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Studies

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Leaf temperatures exceed thermal heat tolerances for a community of eastern North America hardwood trees

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Abstract. Changing climates are creating more intense and frequent high-temperature events that could disrupt forest communities. In temperate forests, we have a relatively limited understanding of how trees are impacted by heat events, hindering our ability to predict the impacts of future heat waves. We conducted a community-level assessment of thermal safety margins in 11 hardwood tree species native to eastern North America. We used chlorophyll fluorescence to determine the critical heat tolerance of photosystem II (PSII) across 2 years in central Tennessee, USA. We focus on the temperature at which PSII first starts to decline (T_{crit}) as this is the temperature where membranes become unstable, resulting in permanent damage to these tissues. T_{crit} varied within the season and between years, being higher in July than June and in 2022 than 2023. T_{crit} also varied among species with species like *Ulmus rubra* and *Ostrya virginiana* showing consistently lower heat tolerances. When compared to the record high temperature for our study site, 10 of 11 species would have experienced heat stress during at least one sample period. When compared to current year high temperatures, the risk was variable and lower across all species and sample periods. However, we found that leaf temperatures often exceeded air temperatures many species were likely heat stressed as heat tolerances were often below species-specific leaf temperatures. Indeed, four species were potentially heat stressed during every sample period. Our data highlights the importance of using leaf temperature, not air temperature to assess thermal safety margins and that community-wide stress may already occur under extreme heat conditions. As climate change intensifies, leaf temperatures will likely approach critical thresholds that lead to damage across the tree community. Understanding species-specific responses to heat stress is essential to predicting future forest dynamics and ecosystem functioning.

Keywords: Chlorophyll fluorescence; critical temperature; heat stress; thermal maxima; thermal safety margin.

Introduction

Record high temperatures are forecasted to increase in frequency, duration and severity in the coming decades (Schoof *et al.* 2019). Indeed, 2023 was the warmest year ever recorded with mean and extreme temperatures breaking records globally (Copernicus 2024; NOAA 2024). Current and future extreme heat events will push some plant communities outside of their optimal temperature ranges and potentially into negative carbon balance (Settele *et al.* 2014). Extreme high temperatures have the potential to irreversibly damage the photosynthetic apparatuses (Berry and Bjorkman 1980), leaving a long-lasting impact on carbon capture that can scale from individuals up to entire ecosystems.

Despite their importance for global biodiversity and ecosystem functioning, our understanding of thermotolerances has largely focussed on commercially important species (Geange *et al.* 2021). Recent technological advances (Geange *et al.* 2021) and a renewed interest in understanding upper thermal tolerances of plants under climate change have revealed some broad patterns of heat tolerances. In one of the most comprehensive global analyses to date, O'sullivan *et al.* (2017) showed an 8 °C decrease in the temperature at which photosystem II (PSII) is disrupted (often referred to as T_{crit}) with latitude, despite a 20 °C shift in high temperatures across the same gradient. Mid-latitude species, such as those found in temperate forest, may be at most risk to rising temperatures since they have moderate heat tolerances but experience high temperatures. While recent studies reveal some broad-scale patterns in heat tolerance, additional work is needed to understand overheating risk across latitude, bioclimatic regions and ecosystem types. Such understanding is critical in trees as they form the backbone of forests and are important for global carbon cycling.

To respond to elevated temperatures, plants have mechanisms to keep their photosynthetic tissues from being damaged. Cooling by evapotranspiration is one of the most widely cited tools foundational plants like trees use to combat high temperatures (Wahid *et al.* 2007). However, extreme heat events are often in concert with dry periods, and future extreme heat events are predicted to regularly occur during periods of drought (Ashfaq *et al.* 2016), limiting the capacity of trees to cool via transpiration. In addition, leaf temperatures frequently become decoupled from air temperatures. In the Atlantic Forest of Brazil, leaf temperatures were observed to

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be up to 18 °C above ambient air temperature (Fauset *et al.* 2018). In a broader analysis of plots distributed across latitude, Still *et al* (2022) found that midday canopy temperatures were often several degrees warmer than the air temperature, even in moist tropical forest where water availability likely does not limit the transpirational cooling of leaves. These studies highlight that trees are often unable to cool leaves below air temperature during the warmest periods of the day, resulting in dangerously high temperatures in sun-exposed leaves. Therefore, both leaf temperature and species-specific heat tolerance data are needed to accurately predict current and future thermal safety margins, the difference between leaf temperature and critical temperature and overheating risk (Perez and Feeley 2020; Kitudom *et al.* 2022; Marchin *et al.* 2023).

Previous work on high temperature thermal safety margins in trees has a large geographic bias towards tropical forests due to the *a priori* assumption that these species already exist at or near their thermal maxima (Perez and Feeley 2020; Perez *et al.* 2021; Slot *et al.* 2021; Doughty *et al.* 2023). Investigations into thermal safety margins of temperate trees are rare, with most conducted in restricted geographic regions (e.g. Australia; O'sullivan *et al.* 2017; Drake *et al.* 2018), investigating only heat tolerance and not thermal safety margins (Münchinger *et al.* 2023), or have focussed on cultivated species (Wahid *et al.* 2007). This previous work leaves wild temperate species chronically understudied (Geange *et al.* 2021). Moreover, studies that pair heat tolerance and leaf temperature through time are limited, further limiting our understanding of how heat tolerances may change throughout a single or successive growing season. Given temperate forests have been identified as high risk of heat damage due to their potentially small thermal safety margins (O'sullivan *et al.* 2017) and the lack of heat tolerance studies on wild trees in temperate systems, there remains a critical gap in our understanding how these systems will respond to current and future heat waves.

The potential alterations to community composition by heat exposure (Meehl *et al.* 2016; Schoof *et al.* 2019) may alter community composition due to differing levels of tolerance in forest species, with some species better able to cope with extreme heat events (Knight and Ackerly 2002). As such, our ability to understand future forest communities is limited by our lack of understanding of variable heat tolerances and thermal safety margins. Here, we address limitations to our understanding of heat tolerance in a North American temperate forest community. For this study, our goals were to (i) determine heat tolerances for 11 commonly occurring eastern United States hardwood tree species, (ii) test for temporal shifts in heat tolerance within and across study years and (iii) determine thermal safety margins of leaves by comparing heat tolerance to air and leaf temperatures.

Methods

Study site

We conducted this study in Clarksville, TN, USA (36.5701, –87.3385°) at an 80 ha second-growth forest dominated by *Fagus grandifolia*, *Acer spp* and *Quercus spp*. Climate for the site is typical of the mid-south United States with summer temperatures highest in July and August, summer air temperatures often exceeding 30 °C, and a record high of 44.4 °C. Precipitation falls throughout the year with slightly increased rates during winter compared to summer.

Leaf heat tolerance and temperature

We selected 11 species of broadleaf, deciduous trees [see Supporting Information—Table S1] that are commonly occurring in secondary forests in the eastern United States. Species spanned across nine families and functional traits like successional status (early to late) and leaf type (simple or compound). Individuals were located along a forest-open field edge that ensured sample leaves were receiving the maximum amount of solar radiation.

From each of the 11 species, we sampled 6 individuals during June and July in 2022 and 2023. During all four sampling events, we collected samples between 8:00 and 10:00 am by clipping a roughly 50 cm branch tip with at least five fully developed leaves from branches that were fully sun exposed and from 2 to 5 m off the ground. After collection, we made 11, 2.5 cm diameter leaf disks from multiple leaves from each individual that were then assigned to 1 of 11 temperature treatments: 23 °C, 38 °C, 40 °C, 42 °C, 44 °C, 46 °C, 48 °C, 50 °C, 52 °C, 54 °C and 60 °C. The six-leaf disks per species assigned to the same temperature were placed into a packet constructed of two layers of Mira-cloth to prevent desiccation. The packet was then placed into a polyethylene bag and submerged in hot water baths controlled by sous vide cookers (Anova model AN400-10). The baths were preheated to the target temperatures, after which the samples were submerged for 15 min. After removal from the water baths, leaf discs were placed into petri dishes lined with wet paper towels to avoid any desiccation. The leaf discs were kept at room temperature (~23 °C) in a dark cabinet. After 24 hr, we dark adapted each disk for 20 min and measured fluorescence using an Opti-Sciences OS30p + (Hudson, New Hampshire) fluorometer (following Maxwell and Johnson 2000). Measurements are made 24 hr after heating to assess intermediate to long-term damage that occurs in the leaf tissue and therefore has a greater impact on whole leaf and plant carbon dynamics.

Following the approach of Perez and Feeley (2020), we modelled the relationship of F_V/F_M for each of the species and temperature treatments using an nls function in R 4.3.0 (The R Project for Statistical Computing 2023). F_V/F_M is the maximum quantum yield of PSII and is a relative index of PSII function where F_V is calculated as $F_V = F_M – F_0$, where F_M is the number of closed reaction centres and F_0 is initial fluorescence. F_V/F_M in a healthy leaf is generally between 0.75 and 0.85 with lower values representing a decline in PSII quantum yield resulting from heat stress. This approach provided us with three thresholds of heat stress, such as T_{crit}, T_{50} and T_{95}. T_{crit} is the temperature threshold where the initial decline in F_V/F_M occurs relative to controls and the slope of the F_V/F_M – temperature curve is 15 % of the maximum. T_{50} and T_{95} are the temperatures where there is a 50 % and 95 % reduction in F_V/F_M relative to controls (Curtis *et al.* 2014). We then used a bootstrapping approach to generate means and 95 % confidence intervals for each of the three thresholds by reapplying the nls model 1000 times while randomly resampling with replacement.

We measured leaf surface temperatures in July 2022 and August 2023 during high-temperature events with a handheld infrared thermometer. We measured the same individuals of

Table 1. High leaf temperatures and *P* values comparing T_{crit} from individual sample periods to leaf temperatures for 11 species of hardwood eastern North American trees. *P* values were determined using a bootstrap analysis and bold values represent sample periods where species heat tolerance was lower than observed leaf temperatures ($P > 0.05$).

	Leaf temperatures		Sample period			
Species	2022	2023	June 2022	July 2022	June 2023	July 2023
Acer saccharum	38.8	43.5	<0.001	<0.001	**0.507**	**0.505**
Celtis laevigata	38.9	38.7	0.003	<0.001	0.004	<0.001
Fagus grandifolia	45.3	41.0	**0.792**	0.026	**0.480**	**0.084**
Juglans nigra	37.6	40.8	<0.001	<0.001	<0.001	**0.137**
Liquidambar styraciflua	36.5	38.7	0.001	<0.001	0.001	0.014
Liriodendron tulipifera	44.5	44.8	**0.739**	**0.620**	**0.996**	**0.987**
Ostrya virginiana	40.6	40.7	<0.001	<0.001	**0.558**	**0.608**
Prunus serotina	40.2	39.5	0.021	0.007	**0.321**	<0.001
Quercus falcata	45.7	43.5	**0.126**	**0.660**	**>0.999**	**0.225**
Quercus montana	41.9	45.3	**0.162**	**0.242**	**>0.999**	**0.996**
Ulmus rubra	45.9	42.1	**0.967**	**0.228**	**0.545**	**0.252**

each species that were used for heat tolerance. To obtain leaf temperatures during maximum solar exposure, we measured leaves between 11:00 am and 3:00 pm. Temperatures were measured on leaves that were roughly perpendicular to solar maximum and unobstructed from any branches occurring higher on the tree. We compared these leaf surface temperatures to air temperature data from NOAA Climate Data Online (National Oceanic and Atmospheric Administration 2023).

Data analysis

We conducted analyses in R 4.3.0 (The R Project for Statistical Computing 2023). Using daily temperature and precipitation data from NOAA Climate Data Online (National Oceanic and Atmospheric Administration 2023; www.ncei.noaa.gov/cdo-web), we investigated changes to high-temperature extremes and means between 1980 and 2023. We used a linear model to examine if daily high temperatures during the hottest season (defined as 1 June to 30 September) were dependent on Julian's date and year. To evaluate temperature patterns at a finer scale, we conducted the same analysis but looked at temperature within the months of June, July, August and September separately. The definition of extreme heat varies by region and agency, but for this study, we used the threshold set by many US government agencies of 32.2 °C (i.e. Ready.gov 2024). We also used a linear model to determine if the number of days above this 32.2 °C threshold has increased since 1980.

We focus all analyses on T_{crit}, as this is the threshold temperature closest to historic and contemporary air temperatures and is a sensitive measure that documents the beginning of PSII membrane damage and carbon capture disruption (Zhang *et al.* 2024). We present T_{50} and T_{95} analyses and results when noted and provide outputs in the Supplementary Material. We used a mixed effects model to determine any community-level temporal variation in estimated mean T_{crit}. The model included fixed effects of month, year, their interaction and species as a random effect. We then performed model selection by comparing all subsets of the global model using Akaike Information Criterion (AIC) values (Burnham and Anderson 2002). Models were considered competing if they were within two AIC units of the lowest-scoring model and when multiple models were competing, we chose the simplest model.

To evaluate overheating risk, we compared T_{crit} to both air and leaf temperatures. Air temperature is often used to estimate heat damage as these data are widely available at fine spatial and temporal resolutions. However, leaf temperature provides a more accurate measure of tree tissue temperature (Marchin *et al.* 2023). For each species and each sample period, we compared the 1000 bootstrap estimates of T_{crit} to a given temperature threshold. We determined significance by dividing the total number of bootstrap estimates that were greater than a given threshold temperature by 1000. To provide a coarse understanding of heat tolerances to historic temperatures we first compared all T_{crit} estimates to the highest temperature recorded in Clarksville, TN, USA since 1980 (44.4 °C). To determine overheating risk for species in their current year, we do the same analysis comparing T_{crit} to the high temperature for that month and year (38.3 °C for June 2022, 38.9 °C for July 2022, 38.3 °C for June 2023, 37.2 °C for July 2023). Finally, we compared each species to the species-specific high leaf temperature recorded during the same year of sampling (Table 1). We repeated this analysis for T_{50} and T_{95} but limited our discussion to T_{crit}, as T_{50} and T_{95} values were above all threshold temperatures for all comparisons [see Supporting Information—Tables S2–S4, Figs S1–S4).

To provide a more explicit comparison of thermal safety margins calculated using air and leaf temperatures, we subtracted the 1000 bootstrap estimates of T_{crit} for each species during each sample period from leaf temperature and sample period high air temperature. We refer to these two measures as leaf thermal safety margin and air thermal safety margin. Negative thermal safety margins represent estimates where T_{crit} is below the critical temperature (leaf or air), whereas positive thermal safety margins represent a T_{crit} that is above the critical temperature. To determine if calculated safety margins differ, we compared leaf and air thermal safety margins across species, months and years using a paired *t* test. We then investigated temporal variation in thermal safety margins similar to our approach above with T_{crit}. We first build a global model of the mean thermal safety margin per species predicted by fixed effects of month, year, their interaction and a random effect of species. We then performed model selection (Table S6) as outlined above.

Results

NOAA data show that between 1980 and 2023 at our study site, the mean daily high temperature from 1 June to 30 September was 37.5 °C and the record high temperature was 44.4 °C (National Oceanic and Atmospheric Administration 2023; www.ncei.noaa.gov/cdo-web). For the same period, the mean low temperature was 18.5 °C. During sample years, temperatures were above average, similar between the 2 years, and did not approach all-time records since 1980. The mean daily high temperature for June 2022 was 32.5 °C and July 2022 was 34.5 °C. The mean daily high temperatures for June and July 2023 were 31.5 °C and 32.9 °C, respectively. Mean annual precipitation for 1980–2023 was 1301 mm the driest months spanning from July to September, coinciding with extreme high temperatures. The mean annual precipitation for study years was slightly below average; 2022 was 1250.8 mm and 2023 was 1147.0 mm.

At our study site, both absolute and mean temperatures since 1980 have warmed but with significant interannual variability (Fig. 1). We found that during the heat season (between 1 June and 30 September) mean high temperature has increased by 0.012 °C per year ($P < 0.003$) with high temperatures increasing in June (0.03 per year ($P < 0.001$) and September (0.03 per year ($P < 0.001$)), but not July ($P = 0.867$) and August ($P = 0.686$). The number of days above 32.2 °C showed a high degree of interannual variability and no significant increase in the number of days above this threshold since 1980 (Fig. 1C, $P = 0.54$).

Measuring heat tolerance multiple times throughout the study reveals patterns in how heat tolerance shifts with time. Month had a positive effect on heat tolerance, as measured by T_{crit}, with July heat tolerances being higher than June for most species [Table 2, Supporting Information—Table S2]. We also found differences in T_{crit} across years with values being higher in 2022 than 2023 for most species (Fig. 2).

We found that the record high temperature for the study site since 1980 (44.4 °C) was consistently higher than leaf heat tolerance values for all sample periods excluding July 2022 [see Supporting Information—Table S5, Fig. 2]. The estimated T_{crit} for 10 of 11 sampled species were below 44.4 °C for June 2022 and both June and July in 2023. Conversely, in July 2022, only 6 of the 11 species maintained heat tolerances below the record high temperature. When compared to temperatures experienced during the month and year of sampling, most species had heat tolerances that would be sufficient to withstand ambient air temperatures with the exception of June 2023 (see Supporting Information—Table S5, Fig. 2). During this month 5 of 11 species had heat tolerances lower than the high air temperature. Conversely, in July 2022, no species had heat tolerances lower than the highest temperature, and in both June 2022 and July 2023 only one species during each sample period had a heat tolerance lower than the highest temperatures (*U. rubra* in June 2022 and *O. virginiana* in July 2023; Fig. 2).

Leaf surface temperature readings show that leaf temperatures reach high-temperature extremes that are much higher than ambient air temperature extremes. Consequently, we found that leaf heat tolerance is often lower than leaf surface temperatures during all sample periods (Table 1, Fig. 2). When compared to leaf surface temperature measurements in 2022, five species in June and four in July had leaf surface temperatures exceeding their respective heat tolerances. For 2023, eight species in June and nine in July had leaf temperatures exceeding heat tolerances. Four species had heat tolerances lower than leaf temperatures for all sampling periods: *L. tulipifera*, *Q. falcata*, *Q. montana* and *U. rubra*.

This important difference in leaf and air temperature resulted in air thermal safety margins being significantly higher than leaf thermal safety margins across all months and year ($t = -6.89$, $P < 0.001$). Meaning that air temperature overestimates the thermal safety margin and underpredicts the risk that leaves face when exposed to extreme heat. Like T_{crit}, leaf and air thermal safety margins varied with month and year, with higher thermal safety margins in July than June, and in 2022 than in 2023 (Table 2, Supporting Information—Table S5, Fig. 3). For example, *U. rubra* and *L. tulipifera* had consistently low T_{crit} and both species had thermal safety

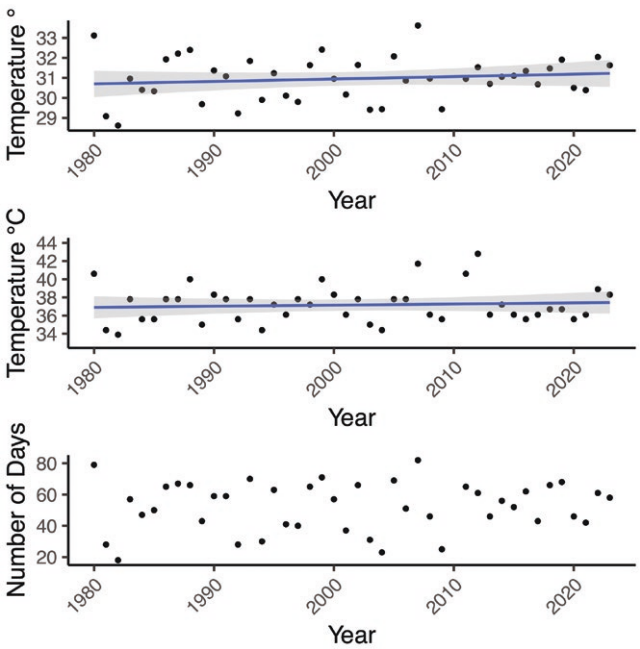

Figure 1. Change in heat season (between 1 June and 30 September) mean annual high air temperatures (A), highest recorded temperature (B) and number of days above 32.2 °C from 1980 to 2023 for Clarksville, TN, USA. Regression lines and confidence intervals represent significant relationships.

Table 2. Model outputs from the best selected model of thermal safety margins predicted by month, sample year and species as a random effect. Thermal safety margins were calculated by subtracting T_{crit} to air and leaf temperature.

Model	Estimate	Std error	DF	*P* value
Thermal safety air				
Intercept	2.7	1.15	31	0.026
Month	2.07	0.74	31	0.009
Year	–2.48	0.74	31	0.002
Thermal safety leaf				
Intercept	5.24	0.54	31	<0.001
Month	2.07	0.61	31	0.002
Year	–1.64	0.61	31	0.012

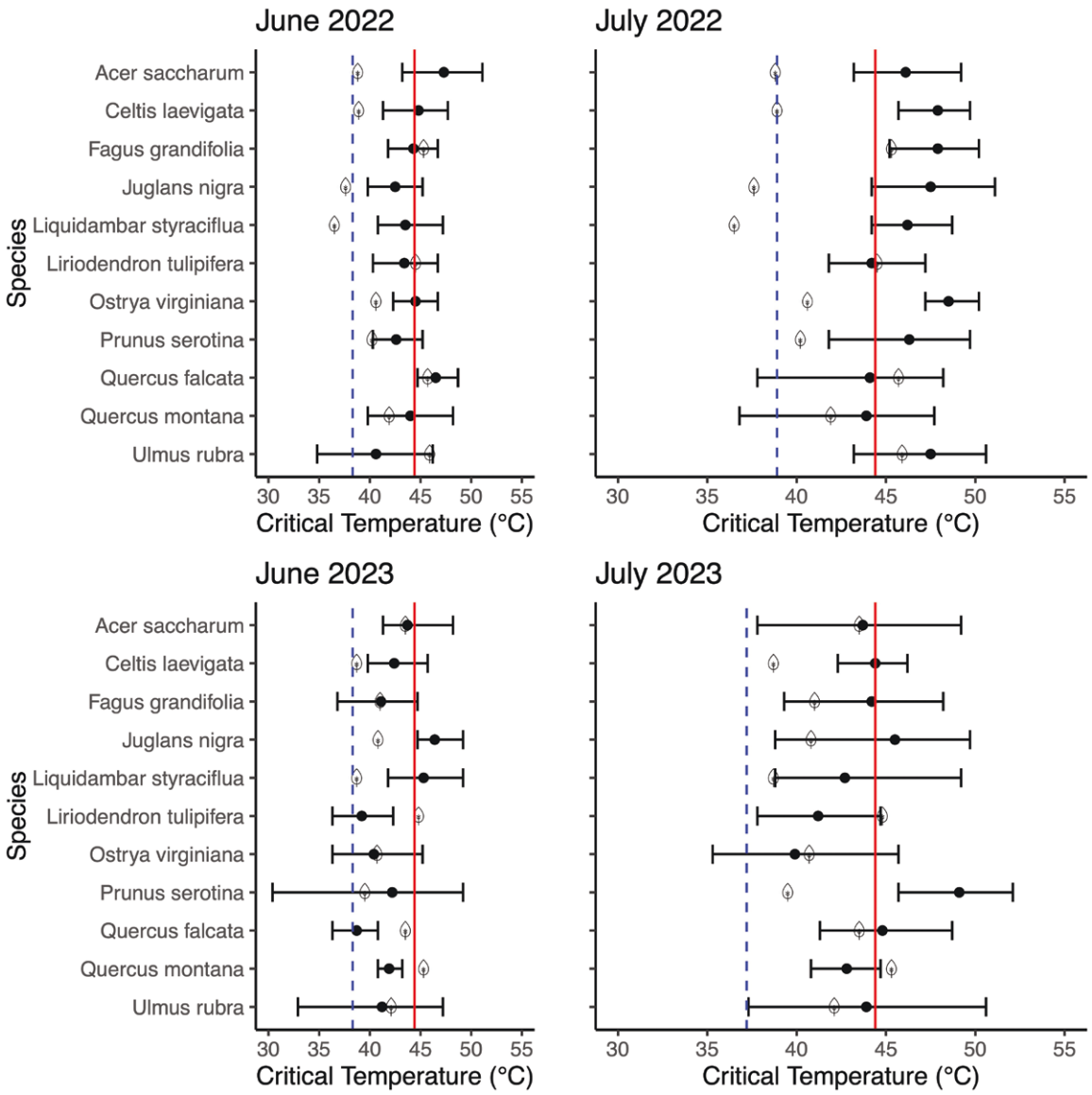

Figure 2. T_{crit} values for 11 species of hardwood eastern North American trees from Clarksville, TN, USA in June/July 2022 and June/July 2023. The dashed line depicts the highest temperature during the respective study month; the solid red line depicts the record high temperature for the location since 1980 and the leaf icon represents the highest leaf temperature recorded for that species in the respective study year.

margin distributions that were largely negative in all sampling periods. Similarly, June 2023 had some of the lowest T_{crit} values across the entire community and likewise thermal safety margins for a majority of species were negative or encompassed zero during this month.

Discussion

Increases in the frequency, intensity and duration of extreme heat events over the last several decades are likely increasing heat-related stress for temperate forests. As climate change intensifies, the importance of heat stress will continue to increase with potential deleterious effects on entire forest tree communities (Wahid *et al.* 2007). In this study, we established the heat tolerances and thermal safety margins for 11 species of hardwood trees common to eastern North America. We show that current heat tolerances vary across season and sample years and would lead to heat stress for most species during historic high-temperature events. When calculating thermal safety margins, we find that using air temperature as a proxy for potential heat stress overestimates leaf thermal safety margins. When using actual leaf temperature, we show that between four and nine species were likely stressed during sample years, with four species having lower heat tolerances than leaf temperature for all sampling periods. Collectively, our results indicate that stress from high temperatures is common across the forest community and that increasing temperatures from climate change will likely increase the number of species that are heat stressed.

Spatially and temporally high-resolution air temperature records are available in many geographic regions, and as such, are commonly used to evaluate plant heat stress (e.g. Zhu *et al* 2018; Perez and Feeley 2020). We limit our historic air temperature data to those after 1980 as the bulk of our sampled trees are likely 20-60 years old and would have established

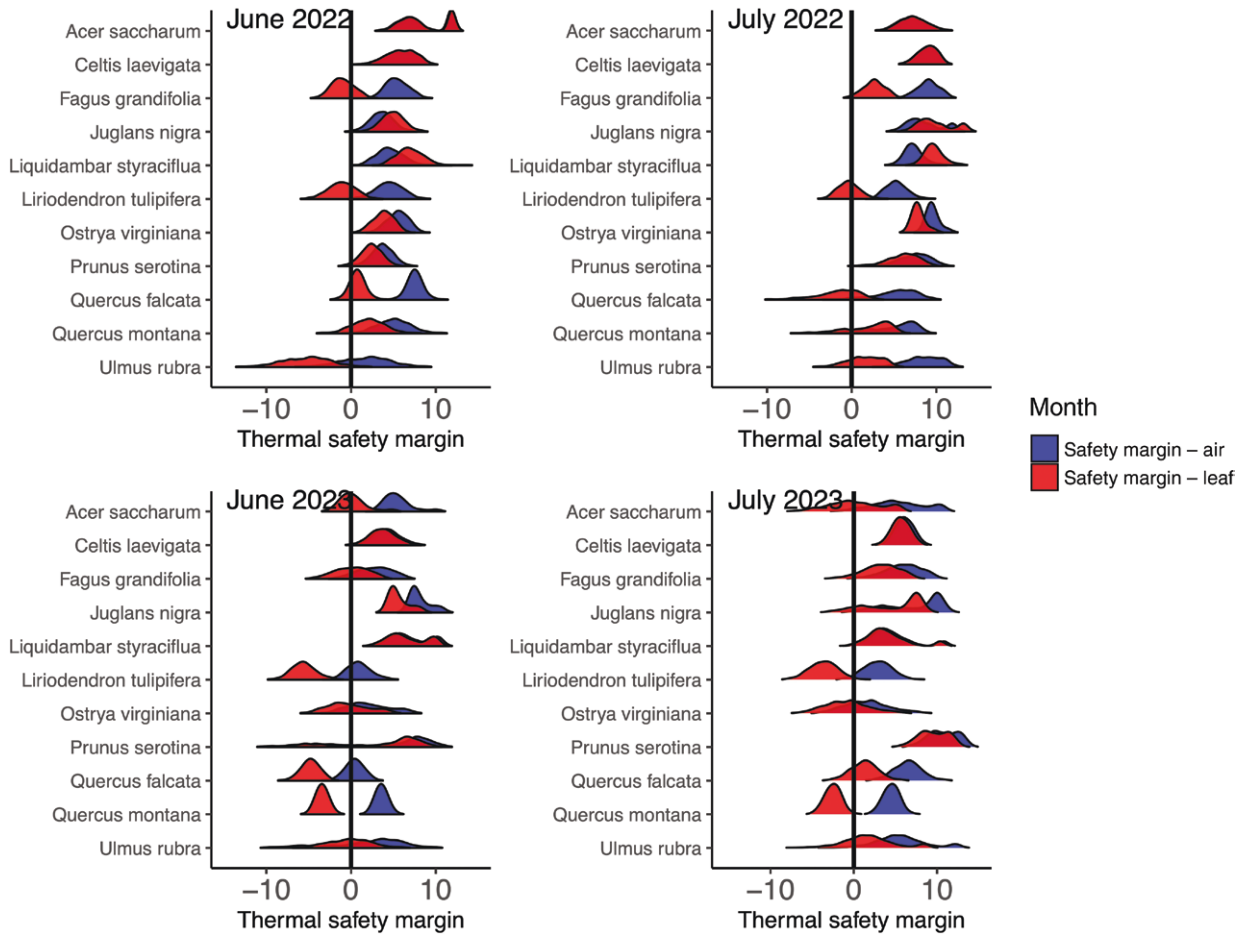

Figure 3. Thermal safety margins of T_{crit} compared to sample month high air temperature (dark grey) and leaf temperature (light grey). Density distributions were calculated as the 1000 bootstrap estimates of T_{crit} minus air/leaf temperature. Negative values represent measurements when T_{crit} was below the air/leaf temperature.

or reached maturity during the last few decades. When comparing heat tolerance (as measured by T_{crit}) to these historic high temperatures, all species would be potentially stressed by the record high temperature during at least one of the four sample periods. These findings match those of other studies that show the variability in heat tolerance across species can lead to large thermal safety margins for some while others are likely experiencing heat stress due to lower thermal tolerances (Perez and Feeley 2020). We do caution on comparing current heat tolerances with historic temperature data as additional research is needed to understand how plant heat tolerances change with life stage, within a season and across years to truly understand a plant's ability to withstand extreme high temperatures over longer time periods.

Comparing heat tolerance to current-year temperatures may provide a better prediction of contemporary overheating risk. In 2022, only one species (*U. rubra*) did not have sufficient heat tolerance to protect against the highest air temperature during the two sampling periods (Fig. 2). However, June 2023 shows an increase of species at risk with 6 of 11 species having heat tolerances below air temperatures. Yet in July 2023, only one species (*O. virginiana*) had heat tolerances below extreme high temperatures. The increase in heat stress in 2023 is due to reduced heat tolerances across all species in 2023 as high temperatures were within 1.7 °C of each other across all sample periods. Collectively, these data show variation in thermal safety margins is strongly based on what air temperature threshold is used (i.e. historic or current temperatures) as well as fluctuations in heat tolerance.

While air temperature is widely available and often used in determining potential overheating dangers, leaf temperature is a better measure of thermal heat stress for individual trees (Kitudom *et al.* 2022; Marchin *et al.* 2023). Indeed, canopy temperatures often exceed ambient air temperature (Rey-Sánchez *et al.* 2016; Fauset *et al.* 2018; Miller *et al.* 2021; Still *et al.* 2022) showing that comparisons of heat tolerance to air temperature do not reflect true thermal safety margins experienced by plants. Our findings highlight that leaf temperatures are often higher than air temperatures and that leaf temperatures are above heat tolerances for more species compared to air temperature. For example, 4 of our 11 species had heat tolerances below leaf temperatures during all sampling periods. Of these four species, only one (*U. rubra*) had heat tolerances consistently below historic and current air temperatures. Furthermore, we found leaf temperature was at least 7 °C higher than species heat tolerances in 2022 for 4 species (*A. saccharum*, *C. laevigata*, *J. nigra* and *L. styraciflua*). Therefore, if we evaluated potential heat stress based on air temperature alone, we would have underestimated true heat stress for the bulk of our species.

Overheating risk may vary through time as previous studies have shown that plant heat tolerance can vary seasonally

and with other conditions such as drought or previous heat exposure (Teskey *et al.* 2015; Sastry *et al.* 2018; Zhu *et al.* 2018; Marchin *et al.* 2022; Docherty *et al.* 2023). We find a similar pattern of seasonally varying heat tolerance as T_{crit} increased through both summers, possibly in response to increasing temperatures during the growing season. We also found that heat tolerances vary across seasons, with higher T_{crit} in 2022 than in 2023. This suggests there are additional factors beyond just high air temperatures that control heat tolerance. Drought and water stress have been linked to plant thermotolerances but the evidence is mixed (Teskey *et al.* 2015) with some studies reporting that water stress results in higher (Cook *et al.* 2021; Sumner *et al.* 2022) or lower (Marchin *et al.* 2022; Docherty *et al.* 2023) heat tolerance.

According to the Standardised Precipitation-Evapotranspiration Index (SPEI) (2024), which is a useful measure of plant water demands as it accounts for both precipitation and evapotranspiration, April–June of both years showed water limitations, with 2022 being drier than 2023. However, by July in both years, water shortages appear to dissipate. Yet our data show contrasting and opposing trends in heat tolerance with water status across season and year. For example, heat tolerances were higher in 2022 than in 2023 when water stress was more severe, suggesting a positive correlation between heat and drought stress. However, heat tolerance increased from June to July in both years while water stress decreased, suggesting a negative relationship within a given year. Additional work on how heat tolerance and thermal safety margins are linked to water stress is certainly warranted and relevant under future climate change scenarios as high temperature events often coincide with drought.

Using T_{crit} as a measure of heat tolerance provides a good understanding of when heat stress impacts are first expressed in a leaf, but T_{50} and T_{95} may be stronger indicators of irreversible damage and loss of tissues. Both T_{50} and T_{95} thresholds for all studied species are beyond current air and leaf temperature extremes for all sample periods showing that high temperatures are likely not reaching critical thresholds to cause permanent damage in trees [see Supporting Information—Figs S1 and S2]. Our findings align with other community studies that show temperatures do not approach T_{50} or T_{95} (Perez and Feeley 2020; Slot *et al.* 2021) and which report leaf temperature-T_{50} thermal safety margins between 6 °C and 14 °C. We find a very similar range of thermal safety margins as T_{50} was 3–10 °C greater than the measured leaf temperature for all species across all survey periods. This shows that most species have a significant buffer to avoid permanent and significant damage to leaf tissues, but climate extremes may possibly reach critical thresholds in the near future. In addition, our leaf temperature measurements are likely conservative and underestimate high-temperature extremes as we sampled only once per year and did not sample upper canopy leaves that may get hotter due to more solar irradiance exposure. With additional temporal data on leaf level temperatures, it is likely we would find temperatures higher than we recorded and which may approach T_{50} values for some species. For example, certain leaf functional traits like size and leaf thickness correlate with heat dissipation (Leigh *et al.* 2017), so large leaves like *L. tulipifera* may have more difficulty dissipating heat than compound or small leaves. As climate change continues to increase the intensity of extreme temperature events, it is likely that more species will be exposed to temperatures approaching T_{50} thresholds.

Exposure to extreme heat can result in species distributional shifts and changes to forest community composition (Gazol and Camarero 2022). While most eastern US tree species are likely to be negatively impacted from extreme heat, some species will be affected more than others. Our data show that *O. virginiana* and *U. rubra* are routinely heat stressed in current conditions (i.e. largely negative thermal safety margins) that could lead to higher stress under future climate models relative to other species. Of note, in 2023 *O. virginiana* showed drying and discolouration of leaves consistent with heat stress signals, but it is unclear if it was solely due to heat stress. In comparison, other species such as *J. nigra* and *C. laevigata* maintain higher heat tolerances and may not face the same levels of stress as other species. However, additional work is needed to understand how additional factors like water stress, leaf traits, and acclimation to elevated temperatures lead to differential responses to heat tolerance and adjustments to leaf temperature in temperate trees. Such species-specific information would likely reveal highly variable strategies to maintain thermal safety margins across diverse forest communities. Without such detailed information, making predictions on how increasingly intense heat events impact community compositional change will remain difficult.

Supporting Information

The following additional information is available in the online version of this article –

Table S1. Scientific names, common names and authority for the 11 species included in the study.

Table S2. Mean T_{crit} values for 11 species of eastern North American hardwood tree species during 2022 and 2023.

Table S3. Mean T_{50} values for 11 species of eastern North American hardwood tree species during 2022 and 2023.

Table S4. Mean T_{95} values for 11 species of eastern North American hardwood tree species during 2022 and 2023.

Table S5. *P* values for comparing T_{crit} to various temperature thresholds for 11 species of hardwood eastern North American trees. Temperature thresholds correspond to record high temperatures since 1980 and the high temperature of the respective study month. *P* values were determined by bootstrap analysis and a *P* value <0.05 represents a sample where heat tolerances for that species were deemed to be significantly greater than the temperature threshold it was compared against. Bold *P* values indicate either the record high temperature or the high temperature for the study month exceeded heat tolerances.

Table S6. Model selection of thermal safety margin with fixed effects of sample month, sample year, their interaction and including species as a random effect. The best-chosen model is highlighted in bold.

Figure S1. T_{50} values for 11 species of hardwood eastern North American trees from Clarksville, TN, USA in June/July 2022 and June/July 2023. The dashed blue line depicts the highest temperature during the respective study month, the solid red line depicts the record high temperature for the location since 1980 and the leaf icon represents the highest leaf temperature recorded for that species in the respective study year.

Figure S2. T_{95} values for 11 species of hardwood eastern North American trees from Clarksville, TN, USA in June/July 2022 and June/July 2023. The dashed blue line depicts the highest temperature during the respective study month, the

solid red line depicts the record high temperature for the location since 1980 and the leaf icon represents the highest leaf temperature recorded for that species in the respective study year.

Figure S3. T_{50} thermal safety margin for 11 species of hardwood eastern North American trees from Clarksville, TN, USA in June/July 2022 and June/July 2023.

Figure S4. T_{95} thermal safety margin for 11 species of hardwood eastern North American trees from Clarksville, TN, USA in June/July 2022 and June/July 2023.

Contributions by the Authors

E.R. designed the experiment. J.E. conducted the experiment and led the writing of the manuscript. J.E. and E.R. analysed the data. Both authors contributed critically to the drafts and gave final approval for publication.

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Conflict of Interest Statement

None declared.

Data Availability

The data for this study are available on Github at https://github.com/jendris573/Endris_Rehm_2024.

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