a higher T_{50} value does not necessarily equate to the species being less susceptible to thermal damage (Perez TM 2020).

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