RESEARCH ARTICLE

Functional Ecology | BRITISH ECOLOGICAL SOCIETY

Photosynthetic heat tolerances and extreme leaf temperatures

Timothy M. Perez[1,2] | **Kenneth J. Feeley**[1,2]

[1]Department of Biology, University of Miami, Coral Gables, FL, USA

[2]Fairchild Tropical Botanic Garden, Coral Gables, FL, USA

Correspondence
Timothy M. Perez
Email: t.more.perez@gmail.com

Abstract

1. Photosynthetic heat tolerances (PHTs) have several potential applications including predicting which species will be most vulnerable to climate change. Given that plants exhibit unique thermoregulatory traits that influence leaf temperatures and decouple them from ambient air temperatures, we hypothesized that PHTs should be correlated with extreme leaf temperatures as opposed to air temperatures.

2. We measured leaf thermoregulatory traits, maximum leaf temperatures (T_{MO}) and two metrics of PHT (T_{crit} and T_{50}) quantified using the quantum yield of photosystem II for 19 plant species growing in Fairchild Tropical Botanic Garden (Coral Gables, FL, USA). Thermoregulatory traits measured at the Garden and micro-environmental variables were used to parameterize a leaf energy balance model that estimated maximum in situ leaf temperatures (T_{MIS}) across the geographic distributions of 13 species.

3. T_{MO} and T_{MIS} were positively correlated with T_{50} but were not correlated with T_{crit}. The breadth of species' thermal safety margins (the difference between T_{50} and T_{MO}) was negatively correlated with T_{50}.

4. Our results provide observational and theoretical support based on a first principles approach for the hypothesis that PHTs may be adaptations to extreme leaf temperature, but refute the assumption that species with higher PHTs are less susceptible to thermal damage. Our study also introduces a novel method for studying plant ecophysiology by incorporating biophysical and species distribution models, and highlights how the use of air temperature versus leaf temperature can lead to conflicting conclusions about species vulnerability to thermal damage.

KEYWORDS
adaptation, climate change, energy balance, microclimate, quantum yield

1 | INTRODUCTION

The heat tolerance of plants can be defined as the maintenance of essential plant functions that contribute to the fitness of a genotype (Hall, 1992; Porch & Hall, 2013) and is commonly quantified as a temperature that causes cell death (i.e. necrosis) or injury to photosystem II (PSII). Several studies have documented positive correlations between species' heat tolerances and regional climates (Feeley et al., 2020; Knight & Ackerly, 2002; O'Sullivan et al., 2017; Smillie & Nott, 1979; Zhu et al., 2018), which has reinforced the assumption that higher heat tolerances promote greater relative fitness in hotter environments (Araújo et al., 2013; Osmond et al., 1987; Zhu et al., 2018). Consequently, heat tolerances have been assumed to constrain leaf morphology (Beerling, Osborne, & Chaloner, 2001; Lee, Upchurch, Murchie, & Lomax, 2015; Wright et al., 2017), used to screen for heat-resistant crops (Hall, 1992; Weng & Lai, 2005; Yamada, Hidaka, & Fukamachi, 1996) and are of increasing interest for understanding and predicting species' responses to climate change.

However, the use of heat tolerances to understand plant ecology is complicated by the fact that tolerances can vary markedly among co-occurring species. For example, 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 et al., 2020; O'Sullivan et al., 2017). In other words, some arctic plants have higher heat tolerances than some tropical plants (O'Sullivan et al., 2017). Clearly, a greater understanding of how variation in heat tolerances correspond to environmental variation is needed before they can be used to predict plant responses to climate change.

Common garden experiments can control for environmental variables that may obfuscate sources of variation in heat tolerances. A recent common garden experiment used cooling and warming treatments to show that plant communities from warmer climates generally exhibit higher mean heat tolerances than communities from colder climates, but that climate did not explain variation in heat tolerances among individual species (Zhu et al., 2018). In a separate study, the variation in the heat tolerances of 42 species grown in a botanical garden were attributed to physiological adaptions to microhabitat, and not climate (Curtis, Knight, & Leigh, 2019). Similarly, Knight and Ackerly (2002) speculated that differences between heat tolerances of congeneric species measured in situ, and those measured in a common environment were attributable to changes in leaf temperature. These latter studies highlight the potential importance of leaf physiology and leaf temperatures for understanding variation in heat tolerances.

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 (Leigh, Sevanto, Close, & Nicotra, 2017; Smith, 1978), stomatal conductance (Lambers, Chapin, & Pons, 2008) and thermal absorptivity (Meinzer & Goldstein, 1985; Smith & Nobel, 1977), different species should experience different leaf temperatures even in identical environmental conditions. 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 et al., 2016). Importantly, this hypothesis could help explain the variation in heat tolerances of plants in common garden experiments and among communities.

Heat tolerance is commonly determined by measuring the responses of cell death, membrane leakage or fluorescence to heat treatments (Hall, 1992; Porch & Hall, 2013). Heat tolerance determined using fluorometry is referred to as *photosynthetic heat tolerance* (PHT) because it provides insight into the effect of high temperatures on the function of PSII, but does not directly measure carbon assimilation (Baker, 2008). Two fluorometric variables widely used to assess the function of PSII in response to heat are the initial fluorescence (F_0) and the maximum quantum yield (F_V/F_M). Changes in F_0 signal changes in the number of open reaction centres of PSII, but are subject to error when measured during stress treatments (e.g. heating) that alter the optical properties of leaves (Baker, 2008). Alternatively, F_V/F_M provides

a relative index of PSII function (where $F_V = F_M - F_0$; F_M = closed reaction centres) that is not biased by confounding effects of changing leaf optical properties during stress treatments (Baker, 2008).

Regardless of the method used to monitor function of PSII, there are two widely reported metrics of PHT. One metric of PHT is termed the critical temperature (T_{crit}) and represents the temperature that causes initial damage in a given response variable (e.g. Offord, 2011; Schreiber & Berry, 1977; Slot, Krause, Krause, Hernández, & Winter, 2018). The second metric of PHT is generally termed T_{50} and is calculated as the temperature that causes 50% damage in the chosen response variable (e.g. Knight & Ackerly, 2002; Krause et al., 2010).

Although T_{crit} and T_{50} indicate different levels of damage to the function of PSII, both can be useful for predicting vulnerability to heat damage. This vulnerability is termed the thermal safety margin and is generally quantified as the difference between maximum air temperatures and the species' heat tolerance (Curtis, Gollan, Murray, & Leigh, 2016; O'Sullivan et al., 2017). Small thermal safety margins signify a high susceptibility to heat damage. Given that leaf temperatures are often decoupled from air temperatures (Michaletz et al., 2016), thermal safety margins calculated using maximum leaf temperatures, rather than air temperatures, should provide better predictions of leaf vulnerability to heat damage.

In this study, we move beyond the use of coarse-scale climatic data for understanding variation in PHTs by testing if T_{crit}, and T_{50} are correlated with extreme leaf temperatures. We first investigate the relationship between both PHTs and maximum observed leaf surface temperatures in 19 plant species growing together at the Fairchild Tropical Botanic Garden (Coral Gables, Florida, USA). Since maximum leaf temperatures observed within a single location may not represent the thermal extremes experienced by species across their full ranges, we next use a novel modelling procedure to estimate the maximum leaf temperatures throughout the known distributions of 13 species and compared these estimates to PHTs measured at Fairchild Garden. These maximum in situ leaf temperature were estimated using a leaf energy balance model parameterized with thermoregulatory traits collected at Fairchild Garden and microhabitat data at the species' known occurrence locations. We then test the assumption that species with higher heat tolerances are less vulnerable to heat damage by calculating the thermal safety margin for each species using extreme leaf temperatures and PHTs measured in the garden.

2 | MATERIALS AND METHODS

2.1 | Study site and species selection

This study was conducted using the living plant collections of the Fairchild Tropical Botanic Garden in Coral Gables, Florida, USA (25.677 N, −80.275 W). Fairchild Garden has a subtropical monsoonal climate with a mean annual temperature of 24.1°C and an average annual rainfall of roughly 130 cm (Figure S1). Fairchild Garden

curates over 12,000 individual plants representing >2,500 species of >175 families from tropical and subtropical biomes all over the world (Perez et al., 2019). We selected 19 woody plant species from 18 different families that naturally occur in tropical rainforest or seasonal forest habitats (Figure S1; Table 1). The study species were chosen based on the availability of individuals with accessible canopies to facilitate measurements of leaf temperatures and leaf traits. For each study species, we selected one focal plant that received direct sunlight for measuring maximum leaf temperatures.

2.2 | Heat tolerances

For this study, PHTs were estimated using the F_v/F_m method as prescribed by Krause et al. (2010). Fresh sun-exposed leaves of the study plants were collected in the mornings before 10:00 a.m. on 5 and 6 September 2017 and brought immediately to onsite laboratory facilities for processing. Leaf discs (~1.9-cm diameter) were cut from several of the leaves (roughly 10–70 leaves per species). A minimum of six leaf discs were selected for heat treatment, but up to 12 discs were used per species per heat treatment if leaf material was sufficient (a total of 66–122 leaf discs per species). Leaf discs were shuffled, placed abaxial side-down on three layers of Miracloth fabric and covered with an additional layer of fabric to prevent anaerobiosis (Krause et al., 2010), before being inserted into waterproof plastic bags. Air was removed from the bags and bags were submerged in one of 11 water baths maintained at room

TABLE 1 Study species, authorities and their respective families

Species	Family
Adenocalymma comosum (Cham.) DC.[a]	Bignoniaceae
Bauhinia divaricata L.	Fabaceae
Brunfelsia lactea Krug & Urb.[a]	Solanaceae
Cocculus laurifolius DC.[a]	Menispermaceae
Combretum rotundifolium Rich.[a]	Combretaceae
Cordia goeldiana Huber[a]	Boraginaceae
Cryptostegia grandiflora Roxb. ex R.Br.[a]	Apocynaceae
Eugenia coronata Vahl ex DC.	Myrtaceae
Forestiera segregata (Jacq.) Krug & Urb.[a]	Oleaceae
Galphimia gracilis Bartl.[a]	Malpighiaceae
Gardenia taitensis DC.	Rubiaceae
Ginoria glabra Griseb.	Lythraceae
Hamelia patens Jacq.[a]	Rubiaceae
Hibiscus rosa-sinensis L.	Malvaceae
Holmskioldia sanguinea Retz.[a]	Lamiaceae
Justicia brandegeeana Wassh. & L.B.Sm.[a]	Acanthaceae
Maytenus procumbens (L. f.) Loes.[a]	Celastraceae
Moringa stenopetala (Baker f.) Cufod.	Moringaceae
Phaleria macrocarpa (Scheff.) Boerl.[a]	Thymelaeaceae

[a]Species used to estimate T_{MIS}.

temperature (~23), 38, 40, 42, 44, 46, 48, 50, 52, 54 or 60°C. During heat treatments, leaves were kept under dim light that was unlikely to induce violaxanthin and zeaxanthin production and could have led to underestimates of heat tolerances (see Section 4; Krause, Winter, Krause, & Virgo, 2015). After 15 min of heat treatment, leaf discs were removed from the water baths and bags, and were then placed in petri dishes lined with moist paper towels where they were left under dim light (~1 μmol photons m^{-2} s^{-1}) at room temperature (~23°C) to recover for ~24 hr. After this recovery period, the leaf discs were dark-adapted for 20 min and F_v/F_m was measured using an OS30p$^+$ handheld fluorometer (Opti-Science).

To estimate T_{crit} and T_{50}, we modelled the relationship between F_v/F_m and treatment temperature for each species using logistic nonlinear least squares models with the 'nls' function in the R STATS package (R Core Team, 2018; see Feeley et al., 2020 for full model). We defined T_{crit} as the temperature at which F_v/F_m begins to decline, and calculated this value by finding the temperature where the slope of the F_v/F_m versus temperature relationship reached 15% of its most negative value. The second metric, T_{50}, was calculated as the temperature that caused a 50% reduction in F_v/F_m compared to the control treatment. We generated bootstrapped means and 95% confidence level estimates of T_{crit} and T_{50} by reiterating the 'nls' model 100 times for each species while randomly resampling data with replacement before each iteration.

2.3 | Observed (T_{MO})

To measure the maximum observed leaf temperature (T_{MO}) of each species, we monitored five to eight mature sun-exposed leaves per individual on sunny days from 7 August through 2 September 2017, which is the hottest portion of the year in Miami (https://www.weather.gov/mfl/climate#NormM). Over the course of the sampling period we visited a random subset of the focal plants each day. During visits, we took point temperature measurements on the monitored leaves with a MT6 MiniTemp infrared thermometer (Raytek) for a total of 45–104 measurements per individual plant. The upper 97.5th quantile of temperature for each leaf was calculated, and the average of these values was used as the species' mean T_{MO}.

2.4 | Estimated maximum leaf temperature (T_{MIS}) across species ranges

The maximum leaf temperatures measured on an individual plant at any single site will not fully represent of the range of leaf temperatures experienced by a species throughout its geographic distribution. To estimate the maximum leaf temperatures across each of our species' distributions, we parameterized a leaf energy balance model with leaf thermoregulatory traits measured on plants in the Fairchild Garden. We used a well-established leaf energy balance model that assumes the thermal radiation absorbed by a leaf is balanced by

latent heat loss, sensible heat loss, emitted thermal radiation, and that metabolic heat production and storage are negligible. According to this model, leaf temperature (T_l) is:

$$T_l = T_a + \frac{\gamma^*}{s + \gamma^*} \left[\frac{R_{ni}}{g_{HR} c_p} - \frac{D}{\rho \gamma^*} \right], \tag{1}$$

where T_a is ambient air temperature, γ^* is the psychrometric constant determined from the ratio of combined boundary layer and radiative conductances to stomatal conductance, s is the slope of the saturation pressure curve, R_{ni} is the isothermal net radiation incident upon a leaf, g_{HR} is the combined boundary layer and radiative conductances, c_p is the heat capacity of dry air, D is the vapour pressure deficit and ρ is atmospheric pressure (Campbell & Norman, 1998).

The thermoregulatory leaf traits we used to parameterize the leaf energy balance model were effective leaf width (w_e), leaf absorptance to shortwave radiation (a) and stomatal conductance (g_s). Effective leaf width and leaf absorptance were measured on the focal species at the Garden in September 2017 at the end of the study period.

Effective leaf width (w_e) is the diameter of the largest circle capable of fitting within a leaf margin (e.g. see Leigh et al., 2017) and is used as the characteristic dimension (d) when determining boundary layer conductance ($\sqrt{u/d}$ where u is the wind speed, ms^{-1}) following the convention of Campbell and Norman (1998). When other leaf thermoregulatory traits are held constant, larger w_e reduces leaf boundary layer conductance resulting in higher temperatures (Campbell & Norman, 1998; Jones, 2014; Leigh et al., 2017). We scanned five to eight of the monitored leaves of each study species in a flatbed scanner and then used ImageJ (Rueden, Schindelin, & Hiner, 2017) to measure w_e.

Higher values of leaf absorptance (a) indicate that a larger proportion of radiant thermal energy is being absorbed by a leaf, causing hotter leaf temperatures. Leaf absorptance (a) is calculated as: $a = 1 -$ leaf reflectance − leaf transmittance. Leaf reflectance and transmittance were measured with a CI-710 Miniature Leaf Spectrometer (Bio-Science) over 400–1,000 nm wavebands following Smith and Nobel (1977) on five fully expanded mature leaves chosen at random for each species.

Stomatal conductance (g_s) influences leaf temperatures through evaporative heat loss (Lambers et al., 2008), and the sum of g_s in series with the boundary layer conductance is used to determine the boundary layer conductance of water vapour (g_{WV}). The term γ^* can then be calculated by multiplying the psychrometric constant (6.66×10^{-4} C^{-1}) by the ratio the radiative conductance (g_{Hr}) to g_{WV}, where g_{Hr} is the sum of boundary and radiative conductance (see Campbell & Norman, 1998). Stomatal conductance was measured with a SC-1 leaf porometer (Meter Devices) on the monitored leaves of each species immediately after temperature measurements. Stomatal conductance was measured in middle of the leaf blades of at least five leaves per visit to an individual plant (40–70 total measurements per individual). To calculate T_{MIS} we parameterized our leaf energy balance model with constant but realistic values of

g_s equal to the g_s observed at the maximum observed temperature (T_{MO}) of each leaf.

Before calculating maximum leaf temperatures (T_{MIS}) across the species' distributions, we validated the leaf energy balance model by using the leaf temperature measurements and environmental data collected at Fairchild Garden. The environmental data we used to parameterize the leaf energy balance model included surface albedo, solar irradiance, air temperature, relative humidity and wind speed. We used a constant surface albedo of 0.26 due to the mixed grass cover at Fairchild Garden (Campbell & Norman, 1998). We measured environmental data and stomatal conductance simultaneously with leaf temperatures. We measured solar irradiance incidental to the leaf blades and over wavebands 400–1,100 nm using a Model 10.0 Digital Pyrometer (Solarmeter). Ambient air temperature, wind speed and relative humidity were measured with a Kestrel 3000 (Kestrel Instruments). Due to the inability of our equipment to measure very low wind speeds, wind speeds were assumed to be 0.1 ms^{-1} when not registered by our anemometer. Even at low wind speeds of 0.1 ms^{-1}, leaves are likely to experience forced convection—one assumption of our energy balance model (Nobel, 1999). We compared the predicted leaf temperatures for Fairchild Garden and each species' T_{MO} using ordinary least squares regression. We also performed a sensitivity analysis to identify the variables that have the greatest effects on modelled leaf temperatures with R's PSE package, which uses a Latin hyper cube sampling technique to randomly sample the multivariate space encompassing the leaf trait and environmental data used to parameterize our leaf energy balance model (Chalom & Prado, 2017). We performed 100,000 sampling iterations based on random uniform distributions of each parameter constrained to our observed trait ranges. We report the partial rank correlation coefficients showing the linear effect of each independent parameter on the modelled leaf temperatures.

To model T_{MIS} across species' distributions, we downloaded all available geo-referenced coordinates for each study species from the Global Biodiversity Information Facility (GBIF; http://www.gbif.org/, accessed 3/24/19). Records were filtered to exclude obvious geo-referencing errors (e.g. coordinates in oceans) and duplicate entries. At each of the remaining collection locations, we extracted estimates of the mean annual temperature (BIO1), maximum temperature of the warmest month (BIO5), minimum temperature of the coldest month (BIO6) and mean annual precipitation (BIO12) from the WorldClim database (2.5 arc-min resolution; Fick & Hijmans, 2017). To minimize the influence of outliers (caused, for example, by geo-referencing or taxonomic errors), we removed any records that fell outside the 95% quantile of a given climatic variable for each species. Only species with ≥15 records in the cleaned occurrence dataset were used in subsequent analyses.

We next used the energy balance model to estimate the maximum leaf temperatures for each species at all of the remaining occurrence locations. To make these estimates, we assumed a constant surface albedo of 0.15 for forest cover (Loescher, Gholz, Jacobs, & Oberbauer, 2005), a low wind speed of 0.1 ms^{-1}, and assumed that all leaves were oriented horizontally. We justify the assumption of

horizontal leaf orientations for modelling purposes in the Discussion section and in our Supplemental Information (Figure S2). The occurrence locations were passed to R's NICHMAPR package (v1.1.4; Kearney & Porter, 2017) to determine ambient air temperature, relative humidity and solar irradiance during the hottest time of the year. Each species' T_{MIS} was then calculated as the mean of the upper 97.5th quantile of the maximum leaf temperatures estimated at the occurrence locations using the leaf energy balance model parameterized with the thermoregulatory traits measured on plants at Fairchild Garden. Our modelling procedure assumes that only environmental variables, and not thermoregulatory traits or PHTs, change throughout the species' distributions. As a point of comparison with traditional methods, we calculated the average of the mean maximum monthly temperature (BIO5; 2.5 arc-min resolution) for each location occurrence in our cleaned dataset and tested for any relationship with T_{crit} and T_{50}.

We did not measure any aspect of plant fitness, but we explored the possibility that heat tolerances could be adapted to leaf temperatures by testing for correlations between measures of maximum leaf temperatures (T_{MO}, T_{MIS}), and metrics of PHT (T_{50} and T_{crit}) using Pearson's correlation coefficient. To better understand which species may be more vulnerable to heat damage, we calculated species' thermal safety margins as the difference between T_{50} and T_{MO}, then tested the a priori hypothesis that species with higher heat tolerances are more likely to be protected from thermal damage using

ordinary least squares regression. All analyses were conducted using R version 3.5.1 (R Core Team, 2018).

3 | RESULTS

3.1 | Heat tolerances

The mean T_{crit} for all 19 study species was 42.8°C and ranged from 37 to 48°C. The mean T_{50} for all species was 49.6°C and ranged from 47 to 52°C (Figure 1). Overall, T_{crit} was more variable across species ($SD = 2.6$) than T_{50} ($SD = 1.1$). T_{crit} and T_{50} were positively correlated but their relationship was not significant (Pearson's $r = 0.403$, $p = 0.087$, $df = 17$).

3.2 | T_{MO}, leaf energy balance model parameterization and T_{MIS}

Mean maximum observed leaf temperatures (T_{MO}) ranged from 33 to 45°C, and differences between species' mean maximum leaf temperatures and concurrent air temperatures ranged from 0.8 to 11.5°C. Leaf temperatures never exceeded T_{50}, but T_{MO} exceeded T_{crit} for three species. T_{MO} was not correlated with T_{crit} (Pearson's $r = 0.364$, $df = 17$, $p = 0.125$; Figure 2a), but was positively
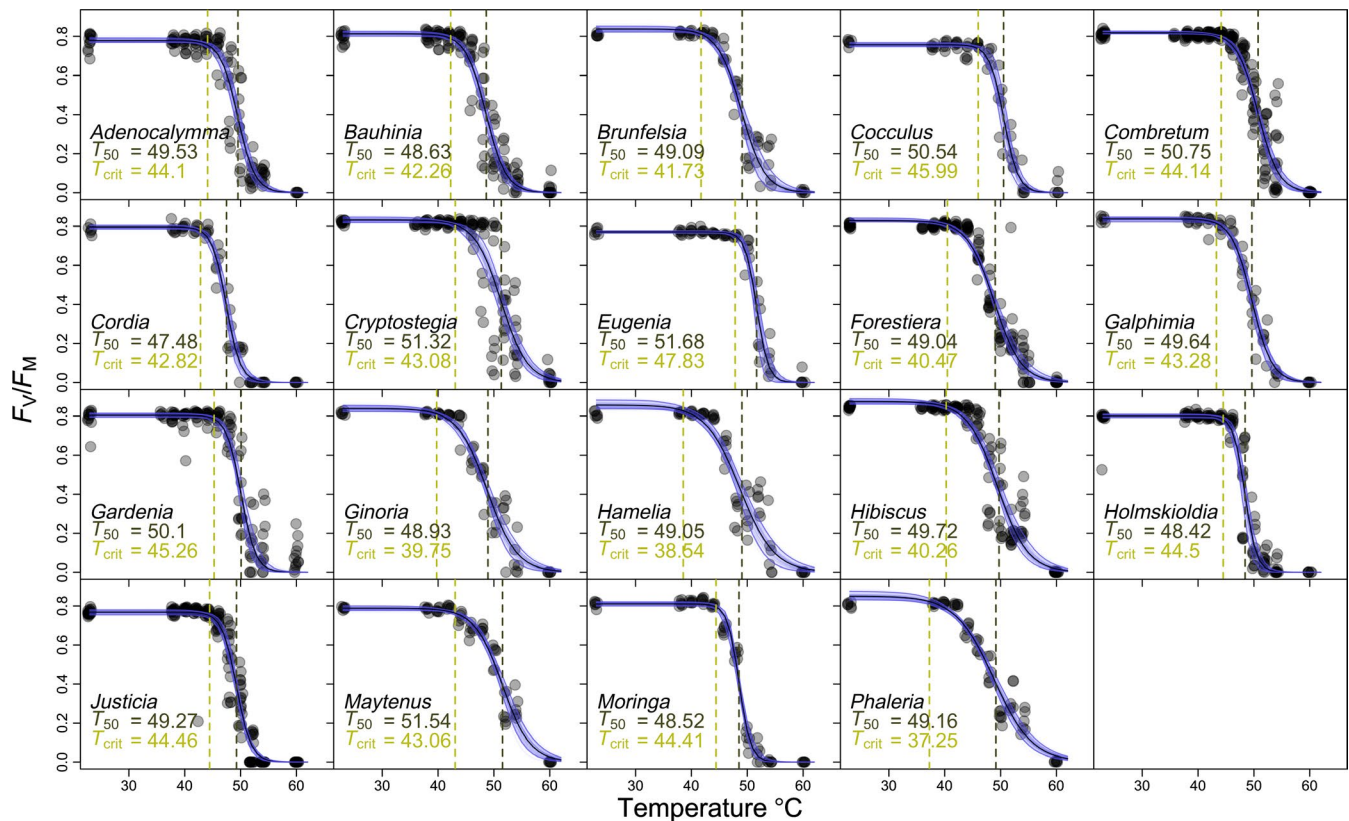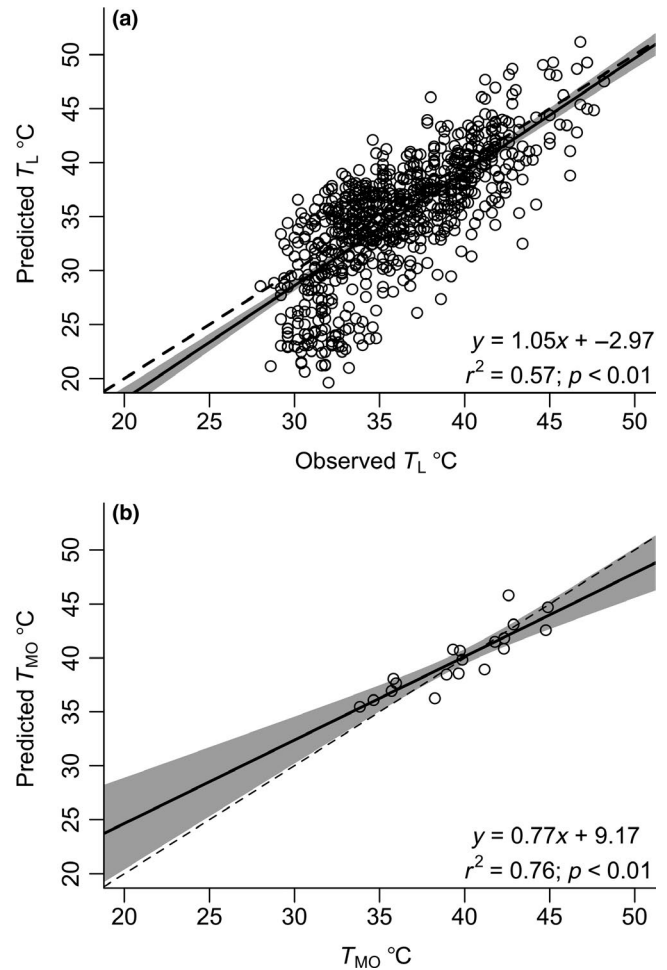

FIGURE 1 The temperature–F_V/F_M relationship used to determine photosynthetic heat tolerances for 19 study species. Grey dots represent individual leaf discs, blue lines indicate one of 100 bootstrapped temperature–F_V/F_M nls models. The vertical light and dark green lines show T_{crit} and T_{50}, respectively
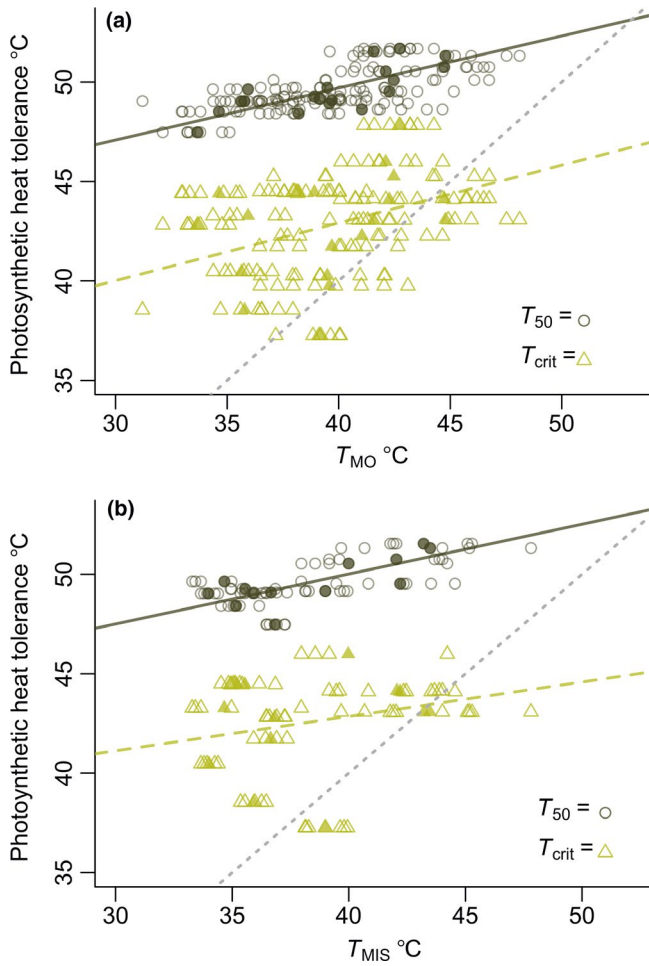
FIGURE 2 The relationship between T_{crit}, T_{50} and (a) maximum observed leaf temperature (T_{MO}), and (b) maximum in situ leaf temperature (T_{MIS}). Open symbols represent individual leaf T_{MO} or T_{MIS} and closed symbols correspond to species mean T_{MO} or T_{MIS}. Horizontally arranged points indicate there is one estimate for photosynthetic heat tolerance, but 5–8 different T_{MO} per monitored leaf per species. Solid lines indicate significant correlations; long-dashed lines indicate insignificant correlation; short-dashed grey lines indicate the 1:1 relationship between variables. Coefficients for all linear models are provided in the Table S1

FIGURE 3 (a) Model validation for the relationship between observed leaf temperature (T_L) and predicted leaf temperature; and (b) the relationship between maximum observed (T_{MO}) and predicted T_{MO}. Only point measurements where solar irradiance was >100 Wm^{-2} were used. The dashed line indicates a 1:1 relationship between variables, the solid line shows the modelled relationships, and shaded regions show the 95% confidence interval of the model. Note that this figure shows the model validation and *not* T_{MIS}

correlated with T_{50} (Pearson's $r = 0.763$, $df = 17$, $p < 0.001$; Figure 2a).

The leaf temperatures of Fairchild Garden plants that we predicted with the leaf energy balance model ranged from 19 to 51°C. The energy balance model predicted substantially more variation (Figure 3a; $p < 0.001$, $r^2 = 0.558$, $df = 742$) in leaf temperature than did ambient air temperature ($r^2 = 0.28$) or solar irradiance alone ($r^2 = 0.34$). Our leaf energy balance model tended to underestimate leaf temperatures (low leaf temperatures were particularly susceptible to underestimation). The leaf energy balance model was successful in predicting species' T_{MO} at Fairchild Garden (Figure 3b; $p = 0.003$, $r^2 = 0.763$, $df = 17$), but tended to underestimate high maximum leaf temperatures, overestimate low maximum leaf temperatures and resulted in up to a 3.33°C difference between in maximum predicted and maximum observed leaf temperatures.

Our sensitivity analysis indicated that solar irradiance, air temperature and relative humidity had the largest positive effects on estimated leaf temperatures (Figure 4). Wind speed had a positive negligible effect on leaf temperature, while leaf width had a negative (cooling) negligible effect on leaf temperature (Figure 4). Stomatal conductance was the leaf trait with the largest effect on leaf temperature, followed by leaf absorptivity; the former had a negative effect, while the latter had a positive effect (Figure 4).

Our final dataset included the estimates of T_{MIS} across the known geographic ranges of 13 species (Table 1). Estimates of T_{MIS} for these species ranged from 35 to 46°C. T_{MIS} was not significantly correlated with T_{crit} (Pearson's $r = 0.25$, $p = 0.42$, $df = 11$; Figure 2b) and T_{MIS} exceeded T_{crit} in four species. T_{MIS} was significantly positively correlated with T_{50} (Pearson's $r = 0.74$, $p < 0.01$; $df = 11$; Figure 2b) and T_{MIS} did not exceed T_{50} in any species. We found
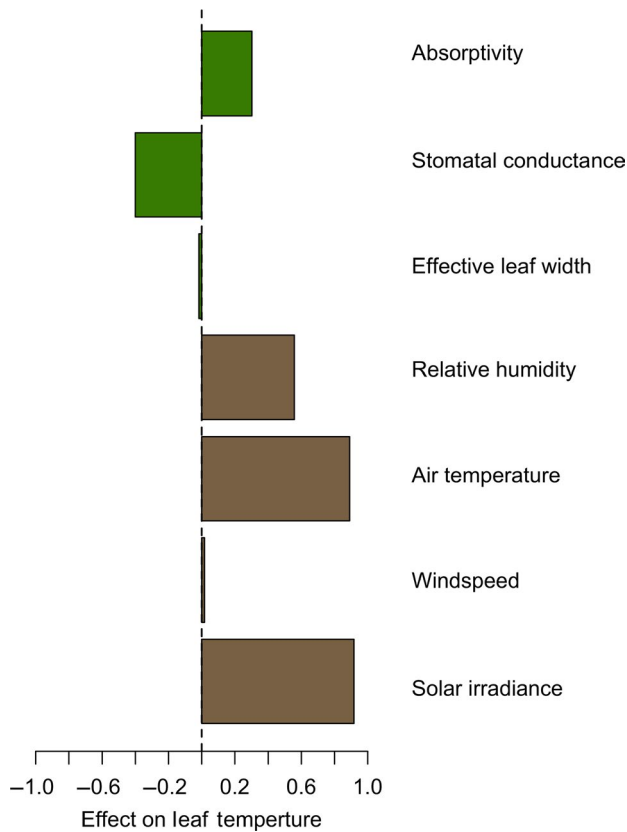
FIGURE 4 Results of the sensitivity analysis performed on the leaf energy balance model show the relative positive or negative effect that the corresponding variables have on predicting leaf temperature. Brown colours indicate environmental variables while green colours indicate leaf traits
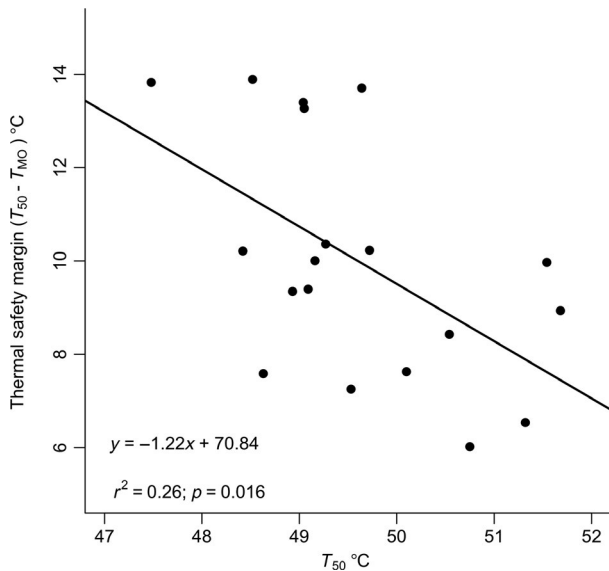
FIGURE 5 The relationship between thermal safety margin and T_{50} heat tolerance

no significant relationship between the average mean maximum monthly temperature (BIO5) where species occur and the species' heat tolerances (Figure S3).

Lastly, the thermal safety margins of our study species ranged from 6 to 14°C. The results of our linear model indicated a negative correlation between PHTs and thermal safety margins ($r^2 = 0.256$, $p = 0.0156$, $df = 17$; Figure 5).

4 | DISCUSSION

We found two separate lines of evidence to support our hypothesis that extreme leaf temperatures drive interspecific variation of photosynthetic heat tolerances (PHTs). First, we found a significant correlation of observed maximum leaf temperatures (T_{MO}) with T_{50}, but not with T_{crit}, in our common garden environment. Second, estimates of maximum in situ leaf temperatures (T_{MIS}) across species' geographic distributions were also correlated with T_{50}, but not with T_{crit}. We also tested the hypothesis that species with higher heat tolerances are less susceptible to thermal damage than are species with lower tolerances. Contrary to expectation, we found a significant negative relationship between T_{50} and thermal safety margins, which suggests that species with higher heat tolerances may actually be more susceptible to heat damage. T_{crit} was not significantly correlated with species' T_{MO}, T_{MIS} or thermal safety margins, and thus its ecophysiological importance and potential link to plant fitness remains unclear.

Many methods are used to determine photosynthetic heat tolerances, which make direct comparisons among studies difficult. Our estimates of T_{crit} and T_{50} were generally comparable to those reported in other studies using similar methods (Curtis, Knight, Petrou, & Leigh, 2014; Krause et al., 2010; Slot et al., 2018). Our estimates of T_{crit} tended to be lower and exhibit less variability than reported for other tropical species with T_{crit} estimated from changes initial fluorescence (F_0; O'Sullivan et al., 2017). However, the greater variation in our T_{crit} values relative to T_{50} may explain why T_{50}, but not T_{crit}, was correlated with extreme leaf temperature. For example, less variation in T_{50} is consistent with the hypothesis that larger reductions in the quantum yield (F_V/F_M) have larger negative effects plant carbon economics than small reductions represented by T_{crit}. This possibly reduces biophysical selection for T_{crit}, explains the greater variability in T_{crit} and explains why it is not coordinated with leaf temperature regardless of assessment method (e.g. F_0 in Knight & Ackerly, 2002).

We found that the leaf temperature estimates for plants at Fairchild Garden based on the energy balance model did a good job of predicting the observed leaf temperatures. However, our modelled leaf temperatures were not as precise or accurate as those in some previous studies (e.g. Fauset et al., 2018; Lee et al., 2015; Meinzer & Goldstein, 1985), possibly because we validated our model outdoors and for several species while other studies have validated their models for fewer species and under controlled laboratory settings. Additional model inaccuracy may be attributable to the low sensitivity of our anemometer and our assumption of a minimum wind speed of 0.1 ms⁻¹. At very low wind speeds (<0.1 ms⁻¹), free convection may dominate over forced convection, thus violating an assumption of our model and explaining our model's under-prediction of lower

leaf temperatures. Our model's accuracy was greatly improved when it was used to predict species' T_{MO}. That said, our predicted T_{MO} differed from observed T_{MO} by up to 3.33°C, which may have non-negligible consequences for some leaf physiological processes including when modelling T_{MIS}.

The positive correlation between T_{50} and the maximum leaf temperatures modelled across species' geographic ranges (T_{MIS}) provides additional support for the hypothesis that extreme leaf temperatures are an important driver of interspecific variation in PHTs. A comparison of our predicted and observed T_{MO} at Fairchild Garden suggests that high leaf temperatures are more likely to be underestimated and low leaf temperatures are more likely to be overestimated. However, given the observed deviations in predicted maximum leaf temperatures of T_{MO}, we are confident the observed relationship between T_{MIS} and T_{50} is robust and is not the consequence of Type I error.

Our results did not support the hypothesis that species with higher heat tolerances have larger thermal safety margins and thus are less susceptible to heat damage (O'Sullivan et al., 2017; Sastry & Barua, 2017). Conversely, our results suggest that species with higher T_{50} are actually more vulnerable to heat damage because they have hotter leaves and smaller corresponding thermal safety margins. We expect this pattern to hold across species' distributions since very large errors in our estimates of T_{MIS} would be needed reverse the slope of the relationship between T_{MIS} and T_{50}. Such large errors are extremely unlikely given the coordination between our predicted and observed T_{MO} values. These results highlight how the use of air temperatures versus leaf temperatures can lead to conflicting conclusions about which species are most vulnerable to thermal damage.

A potential source of error in our results is that we did not illuminate our leaf tissues during heat treatments. Leaf tissue exposed to light activates the production of violaxanthin and zeaxanthin photoprotective pigments, which have been shown to increase the estimates of T_{50} by up to 1°C (Krause, Winter, Krause, & Virgo, 2016). To test if potentially underestimated heat tolerance could have influenced our results, we randomly introduced error of up to +1°C in T_{50}; this did not have any significant effect on the observed relationships between T_{50} and T_{MO} or T_{MIS} or their significance.

The results of our garden and modelling experiments support our hypothesis that extreme leaf temperatures drive variation in T_{50}, and suggest that species with high heat tolerances are more vulnerable to rising temperatures due to climate change. However, we made three important assumptions in our procedure for calculating T_{MIS} that warrant discussion.

Our first assumption was that all leaves were horizontal. In reality, plant canopies exhibit a large distribution of leaf angles. While steeper angles will greatly reduce sun exposure and hence leaf temperatures, theoretical and observed probability distributions of leaf angles show that a non-negligible proportion of leaves are horizontal or have angles ≤15° (corresponding to <1°C of cooling compared to horizontal). Given that we were interested in maximum leaf temperatures, our results are unlikely to be invalidated by our assumption of horizontal leaves (Figure S2).

The second assumption we made was that leaf thermoregulatory traits did not vary across species' geographic distributions. Since we did not incorporate intraspecific trait variation in our models, variation in T_{MIS} was driven entirely by changes in environmental conditions, and there is no reason to expect that variation in environmental conditions would produce patterns in T_{MIS} that should exhibit any relationship with T_{50}. The effects of geographic variation in thermoregulatory leaf traits on leaf temperature should be negligible given their concomitant changes with environmental conditions or with other traits. For example, the leaf widths of *Dodonaea viscosa* were observed to decrease by 0.6 mm per degree latitude (Guerin, Wen, & Lowe, 2012), but decreases in leaf widths of this magnitude would cause minor decreases in leaf temperature and could be counteracted by increases in air temperatures associated with lower latitudes. Similarly, intraspecific changes leaf size that increased along precipitation gradients were accompanied by increases in stomatal conductance (McLean et al., 2014), which indicates that changes among thermoregulatory traits may be coordinated and have a limited effect on our observed relationship between T_{MIS} and T_{50}.

Our third assumption was that PHTs did not vary across species' distributions. Variation in PHTs across a species' distribution could be caused by acclimation, plasticity or local adaptation. Our justification for not incorporating variation in PHTs across the species' distributions is supported by the theory that tropical species generally have limited capacities to acclimate their photosynthetic traits (Cunningham & Read, 2003). Consistent with this hypothesis, previous studies found that experimental warming of tropical tree seedlings had no effect on their PHTs (Krause et al., 2010). Indeed, many tropical species are already operating above their temperature optima and maxima for carbon assimilation (Doughty & Goulden, 2009; Mau, Reed, Wood, & Cavaleri, 2018), which is partially influenced by PSII function and provides additional indirect evidence of their limited capacity to acclimate to higher temperatures. Nevertheless, we did measure PHTs during the hottest part of the year which should have minimized any potential effect of cool-to-warm season acclimation on our heat tolerances.

Although not supported by common garden data, it is possible that local adaptation could cause PHTs to vary independently of leaf temperatures, causing Type I error and leading us to incorrectly conclude there is a relationship between T_{MIS} and T_{50}. Even if PHTs vary across species' distributions, this variation should be relatively small as PHTs are known to increase by just 0.16–0.25°C per degree in air or leaf temperature (Drake et al., 2018; Feeley et al., 2020; Zhu et al., 2018). Despite its limitations, we contend that our method for estimating T_{MIS} is a promising approach for studying the thermal ecology and geographic distributions of plants.

Coarse climatic variables may explain some of the variation in heat tolerances among different communities, but our study implicates extreme leaf temperature as an important ecophysiological driver of interspecific variation in T_{50}. This hypothesis is supported by field observations as well as theoretical predictions from biophysical models. The techniques we employed here have potentially

widespread applications for making physiologically informed predictions of species' distributions and their responses to climate change. Notably, our findings indicate that species with higher heat photosynthetic tolerances may actually be at greater risk of thermal damage since they often have hotter leaf temperatures and narrower thermal safety margins. Our study also highlights the need for more information on intraspecific variation in leaf thermoregulatory traits and PHTs across species' distributions to help improve accuracy in future models. We did not test the adaptive significance of PHTs, but our results provide an ecophysiological mechanism for testing if heat tolerances are an adaptation to extreme leaf temperatures and influence fitness. Photosynthetic heat tolerances are presumed to be adaptations that contribute to plant productivity, but this has not been rigorously tested and should be a priority for future research (Hall, 1992; Porch & Hall, 2013).

ACKNOWLEDGEMENTS

AUTHORS' CONTRIBUTIONS

T.M.P. conceived and designed the experiments; T.M.P. performed the experiments; T.M.P. analysed the data; T.M.P. and K.J.F. wrote the manuscript.

DATA AVAILABILITY STATEMENT

All data needed to replicate this study can be located at https://doi/10.5061/dryad.1rn8pk0q3 (Perez & Feeley, 2020).

ORCID

Timothy M. Perez 🆔 https://orcid.org/0000-0002-3707-7285
Kenneth J. Feeley 🆔 https://orcid.org/0000-0002-3618-1144

REFERENCES

Araújo, M. B., Ferri-Yáñez, F., Bozinovic, F., Marquet, P. A., Valladares, F., & Chown, S. L. (2013). Heat freezes niche evolution. *Ecology Letters*, *16*(9), 1206–1219. https://doi.org/10.1111/ele.12155

Baker, N. R. (2008). Chlorophyll fluorescence: A probe of photosynthesis in vivo. *Annual Review of Plant Biology*, *59*(1), 89–113. https://doi.org/10.1146/annurev.arplant.59.032607.092759

Beerling, D. J., Osborne, C. P., & Chaloner, W. G. (2001). Evolution of leaf-form in land plants linked to atmospheric CO_2 decline in the Late Palaeozoic era. *Nature*, *410*(6826), 352–354. https://doi.org/10.1038/35066546

Campbell, C. S., & Norman, J. M. (1998). *Introduction to environmental biophysics* (2nd ed.). https://doi.org/10.1007/978-1-4612-1626-1

Chalom, A., & Prado, P. I. K. L. D. (2017). pse: Parameter space exploration with Latin Hypercubes. Retrieved from https://arxiv.org/pdf/1210.6278v2.pdf?

Cunningham, S. C., & Read, J. (2003). Do temperate rainforest trees have a greater ability to acclimate to changing temperatures than tropical rainforest trees? *New Phytologist*, *157*(1), 55–64. https://doi.org/10.1046/j.1469-8137.2003.00652.x

Curtis, E. M., Gollan, J., Murray, B. R., & Leigh, A. (2016). Native microhabitats better predict tolerance to warming than latitudinal macroclimatic variables in arid-zone plants. *Journal of Biogeography*, *43*(6), 1156–1165. https://doi.org/10.1111/jbi.12713

Curtis, E. M., Knight, C. A., & Leigh, A. (2019). Intracanopy adjustment of leaf-level thermal tolerance is associated with microclimatic variation across the canopy of a desert tree (*Acacia papyrocarpa*). *Oecologia*, *189*(1), 37–46. https://doi.org/10.1007/s00442-018-4289-x

Curtis, E. M., Knight, C. A., Petrou, K., & Leigh, A. (2014). A comparative analysis of photosynthetic recovery from thermal stress: A desert plant case study. *Oecologia*, *175*(4), 1051–1061. https://doi.org/10.1007/s00442-014-2988-5

Doughty, C. E., & Goulden, M. L. (2009). Are tropical forests near a high temperature threshold? *Journal of Geophysical Research: Biogeosciences*, *114*(1), 1–12. https://doi.org/10.1029/2007JG000632

Drake, J. E., Tjoelker, M. G., Vårhammar, A., Medlyn, B. E., Reich, P. B., Leigh, A., … Barton, C. V. M. (2018). Trees tolerate an extreme heatwave via sustained transpirational cooling and increased leaf thermal tolerance. *Global Change Biology*, *24*(6), 2390–2402. https://doi.org/10.1111/gcb.14037

Fauset, S., Freitas, H. C., Galbraith, D. R., Sullivan, M. J. P., Aidar, M. P. M., Joly, C. A., … Gloor, M. U. (2018). Differences in leaf thermoregulation and water use strategies between three co-occurring Atlantic forest tree species. *Plant, Cell & Environment*, *41*(7), 1618–1631. https://doi.org/10.1111/pce.13208

Feeley, K., Martinez-Villa, J., Perez, T., Silva Duque, A., Triviño Gonzalez, D., & Duque, A. (2020). The thermal tolerances, distributions, and performances of tropical montane tree species. *Frontiers in Forests and Global Change*, *3*(March), 1–11. https://doi.org/10.3389/ffgc.2020.00025

Fick, S. E., & Hijmans, R. J. (2017). WorldClim 2: New 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*, *37*(12), 4302–4315. https://doi.org/10.1002/joc.5086

Guerin, G. R., Wen, H., & Lowe, A. J. (2012). Leaf morphology shift linked to climate change. *Biology Letters*, *8*(5), 882–886. https://doi.org/10.1098/rsbl.2012.0458

Hall, A. E. (1992). Breeding for heat tolerance. In J. Janick (Ed.), *Plant breeding reviews* (Vol. 10, pp. 129–168). New York, NY: John Wiley & Sonc Inc.

Jones, H. G. (2014). *Plants and microclimate: A quantitative approach to environmental plant physiology* (3rd ed.). New York, NY: Cambridge University Press.

Kearney, M. R., & Porter, W. P. (2017). NicheMapR – An R package for biophysical modelling: The microclimate model. *Ecography*, *40*(5), 664–674. https://doi.org/10.1111/ecog.02360

Knight, C. A., & Ackerly, D. D. (2002). An ecological and evolutionary analysis of photosynthetic thermotolerance using the temperature-dependent increase in fluorescence. *Oecologia*, *130*(4), 505–514. https://doi.org/10.1007/s00442-001-0841-0

Krause, G. H., Winter, K., Krause, B., Jahns, P., García, M., Aranda, J., & Virgo, A. (2010). High-temperature tolerance of a tropical tree, *Ficus insipida*: Methodological reassessment and climate change considerations. *Functional Plant Biology*, *37*(9), 890. https://doi.org/10.1071/FP10034

Krause, G. H., Winter, K., Krause, B., & Virgo, A. (2015). Light-stimulated heat tolerance in leaves of two neotropical tree species, *Ficus insipida* and *Calophyllum longifolium*. *Functional Plant Biology*, *42*, 42–51. https://doi.org/10.1071/FP14095

Krause, G. H., Winter, K., Krause, B., & Virgo, A. (2016). Protection by light against heat stress in leaves of tropical crassulacean acid metabolism plants containing high acid levels. *Functional Plant Biology*, *43*(11), 1061. https://doi.org/10.1071/fp16093

Lambers, H., Chapin, F. S., & Pons, T. L. (2008). *Plant physiology* (2nd ed.). https://doi.org/10.1007/978-0-387-78341-3

Lee, A. P., Upchurch, G., Murchie, E. H., & Lomax, B. H. (2015). Leaf energy balance modelling as a tool to infer habitat preference in the early angiosperms. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1803), 20143052. https://doi.org/10.1098/rspb.2014.3052

Leigh, A., Sevanto, S., Close, J. D., & Nicotra, A. B. (2017). The influence of leaf size and shape on leaf thermal dynamics: Does theory hold up under natural conditions? *Plant, Cell & Environment*, *40*, 237–248. https://doi.org/10.1111/pce.12857

Loescher, H. W., Gholz, H. L., Jacobs, J. M., & Oberbauer, S. F. (2005). Energy dynamics and modeled evapotranspiration from a wet tropical forest in Costa Rica. *Journal of Hydrology*, *315*(2005), 274–294. https://doi.org/10.1016/j.jhydrol.2005.03.040

Mau, A., Reed, S., Wood, T., & Cavaleri, M. (2018). Temperate and tropical forest canopies are already functioning beyond their thermal thresholds for photosynthesis. *Forests*, *9*(2), 47. https://doi.org/10.3390/f9010047

Mclean, E. H., Prober, S. M., Stock, W. D., Steane, D. A., Potts, B. M., Vaillancourt, R. E., & Byrne, M. (2014). Plasticity of functional traits varies clinally along a rainfall gradient in *Eucalyptus tricarpa*. *Plant, Cell & Environment*, *37*, 1440–1451. https://doi.org/10.1111/pce.12251

Meinzer, F., & Goldstein, G. (1985). Some consequences of leaf pubescence in the andean giant rosette plant espeletia timotensis. *Ecology*, *66*(2), 512–520. https://doi.org/10.2307/1940399

Michaletz, S. T., Weiser, M. D., Mcdowell, N. G., Zhou, J., Kaspari, M., Helliker, B. R., & Enquist, B. J. (2016). The energetic and carbon economic origins of leaf thermoregulation. *Nature Plants*, *2*(9), 1–8. https://doi.org/10.1038/nplants.2016.129

Nobel, P. S. (1999). *Physicochemical and environmental plant physiology* (2nd ed.). San Deigo, CA: Academic Press.

Offord, C. A. (2011). Pushed to the limit: Consequences of climate change for the Araucariaceae: A relictual rain forest family. *Annals of Botany*, *108*(2), 347–357. https://doi.org/10.1093/aob/mcr135

Osmond, C. B., Austin, M. P., Berry, J. A., Billings, W. D., Boyer, J. S., Dacey, J. W. H., … Winner, W. E. (1987). Stress physiology and the distribution of plants. *BioScience*, *37*(1), 38–48. https://doi.org/10.2307/1310176

O'Sullivan, O. S., Heskel, M. A., Reich, P. B., Tjoelker, M. G., Weerasinghe, L. K., Penillard, A., … Atkin, O. K. (2017). Thermal limits of leaf metabolism across biomes. *Global Change Biology*, *23*(1), 209–223. https://doi.org/10.1111/gcb.13477

Perez, T., & Feeley, K. (2020). Data from: Photosynthetic heat tolerances and extreme leaf temperatures. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.1rn8pk0q3

Perez, T. M., Valverde-Barrantes, O., Bravo, C., Taylor, T. C., Fadrique, B., Hogan, J. A., … Feeley, K. J. (2019). Botanic gardens are an untapped resource for studying the functional ecology of tropical plants. *Philosophical Transactions of the Royal Society of London Series B: Biological Sciences*, *374*(1763), 20170390. https://doi.org/10.1098/rstb.2017.0390

Porch, T. G., & Hall, A. E. (2013). Heat tolerance. In C. Kole & B. C. K. Viswavidyalaya (Eds.), *Genomics and breeding for climate resilient crops: Vol. 2 target traits* (pp. 167–202). https://doi.org/10.1007/978-3-642-37048-9

R Core Team. (2018). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.r-project.org/

Rueden, C. T., Schindelin, J., & Hiner, M. C. (2017). ImageJ2: ImageJ for the next generation of scientific image data. *BMC Bioinformatics*, *18*(1). https://doi.org/10.1186/s12859-017-1934-z

Sastry, A., & Barua, D. (2017). Leaf thermotolerance in tropical trees from a seasonally dry climate varies along the slow-fast resource acquisition spectrum. *Scientific Reports*, *7*(1), 1–11. https://doi.org/10.1038/s41598-017-11343-5

Schreiber, U., & Berry, J. A. (1977). Heat-induced changes of chlorophyll fluorescence in intact leaves correlated with damage of the photosynthetic apparatus. *Planta*, *136*(3), 233–238. https://doi.org/10.1007/BF00385990

Slot, M., Krause, G. H., Krause, B., Hernández, G. G., & Winter, K. (2018). Photosynthetic heat tolerance of shade and sun leaves of three tropical tree species. *Photosynthesis Research*, *141*(1), 119–130. https://doi.org/10.1007/s11120-018-0563-3

Smillie, R. M., & Nott, R. (1979). Heat Injury in leaves of alpine, temperate and tropical plants. *Australian Journal of Plant Physiology*, *6*(1), 135–141. https://doi.org/10.1071/PP9790135

Smith, W. K. (1978). Temperatures and desert plants: Another perspective on adaptability of leaf size. *Science*, *201*, 614–616.

Smith, W. K., & Nobel, P. S. (1977). Temperature and water relations for sun and shade leaves of a desert broadleaf, *Hyptis emoryi*. *Journal of Experimental Botany*, *28*(102), 169–183.

Weng, J., & Lai, M. (2005). Estimating heat tolerance among plant species by two chlorophyll fluorescence parameters. *Photosynthetica*, *43*(3), 439–444. https://doi.org/10.1007/s11099-005-0070-6

Wright, I. J., Dong, N., Maire, V., Prentice, I. C., Westoby, M., Díaz, S., … Wilf, P. (2017). Global climatic drivers of leaf size. *Science*, *12*(September), 917–921. https://doi.org/10.1126/science.aal4760

Yamada, M., Hidaka, T., & Fukamachi, H. (1996). Heat tolerance in leaves of tropical fruit crops as measured by chlorophyll fluorescence. *Scientia Horticulturae*, *67*(1–2), 39–48. https://doi.org/10.1016/S0304-4238(96)00931-4

Zhu, L., Bloomfield, K. J., Hocart, C. H., Egerton, J. J. G., O'Sullivan, O. S., Penillard, A., … Atkin, O. K. (2018). Plasticity of photosynthetic heat tolerance in plants adapted to thermally contrasting biomes. *Plant, Cell & Environment*, *41*(6), 1251–1262. https://doi.org/10.1111/pce.13133

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Perez TM, Feeley KJ. Photosynthetic heat tolerances and extreme leaf temperatures. *Funct Ecol*. 2020;00:1–10. https://doi.org/10.1111/1365-2435.13658