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Kitudom, N

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Thermal safety margins of plant leaves across biomes

Kitudom Nawatbhrist[1,5], Fauset Sophie[1, 4], Zhou Yingying[1,5], Fan Zexin[1,2,3], Li Murong[1, 6], He Mingjian[1, 6], Zhang Shubin[1], Xu kun[7], Lin Hua[1,2 *]

[1]Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Mengla, Yunnan 666303, China.

[2]Center of Plant Ecology, Core Botanical Gardens, Chinese Academy of Sciences, Xishuangbanna 666303, China.

[3]Ailaoshan Station of Subtropical Forest Ecosystem Studies, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Jingdong, Yunnan 676209, China.

[4]School of Geography, Earth and Environmental Sciences, University of Plymouth, Plymouth, UK.

[5]University of Chinese Academy of Sciences, Beijing 100049, China

[6]College of Biology and Chemistry, Puer University, Puer, Yunnan 665000, China.

[7]Yunnan Lijiang Forest Ecosystem National Observation and Research Station, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan 650201, China

*Corresponding author, E-mail: lh@xtbg.ac.cn

Running title: Thermal safety margins of plants across biomes

Abstract

Climate change has great impacts on forest ecosystems, especially with the increasing frequency of heatwaves. Thermal safety margin (TSM) calculated by the difference between body temperature and thermotolerance threshold is useful to predict thermal safety of organisms. It has been widely used for animals, whereas has rarely been reported for plants. Besides, most of the previous studies used only thermotolerance to estimate thermal safety; or used thermotolerance and air temperature (Ta) to calculate TSM. However, leaf temperature (Tl) is the real body temperature of plant leaves. The departure of Tl from Ta might induce large error in TSM. Here, we investigated TSM of photosystem II (thermotolerance of PSII – the maximum Tl) of dominant canopy plants in four forests from tropical to temperate biomes during a heatwave, and compared the TSMs calculated by Tl (TSM.Tl) and Ta (TSM.Ta) respectively. Also, thermal related leaf traits were investigated. The results showed that both TSM.Tl and TSM.Ta decreased from the cool forests to the hot forests. TSM.Tl was highly correlated with the maximum leaf temperature (Tlmax), while had an opposite trend with thermotolerance across biomes. Thus, Tlmax instead of thermotolerance can be used to evaluate TSM. The maximum Ta (Tamax) and Tlmax explained 68% of the variance of thermotolerance in a random forest model, while other leaf traits including morphological, optical, material properties, anatomic and physiological traits only explained 6%. TSM.Ta cannot distinguish thermal safety differences between co-occurring species. The overestimation of TSM by TSM.Ta increased from the tropical to the temperate forest, and increased with Tl within biome. The present study

enriches the dataset of photosynthetic TSMs across biomes, proposes using Tlmax to estimate TSMs of leaves, and highlights the risk of hot dry forest during heatwaves. The results also stress that it is not proper to use TSM.Ta in cold forests.

Keywords： photosynthetic heat tolerance, thermal environment, heatwave, heat stress, thermal stability

1. Introduction

Global warming has caused an increase in the frequency and intensity of extreme climate events, especially droughts and heatwaves (Alexander et al., 2006; Hansen et al., 2012). It is projected that global heatwaves will be quadruple by 2040 (Coumou and Robinson, 2013). Heatwave events have caused severe reduction in forest and agriculture productivity (Ciais et al., 2005; Tatarinov et al., 2016), and large scale tree mortality (Allen et al., 2015; Allen et al., 2010; Chaste et al., 2019). High temperature associated with drought will reduce net photosynthesis by suppressing assimilation rates and promoting respiration (Teskey et al., 2015), and even damage photosynthetic components (Havaux, 1993). Measurements in a Brazil tropical forest showed a rapid decrease in leaf photosynthesis above 37.5 °C (Doughty and Goulden, 2008). Accurately evaluating how plants will be affected by climate change is important to predict species change in plant community and protect natural resources.

Although thermotolerance is an ability of plants to survive under high temperature, it is not enough to assess thermal safety of plants. A leaf with low thermotolerance might control leaf temperature well below its thermal limit by physical and physiological cooling (Lin et al., 2017), while a highly thermotolerant species might experience high leaf temperature. With regard to this, thermal safety margin (TSM) which is defined as the difference between body temperature (leaf temperature for plants) and critical temperatures that represent threshold for function or lethality was proposed to assess thermal safety (Gunderson and Stillman, 2015). TSM has been widely applied in animal studies (Denny and Dowd, 2012; McArley et al., 2017; Pincebourde and Casas, 2019; Sunday et al., 2014; Vinagre et al., 2019), but its application in plants is lacking. Recently, it has begun to catch attention of researchers. There are reports of TSM of plants at particular sites (Araújo et al., 2021; Leon-Garcia and Lasso, 2019), across latitude gradients (O'Sullivan et al., 2017), and in common gardens (Curtis et al., 2016; Perez and Feeley, 2020; Sastry and Barua, 2017). However, TSM at one site cannot reveal TSM patterns across biomes. Plants growing in common gardens might have acclimated to local environment, thus had different TSMs from those in natural environments. In addition, most of the studies used air temperature instead of leaf temperature when calculating TSM (Curtis et al., 2016; O'Sullivan et al., 2017; Sastry and Barua, 2017).

Leaf temperature is the real “body temperature” for leaf metabolic processes, influencing leaf carbon economics (Michaletz et al., 2016; Michaletz et al., 2015). It can departure from air temperature up to 15 °C (Ackerly and Stuart, 2009), and varies across species and environment (Leuzinger and Körner, 2007). Even under the same environment, leaf temperature can be very different, depending on leaf physical and physiological traits (Fauset et al., 2018; Lin et al., 2017). Michaletz et al. (2015) have demonstrated limited homeothermy of plants based on energy budget. Both site measurements and isotope analysis showed that leaf temperatures were more stable than air temperatures across biomes (Dong et al., 2017; Song et al., 2011). However, we still have no information of how the decoupling of leaf temperature and air temperature influences the difference between TSM calculated by leaf temperature (TSM.Tl) and air temperature (TSM.Ta) across biomes. Leaf traits including morphological traits, optical traits, physiological traits all have great impacts on leaf temperature (Gates, 2003). Convective cooling can be enhanced by reducing leaf size (Okajima et al., 2012; Smith, 1978); compound or dissected leaves have advantage of heat exchange by increasing the contact edge with air (Stokes et al., 2006); high water content or leaf mass area (LMA) can prolong leaf thermal time constant and delay leaf warming (Leigh et al., 2012; Smith, 1978); high reflectivity reduces radiation loads on leaves; and transpiration is efficient to cool leaves (Crawford et al., 2012; Lin et al., 2017). Leaf temperature is the direct micro-environment for leaf function, thus thermotolerance should acclimate to leaf temperature. Accordingly, leaf traits might affect thermotolerance through the impact on leaf temperature.

Except for leaf temperature, thermotolerance is another important parameter in TSM calculation. Photosystem II is sensitive to temperature, and its thermotolerance can be quantified by heat induced change of chlorophyll fluorescence parameters: the initial fluorescence (F_o) or the ratio of variable to maximum fluorescence (F_v/F_m) (Baker, 2008). The critical temperature of the intersection of lines extrapolated from the slow and fast rise portion of the temperature-dependent fluorescence response (F_o-T curve) indicates the start point of the collapse of Photosystem II (Knight and Ackerly, 2002). The temperatures leading to 50% reduction in F_v/F_m ratios was defined as T_{50} (Knight and Ackerly, 2003; Krause et al., 2010). The two fluorescence parameters are positively correlated (Krause et al., 2010; Lancastera and Humphreys, 2020). Most studies of thermotolerance across biomes used T_{crit}, because F_o can be continuously monitored in a heating bath with the same samples (Dahl et al., 2019; Knight and Ackerly, 2003; Lancastera and Humphreys, 2020; O'Sullivan et al., 2017; Song et al., 2011; Zhu et al., 2018). However, the measurement of F_v/F_m needs new samples at each temperature gradient, and the results vary with the exposure time at the target temperature. Therefore, we used T_{crit} in the present research. Some unified trends have been found for thermotolerances of plants: e.g. species from warmer habitats are inherently higher in thermotolerance; thermotolerance acclimates to growth temperature (Zhu et al., 2018); and plants from dry habitat are more thermotolerant than plants from wet habitat (Curtis et al., 2016; Knight and Ackerly, 2003). However, we don't know whether the maximum leaf temperatures vary proportionately to thermotolerance across biomes, thus how global thermotolerance patterns corresponding to TSM are still unclear. In the present study, we investigated TSM and leaf traits of plants in four forests across biomes (savanna, tropical rain forest, subtropical broad-leaved forest, temperate mixed forest) along precipitation and temperature gradients. Aim to (1) compare the patterns of thermotolerance and TSM across biomes, (2) evaluate thermal risk of plants across biomes, and (3) assess the difference between TSM.Tl and TSM.Ta.

2. Materials and Methods

2.1 Study sites and species selection. Four forests with contrasting temperature and precipitation gradients across biomes were selected in Yunnan province, south western China: savanna (SAV), tropical rain forest (TRF), subtropical broad-leaved forest (STF), temperate mixed forest (TEF). Four dominant upper canopy species and three individuals for each species were chosen in each forest, considering both richness in the upper canopy and reachability. They covered all the species of emergent trees in TRF, all the canopy species in TEF, and the most important canopy species in STF and SAV. Healthy, sun-exposed, and fully mature leaves in the upper canopy were sampled for temperature and leaf traits measurement. We accessed to the tall canopy using canopy cranes at TRF and STF, and using ladders in SAV and TEF. Detailed information of the sites and species were given in Table 1 and 2. All field measurements were conducted at the end of dry season in 2019 from May 13 ~ May 16 at TRF, May 19 ~ May 23 at STF, May 25~ May 28 at SAV, June 4 ~ June 7 at TEF. This period was the most severe heatwave in recent 10 years, which was widely spread in Yunnan province (Fig S1).

2.2 Temperature measurement. Temperatures were measured by Type-T thermocouples (TT-T-30-SLE-1000, OMEGA, USA; diameter = 0.25 mm). To avoid thermocouples falling from leaves, we hung them on the adaxial surfaces of leaves and fixed the tips with heat-conducting glue (Fig S2). The glue can strengthen the attachment and block direct irradiation on the sensor head. We compared our method with the traditional method (using tape to attach thermocouples on the abaxial sides of leaves) for four species. Two of the species had slightly higher leaf temperatures on abaxial sides than on adaxial sides at noon, while no significant differences were found for the other two species (Fig S3). It demonstrated that the impact of direct sunshine on leaf temperature was not significant. To simulate the extreme drought situation, we selected 2 leaves with similar size, age and orientation beside the leaves with temperature measurements, put Vaseline on the abaxial side of the leaves to stop transpiration (all the leaves are hypostomatous), and recorded their temperatures (Tn) with the same type of thermocouples. Air temperatures were simultaneously measured by the same type of thermocouples near the leaves with temperature measurements, avoiding direct solar radiation. For each individual, we measured one air temperature (Ta), temperatures of 4 sun leaves (Tl) and 2 Vaseline leaves (Tn). All the temperatures were continuously recorded by data logger (UX120-04, HOBO, USA) at one-minute interval from May 13 ~ May 16 at TRF, May 19 ~ May 23 at STF, May 25~ May 28 at SAV, June 4 ~ June 7 at TEF in 2019. On each day, we extracted the intervals that describe the hottest leaf temperatures for at least 10 minutes; these could be several intervals that sum to 10 minutes or a single interval that spans 10 minutes. We took the minimum temperature recorded in these intervals as Tlmax for that day. The maximum Tlmax during these days was Tlmax for each individual. With this method, we confirmed the temperature which was equal or higher than Tlmax lasted for at least 10 minutes.

2.3 Thermotolerance measurement. Thermotolerance was measured with

PlanTherm PT100 (PT100, Czekh). The measurement of thermotolerance was based on the response of initial chlorophyll fluorescence to temperature (F_o-T curve) (Schreiber and Berry, 1977). Three sun leaves similar with the leaves with temperature measurements were sampled from each individual in the morning, dark adapted for at least half an hour in plastic bags with wet tissue paper inside to prevent water loss. Leaves were rinsed with deionized water, cut rectangular segments (2 cm long) from the middle of the leaf avoiding main veins. Leaf segments were immersed into 5 ml deionized water in a cuvette, then set the temperature increasing rate at 2 °C /min from 25 °C - 70 °C. A magnetic stirrer bar was put in the water bath to achieve uniform heating. T_{crit} is calculated by the intersection of lines extrapolated from the slow and fast rise portion of the temperature-dependent fluorescence response (Knight and Ackerly, 2002)

2.4 Leaf traits measurement. We selected leaf traits which might have impact on leaf temperature, including morphological traits, optical traits, material properties, anatomical traits, and physiological traits (Table 3).

The leaves of similar size, age and orientation to the leaves with temperature measurement were collected. Eight to ten leaves for each individual were scanned using a flatbed-scan scanner. Leaf area (Area), leaf perimeter (P), perimeter/area ratio (P/A), leaf length (Length), and leaf width (Width) were analyzed by ImageJ 1.52q based on the scanned image. Optical properties of leaf reflectivity (Ref), transmissivity (Trans), and absorptivity (Abs)) were measured by spectrometer (USB2000, Ocean Optics, USA), using 10 leaves for each individual. These leaves were also used to measure greenness which is proportional to the amount of chlorophyll present in leaves by chlorophyll meter (SPAD-502, Minolta, Japan). Three to ten leaves of each individual (more blades for small leaves) were collected in the morning and stored in the sealed plastic bags with moist paper inside for density and water content (WC) measurements. They were weighed soon after harvesting, and used water displacement to get leaf volumes, then oven dried under 80 ℃ to constant weight. Leaf density was calculated by the ratio of leaf mass (both fresh and dry density) to leaf volume. Water content was calculated by the ratio of weight difference between fresh and dry leaves to the dry mass (Perez-Harguindeguy et al., 2013). Histological technique of Paraffin-fixing (Biosystems, 2021) was used to make cross-sections for the measurements of leaf thickness (Thickness), the thickness of upper and lower epidermis (Thickness_up, Thickness_low), palisade mesophyll (Thickness_ palisade) and spongy mesophyll (Thickness_spongy) (4 leaves for each individual). All the anatomical sections were photographed under a microscope (Leica Microsystems Vertrieb GmbH, Wetzlar, Germany), and then analyzed with ImageJ. Stomatal density and size were measured using paradermal sections. Paradermal sections were cut from the middle part of leaf avoiding main veins and boiled in water for 10-15 min, then immersed in a 1:1 mixture of 30% H_2O_2 and acetic glacial aqueous solution until they became soft and disintegrated. The needle leaves for stomata sections were bleached with 1:1 of HNO_3, and H_2O in saturated $KClO_3$. The samples for vein density analysis were bleached with 5% NaOH until they become transparent. Stain leaves for 15 min in 1% safranin in ethanol. All the sections were mounted on slides and photographed under a Leica DM2500 light microscope. Measure total length of veins in the image and divide this number by the image area to get vein density.

Diurnal transpiration rate, photosynthesis rate and stomatal conductance were measured by Portable Photosynthesis System (LI-6400, LI-COR, USA) for two sunny days at each site, then we combined the two days measurements into one diurnal curve. Three leaves adjacent to the leaves with temperature measurement were selected for each individual. All the leaves were measured one by one alternately from morning to afternoon, the start and end time were dependent on solar radiation and the availability of canopy crane at each forest (SAV: 8:00~17:00; TRF: 9:20~14:40; STF: 9:30~16:30; TEF: 8:30 ~ 17:40).

2.5 Thermal safety margin. Thermal safety margin was calculated based on T_{crit} to represent thermal safety of photosynthetic system II.

$$TSM = T_{crit} - T \qquad (1)$$

In formula (1), three different metrics of temperature (T) were used to assess the impact of different assumptions of body temperature on TSM. For TSM.Tl, T was the maximum leaf temperature of the individual (Tlmax). For TSM.Ta, T was the maximum air temperature beside the individual (Tamax). For TSM.MTa, T was the maximum canopy air temperature of each forest.

2.6 Data analysis

Comparison of TSM and T_{crit} across and within biomes. The difference of TSM and T_{crit} across and within biomes were analyzed by multiple comparison of least significant difference (LSD). P-value < 0.05 was considered as significant difference.

Impact factors on TSM.Tl. TSM.Tl is determined by two parameters —— T_{crit} and Tlmax. We constructed a mixed effects model by setting TSM.Tl as the response variable, T_{crit} and Tlmax as the fixed effects, and species nested in site as random effects. The contribution of T_{crit} and Tlmax to the variance of TSM.Tl were analyzed by partR2 in R package "partR2" (Martin A. Stoffel et al., 2021).

Impact factors on T_{crit}. Impact factors including 27 leaf traits which might have relationships with leaf temperature (Table 3) and Tlmax. Random forest (Breiman, 2001) was used to find the important leaf traits playing strong roles in explaining variation of thermotolerance. This model corrects data overfitting, and allows non-linear relationships and colinear variables (Breiman, 2001). We calculated variation explained rate by setting number of variables randomly sampled as candidates at each split (mtry) from 1 to 27 (the number of variables minus 1), and got the highest variation explained rate when mtry = 20. OOB error converged at number of trees to grow (ntree) = 400. Therefore, we fit the random forest model with mtry = 20 and ntree = 400, then used node purity values to inform the importance of each predictor. Mixed-effects model was also used to confirm the results. Species nest in site was set as random effect, the important leaf traits selected by random forest were fixed effects and Tcrit was the response variable.

Impact factors on the difference between TSM.Tl and TSM.Ta (TSM.Ta-TSM.Tl). Repeated Measures Correlation in R package “rmcorr” (Bakdash and Marusich, 2017) was used to calculate the correlation between temperature traits and TSM.Ta - TSM.Tl by setting site as subject. Temperature traits included maximum leaf temperature (Tlmax), maximum air temperature (Tamax).

All the analyses were performed using R 3.6.1(Team, 2019).

3. Results

3.1 Patterns of thermal safety margin (TSM) across and within biomes

Site mean TSM.Tl ranged from 3.0 ± 0.5°C at SAV to 12.8 ± 0.9°C at STF. The rank of TSM calculated by the maximum air temperature of each individual (TSM.Ta) and the maximum leaf temperature of each individual (TSM.Tl) across biomes followed the same trend: STF = TEF > TRF > SAV, and TSM calculated by the maximum air temperature at each forest (TSM.MTa) followed STF > TEF > TRF > SAV. The patterns within biomes were different. TSM.Tl, TSM.Ta and TSM.MTa had positive correlations at SAV, TSM.Tl and TSM.Ta were positively correlated at TRF, while TSM.Ta and TSM.MTa were positively correlated at STF and TEF (Fig. 1a).

No negative TSM.Tl was found for normal leaves. However, *Woodfodia fruticosa* and *Bauhinia brachycarpa* presented negative and zero TSM respectively, when transpiration was blocked. All the Vaseline leaves of these two species were dried and dropped at the end of the experiment.

3.2 The patterns of T_{crit} across and within biomes

T_{crit} is one of the two parameters in TSM calculation. Site mean T_{crit} range from 42.5

± 0.6 °C in TEF to 48.5 ± 0.5 °C in SAV, and followed the pattern: SAV = TRF > STF > TEF (Fig. 1b). T_{crit} was linearly and negatively correlated with TSM.Tl (Pearson correlation coefficient = -0.46, p-value < 0.001) across biomes. There were no significant correlations between T_{crit} and TSM.Tl within biomes, except for positive correlation at SAV (Pearson correlation coefficient = 0.70, p-value = 0.01). The random forest model including all the leaf traits and the maximum air temperature explained 68% of the variance of T_{crit}, among them, Tlmax and Tamax explained 62%. Considering of the high correlation between Tlmax and Tamax, we used Tamax and Tlmax as a fixed effect respectively, and species nested in site as random forest in mixed effects model. The model used Tamax as fixed effect gave conditional $R^2 = 0.798$ and marginal $R^2 = 0.571$, and the model used Tlmax as fixed effect gave conditional $R^2 = 0.799$ and marginal $R^2 = 0.131$. However, the Pearson correlations between Tamax (or Tlmax) and T_{crit} were not significant within biomes except for Tamax and T_{crit} at TRF. Therefore, T_{crit} increased with environmental temperature across biomes; while the positive relationships between T_{crit} and environmental temperature within biomes was not confirmed.

3.3 Patterns of leaf temperature across and within biomes

Leaf temperature is another parameter in TSM calculation. It linearly increased with air temperature, however, the increasing slope increased from the hot to the cold forest. Except for the SAV species, all the other species had significant higher Tlmax than Tamax (Fig. 2a). During daytime, leaves had strong cooling effects to reduce Tl close to Ta at SAV, while Tl was much higher than Ta for most of the time at TEF.

Thus, the difference between Tl and Ta increased from the hot to the cold forest (Fig. 2a). The variances of Tlmax between species were within 8 ℃ in SAV, STF and TRF, reached to 12.2 ℃ in TEF, while the variances of Tamax between species within biomes were all lower than 5.5 ℃.

Normal leaf temperatures were all below T_{crit} except for two savanna species *L. coromandelica* and *W. fruticosa*, and their temperatures exceeded T_{crit} for less than one minute. When transpiration was blocked, leaf temperature increased, and the increase extent was highest for SAV species (Fig. 2b). Vaseline leaf temperature of all savanna species and one subtropical species exceeded T_{crit}. Among them, the overheating time of the two savanna species *B. brachycarpa* and *W. fruticosa* exceeded 10 minutes (10.9 ± 7.3 minutes and 39.2 ± 11.6 minutes respectively).

3.4 Factors affecting TSM.Tl

TSM.Tl were calculated using Tlmax and T_{crit}. Compared with T_{crit}, Tlmax were highly variable. The range of T_{crit} across biomes was 12.4 °C, while the range of Tlmax were much higher (19.7 °C). In the mixed effects model, marginal R^2 contributed by Tlmax was 87.1%, while marginal R^2 contributed by T_{crit} was 0%. Pearson correlation coefficient between TSM.Tl and Tlmax was -0.93. TSM.Tl can be predicted by Tlmax by the model TSM.Tl = -0.672×Tlmax+33.581 (R^2 = 0.85, p-value < 0.001), and 83% of the residuals were within 1.5 °C (Fig. 3).

3.5 Factors affecting the difference between TSM.Tl and TSM.Ta (TSM.Ta-TSM.Tl)

TSM.Ta - TSM.Tl increased from 0.3 ± 0.9°C at SAV, 1.8 ± 1.1°C at TRF, and 3.2 ± 1.1°C at STF, to 4.7 ± 2.1°C at TEF. Repeated measures correlation revealed that Tlmax (cor 0.87, p-value < 0.001) had the highest correlation with TSM.Ta - TSM.Tl. TSM.Ta - TSM.Tl linearly increase with Tlmax, but the intercept increased from SAV to TEF (Fig. 4).

4. Discussion

4.1 General patterns of TSM and T_{crit}. Patterns of TSM (regardless of the temperature metrics used) followed similar trends with previous studies across biomes: TSM decreased from the cool forests to the hot forests (Curtis et al., 2016; O'Sullivan et al., 2017). During the heatwave, TSMs of savanna species were the lowest, indicating that their photosynthetic systems were more dangerous under heat stress. Especially when transpiration was suppressed, leaves of some species in SAV were dried and dropped at the end of the experiment. A global study also demonstrated that woody productivity in the hottest forests among 590 permanent plots across the tropics are more sensitive to temperature than at cooler sites (Sullivan et al., 2020). The risks of species disappear and reduction of carbon stock in tropical hot forests under heatwave requires high attention (Gallagher et al., 2019; Tiwari et al., 2020).

Site mean T_{crit} ranged from 42.5 °C ± 0.6 °C at TEF to 48.5 °C ± 0.5 °C at SAV. The pattern and values were comparable with T_{crit} of other studies using the same methodology (O'Sullivan et al., 2017; Zhu et al., 2018). T_{crit} values were lower than T_{50} measured using F_v/F_m-T method (Perez et al., 2020). Random forest model showed that Tlmax and Tamax can explain 62% of the variance of T_{crit}. This indicated the important influence of micro-environment on $T_{crit.}$ However, the correlations between T_{crit} and Tlmax or Tamax were not confirmed within biomes. The range of Tamax across biomes was 21.6 ℃, while the maximum range of Tamax within biomes were below 5.1 ℃. Therefore, environmental temperature can explain more than 60% of the variance of T_{crit} across biomes, while leaf traits might be the main explanation on T_{crit} variance within biomes. There are some leaf traits have been reported having relationships with T_{crit}, e.g. LMA (Gallagher, 2014; Sastry et al., 2018), leaf carbon assimilation (Perez et al., 2020), and leaf chemical composition (Zhu et al., 2018). Thus, more samples should be collected to confirm which leaf traits are more related to T_{crit} within biomes.

4.2 Contrary pattern between TSM and thermotolerance.

Traditionally, the plants with higher thermotolerance are considered to be more resilient to heat stress (Wahid et al., 2007). However, our results showed that TSMs might be negatively related to thermotolerance. For example, the SAV species have high thermotolerance, however, they are more vulnerable to heat damage than the TEF and STF species which have low thermotolerance while low leaf temperature. Notably, the negative relationship between TSM.Tl and T_{crit} was only found across biomes, and their relationships within biomes are uncertain. We found positive correlation between TSM.Tl and T_{crit} at SAV, but no significant relationships between them at other forests; while the study of 19 plant species in Fairchild Tropical Botanic garden found negative correlation between thermotolerance and TSM.Tl (Perez and

Feeley, 2020). Accordingly, thermotolerance cannot be used to estimate thermal safety of plant leaves.

4.3 How to detect vulnerable species under heat stress

Our results demonstrated that evaluating thermal safety of plants based on leaf physical traits are not reliable. TSM was determined by T_{crit} and the maximum leaf temperature (Tlmax). T_{crit} increased with Tlmax, whereas only by around one third of a degree per degree increase in Tlmax. A previous study also reported that T_{crit} ranged around 8 °C from arctic to equatorial sites compared with 20 °C ranged in mean maximum daily temperature of the warmest month (O'Sullivan et al., 2017). As a result, the variance of TSM was mainly determined by Tlmax. The calculation of TSM requires the measurements of T_{crit} and thermotolerance simultaneously, which costs time and cannot be done in situ. Considering the high correlation between leaf temperature and TSM, leaf temperature is an efficient substitute to estimate thermal safety of leaves. Thermal camera can quickly and remotely measure temperature of multiple leaves, thus instantly evaluating thermal safety of leaves in the field.

The method to determine Tlmax has great impact on TSM. The damage of high temperature on leaves is determined by both the threshold of temperature and the exposure time. If leaf temperature exceeding T_{crit} lasted for a few seconds, it could not damage leaf. Previous research usually used 15 minutes to treat leaves under water bath when measure the response of F_v/F_m to temperature (Curtis et al., 2014; Krause et al., 2010). In the present study, we observed leaves died when leaf temperature exceeding T_{crit} for more than 10 minutes in one day. Therefore, the calculation of

Tlmax in TSM should consider its duration time.

4.4 Can we use air temperature to measure TSM?

The present research systematically compared TSM calculated by leaf temperature and air temperature of canopy plants across and within biomes using in situ measurements. Generally, both leaf temperature and air temperature based TSM produced similar rank of TSMs across biomes, however TSM.Ta overestimates TSM especially at cool biomes (Fig. 4). Because the differences between Tl and Ta increased from hot biomes to cold biomes (Fig. 2). Within each biome, TSM.Ta - TSM.Tl increased with leaf temperature (Fig. 4). Hence, it will cause larger errors if TSM.Ta was applied in cooler biomes and for species with higher leaf temperatures within biomes.

5. Conclusion

TSM is important to predict thermal safety of organisms under global warming (Sunday et al., 2014). Our results suggest using leaf temperature instead of thermotolerance to evaluate thermal safety of plants. In this way, thermal safety can be instantly and remotely measured by infrared camera in the field. It will greatly improve the detection of threatened species under heat stress. The present research clarifies the differences of TSM.Tl and TSM.Ta across and within biomes, which is helpful to evaluate the reliability of previous reports of TSM based on air temperature. Our results are valuable for understanding the impact of heat stress on vegetation, and can be applied in forest management.

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Data availability statement

Primary data are stored at figshare, https:// 10.6084/m9.figshare.14038478.

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Figure legend

Figure 1 The patterns of (a) thermal safety margin (TSM) and (b) thermotolerance (T_{crit}). TSM.Ta, TSM calculated by the maximum air temperature of each individual; TSM.Tl, TSM calculated by the maximum leaf temperature of each individual; TSM.MTa, TSM calculated by the maximum air temperature at each forest. SAV, savanna; TRF, tropical rain forest; STF, subtropical broad-leaved forest; TEF, temperate mixed forest.

Figure 2 The relationships between (a) leaf temperature and air temperature; and (b) Vasline leaf temperature and air temperature during daytime (9:00 ~ 17:00). SAV, savanna; TRF, tropical rain forest; STF, subtropical broad-leaved forest; TEF, temperate mixed forest. Dash line is the regression line of y = x.

Figure 3 The relationship between TSM.Tl and Tlmax. The regression line can be modeled by TSM.Tl = -0.672×Tlmax+33.581 (R^2 = 0.85, p-value < 0.001).

Figure 4 The relationships between TSM calculated by the maximum air temperature and the maximum leaf temperature of individuals respectively (TSM.Ta - TSM.Tl) and the maximum leaf temperature (Tlmax). SAV, savanna; TRF, tropical rain forest; STF, subtropical broad-leaved forest; TEF, temperate mixed forest.