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Title: Thermal limits of leaf metabolism across biomes

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Abstract

High-temperature tolerance in plants is important in a warming world, with extreme heat-waves predicted to increase in frequency and duration, potentially leading to lethal heating of leaves. Global patterns of high-temperature tolerance are documented in animals, but generally not plants, limiting our ability to assess risks associated with climate warming. To assess whether there are global patterns in high-temperature tolerance of leaf metabolism, we quantified T_{crit} (high temperature where minimal chlorophyll *a* fluorescence rises rapidly, and thus where photosystem II is disrupted) and T_{max} (temperature where leaf respiration in darkness is maximal, beyond which respiratory function rapidly declines) in upper-canopy leaves of 218 plant species spanning seven biomes. Mean site-based T_{crit} values ranged from 41.5 °C in the Alaskan arctic to 50.8 °C in lowland tropical rainforests of Peruvian Amazon. For T_{max}, the equivalent values were 51.0 and 60.6 °C in the Arctic and Amazon, respectively. T_{crit} and T_{max} followed similar biogeographic patterns, increasing linearly (~8 °C) from polar to equatorial regions. Such increases in high temperature tolerance are much less than expected based on the 20 °C span in high temperature extremes across the globe. Moreover, with only modest high-temperature tolerance despite high summer temperature extremes, species in mid-latitude (~20°-50°) regions have the narrowest thermal safety margins in upper-canopy leaves; these regions are at the greatest risk of damage due to extreme heat-wave events, especially under conditions when leaf temperatures are further elevated by a lack of transpirational cooling. Using predicted heat-wave events for 2050 and accounting for possible thermal acclimation of T_{crit} and T_{max}, we also found that these safety margins could shrink in a warmer world, as rising temperatures are likely to exceed thermal tolerance limits. Thus, increasing numbers of species in many biomes may be at risk as heat-wave events become more severe with climate change.

Introduction

Tolerance of heat-wave events can influence the performance and fitness of organisms that experience elevated temperature (T) extremes and, due to climate change, will become an increasingly important factor in the future (Battisti & Naylor, 2009; Hansen *et al.*, 2012; IPCC, 2012). High temperature tolerance (HT_{tol}) studies in animals have found that equatorial and tropical species exhibit higher heat tolerance than those in cooler, high latitude regions (Deutsch *et al.*, 2008; Sunday *et al.*, 2011; Araújo *et al.*, 2013;). Animals in hot, low latitude environments are most at risk, although the ability to move to lower T refugia ameliorates that risk (Sunday *et al.*, 2014). HT_{tol} is also of concern for plants, which are sessile and limited in the extent to which they can ameliorate risks associated with heat waves, with sun-exposed leaves particularly vulnerable (Vogel, 2009; Leigh *et al.*, 2012). Depending on factors such as leaf orientation, reflectance, transpiration rate and wind speed, leaf T can exceed ambient air T (ΔT) (Fuchs, 1990; Luquet *et al.*, 2003; Vogel, 2009; Leigh *et al.*, 2012). Leaf shape may also be crucial, with ΔT likely to be greater in broad, entire (i.e. non-dissected) leaves compared to their highly dissected and/or narrow-leaf counterparts (Givnish, 1988; Nicotra *et al.*, 2011). In nature, large increases in leaf ΔT ranging from +5 to +20°C have been recorded (Ansari & Loomis, 1959; Gates *et al.*, 1968; Miller, 1972; Beadle *et al.*, 1973; Smith, 1978; Tyree & Wilmot, 1990; Valladares & Pearcy, 1997; Singsaas & Sharkey, 1998; Ishida *et al.*, 1999; Trubuzi, 2005; Doughty & Goulden, 2008; Vogel, 2009; Leuzinger *et al.*, 2010), with desert biomes unsurprisingly showing the greatest elevation likely due to the arid conditions and the lesser likelihood of transpirational cooling (Gates *et al.*, 1968; Smith, 1978); see Table S1 for details. Interestingly, in places where water availability is not limiting, such as tropical rainforests, elevated leaf temperatures of >10 °C have been recorded (Trubuzi, 2005). While such events may be rare, they can be sufficiently long in duration (Ganguly *et al.*, 2009; Perkins *et al.*, 2012) to affect plant performance and potentially plant survival (Reyer *et al.*, 2013).

Heat-waves may disrupt many plant processes (Teskey *et al.*, 2014), including photosynthetic (Berry & Björkman, 1980; Hüve *et al.*, 2011) and respiratory (Hüve *et al.*, 2011; O'Sullivan *et al.*, 2013) metabolism. At the whole organism level, severe heat-waves (+12 °C above ambient) have been shown experimentally to significantly reduce biomass accumulation and net photosynthesis in red oak *Quercus rubra* and loblolly pine *Pinus taeda*, particularly in combination with drought conditions (Ameye *et al.*, 2012; Bauweraerts *et al.*, 2013, 2014). At the leaf level, short-term increases in leaf Ts - such as those experienced during heat-wave events - increase rates of respiration, whereas net photosynthetic rates decline beyond an optimal T to which the plant is acclimated (Dewar *et al.*, 1999; Teskey *et al.*, 2014) due to increases in CO_2 release by photorespiration and/or leaf respiration in the light exceeding carboxylation rates (Atkin *et al.*, 2006). Stomatal closure at high *T* (Valladares & Pearcy, 1997; Zweifel *et al.*, 2006) will further limit CO_2 supply and rates of net photosynthetic CO_2 uptake. Acute heat-waves may also damage photosynthetic and respiratory capacity as high *T*s increase the fluidity of leakiness of cell and organelle membranes (Hazel, 1995) leading to disruption of metabolic processes. Depending on the speed/extent of acclimation of heat tolerance - underpinned by induction of heat shock proteins, accumulation of antioxidant enzymes and osmotic agents, and changes in membrane function and chemistry (Björkman *et al.*, 1980; Seemann *et al.*, 1986; Knight & Ackerly, 2003; Sung *et al.*, 2003; Larkindale *et al.*, 2005; Sharkey, 2005; Hüve *et al.*, 2006, 2011; Velikova *et al.*, 2011) - continued exposure to heat stress may cause permanent damage to leaf tissues (Hüve *et al.*, 2011; O'Sullivan *et al.*, 2013), and contribute to hydraulic failure (Schymanski *et al.*, 2013). High *T*-mediated disruption of plant metabolic processes has been linked with adverse effects on productivity, biodiversity and crop production (Reyer *et al.*, 2013). Given these observations, warm-dry habits may provide a selective pressure for plants with a higher HT_{tol}, particularly at drier sites where the potential for evaporative cooling is diminished and where the effects of heat-waves are likely to be most acute (Bauweraerts *et al.*, 2014). Consistent with this prediction, regional studies have reported higher HT_{tol} of photosynthetic metabolism at dry, warmer sites (Knight & Ackerly, 2002, 2003). Moreover, a comparison of metabolic HT_{tol} in a diverse range of marine poikilotherms showed that heat tolerance was lower in cold-acclimated organisms compared to those from warm oceans (Hochachka & Somero, 2002). With the exception of a study documenting thermotolerance in a single forb across a latitudinal gradient (Barua *et al.*, 2008), and the comparison of four congeneric species pairs from desert and coastal environments (Knight & Ackerly, 2002, 2003), the characterization of the HT_{tol} of higher plants, and how it may vary with climate and geography, is largely unknown.

To characterise HT_{tol} of leaf metabolism, many studies have focussed on the *T* response of minimal chlorophyll *a* fluorescence (F_{o}) (Schreiber & Berry, 1977; Berry & Björkman, 1980; Seemann *et al.*, 1984; Knight & Ackerly, 2002; Hüve *et al.*, 2006, 2011; O'Sullivan *et al.*, 2013). Using this approach, the *T* response of F_{o} typically follows a pattern whereby levels remained stable with moderate heating, before increasing sharply at higher *T*s, followed by a sharp decline. The critical *T* for functioning of photosystem II (T_{crit}) is determined at the point of intersection of two lines, representing the flat and steep parts of the F_{o} - *T* response curve (Knight & Ackerly, 2002; O'Sullivan *et al.*, 2013). The increased F_{o} at high *T*s is indicative of a disruption in electron transport due to increased membrane fluidity and dissociation of membrane-bound proteins involved in photosynthesis (Schreiber & Berry, 1977) and is associated with non-catastrophic degradation in chloroplast membranes (Hüve *et al.*, 2011). Recent studies have also characterised HT_{tol} at temperatures above T_{crit} via determination of

T_{max} defined as the high T at which rates of respiratory CO_2 release are maximal (T_{max}) (Hüve *et al.*, 2011, 2012; O'Sullivan *et al.*, 2013; Gauthier *et al.*, 2014; Heskel *et al.*, 2014; Weerasinghe *et al.*, 2014). At leaf *T*s above T_{max}, rates of leaf respiration irreversibly decline (O'Sullivan *et al.*, 2013), reflecting loss of mitochondrial function and the rapid onset of tissue death.

Here, we provide a cross-biome analysis of how high temperature tolerance of photosynthetic and respiratory metabolism (as characterised by T_{crit} and T_{max}) of upper canopy leaves vary with latitude and thermal environment. Our dataset focuses on *T*-responses observed above 45°C – for patterns in the *T*-response of leaf respiration below 45°C from the same dataset, see Heskel *et al*. (2016). By relating HT_{tol} values to the magnitude and frequency of extremes in air and leaf *T*, our study also provides insights into how the *thermal safety margin* of leaf metabolism varies across the Earth's surface. Here, we define thermal safety margin as the difference between measured high *T* at which damage to leaf metabolism is detected and the leaf *T* experienced during heat-wave events – analogous to similar definitions used for animal ectotherms (Deutsch *et al.*, 2008). Our study addresses the following questions: (1) is HT_{tol} greater in plants growing in warmer compared to cooler environments; (2) does the thermal safety margin of upper canopy leaves differ among plants growing in low and high *T* environments and, if so, what regions are most at risk to high *T* stress; and, (3) what are the consequences of a future warmer world with respect to thermal safety margins?

Materials and methods

Field sites and species selection

Measurements were made at 19 globally distributed, thermally contrasting sites spanning seven biomes (Fig. 1; Table 1). The selected sites have mean annual temperatures ranging from -11 to 26 °C; across the sites, mean maximum daily temperature of the warmest month ranges from 16.7 to 36.6 °C, with heat-wave temperatures (defined as the mean maximum temperature of the warmest 3-day period) ranging from 25.2 to 44.8 °C (Table 1, Table S2). Other than one high-altitude site in Peru, the thermal environment of each site was within the 95% confidence interval for their respective 1° latitudinal band (on land) as assessed using mean maximum *T* of the warmest month taken from the *WorldClim* database (Hijmans *et al*. 2005) (Fig. S1, Table S2). Consequently, the sampled sites (other than the high altitude site) were not outliers in relation to thermal environment with latitude; the high altitude site in Peru was therefore used in analyses related to growth *T* but not to latitude.

For each of the 19 sites, measurements were conducted over a 1- to 3-week period during one of the warmest months of the year (Table 1). A single leaf from three to four replicate individuals of a representative species were selected for measurement at each site, with the exception of the two Peruvian sites where only one replicate per species was selected due to time and logistical limitations (corresponding to 16% of the species in the dataset). Upper canopy, sun-lit leaves were sampled and stored in cool, moist dark conditions until measurement of *T*-responses, typically within six hours of sampling. In total, we developed a global HT_{tol} data set comprising 798 individual measurements of 218 plant species representing a range of plant functional types including evergreen and deciduous tree species, evergreen shrubs and forb species. The vast majority of the selected species were woody (203 sp.), with 15 non-woody species being sampled at three sites (Toolik, Alaska and the two sites in Western Australia). Finally, to assess whether T_{crit} and T_{max} seasonally acclimate, additional measurements were made on a three species at the Greater Western Woodland site in Western Australia (*Olearia mulleri, Ptilotus holosericeus* and *Sclerolaena diacantha*), and at the Atherton tropical wet forest site in Far North Queensland (*Alstonia meulleriana, Cryptocarya mackinnoniana* and *Gillbeea adenopetala*); these measurements were made at time points approximately 5-6 months offset from that of the original campaigns. Thus, for the above listed species, T_{crit} and T_{max} values were available during the warm and cool seasons at each site.

Determination of high temperature tolerance (HT_{tol})

We measured two aspects of HT_{tol} previously linked to changes in cell membranes and plant metabolic performance: heat-induced changes to minimal chlorophyll *a* fluorescence (F_o) - T_{crit} (Schreiber & Berry, 1977); and the upper thermal limit of leaf respiratory CO_2 release in darkness - T_{max} (Hüve *et al.*, 2011; O'Sullivan *et al.*, 2013). Whole leaves were placed in a *T*-controlled, well-mixed cuvette; the cuvettes were *T*-controlled via a thermostatically-controlled circulating water bath as in O'Sullivan *et al.* (2013) and Heskel *et al.* (2014), or via a Peltier system (3010-GWK1 Gas-Exchange Chamber, Walz, Heinz Walz GmbH, Effeltrich, Germany). After a 30-min dark adaption period, the cooled cuvette chamber was heated continuously at a rate of 1°C min^{-1}, until T_{max} was reached (generally leaf *T* between 55-70°C). Leaf *T* was measured with a small-gauge wire copper constantan thermocouple pressed against the lower surface of the leaf, and which was attached to a LI-6400 external thermocouple adaptor (LI6400-13, Li-Cor Inc., Lincoln, NE, USA) that enabled leaf *T*s to be recorded by a LI-6400XT portable gas exchange system (Li-Cor Inc.).

During each *T*-response experiment, we measured F_o in the presence of a low intensity far-red light pulse (necessary to maintain PSII in the oxidized state) every 30 s using a MiniPAM portable chlorophyll fluorometer (HeinzWalz, Effeltrich, Germany) fitted to the glass surface of the leaf chamber. T_{crit} was determined at the point of intersection of two regression lines, representing the flat and steep parts of the F_o - T response curve (Knight & Ackerly, 2002; O'Sullivan *et al.*, 2013). The linear parts of each curve were determined by calculating the instantaneous slope of the relationship between F_o (normalised so that maximum value equals 100) and T across a 3°C range centred on the measurement temperature. The regression lines for the flat and steep parts of the curve were calculated using points where the slope values were <1 or >3.5 respectively. These values were chosen arbitrarily but used consistently to ensure comparable T_{crit} values. Respiratory CO_2 release was also recorded at 30s intervals using a LI-6400XT portable gas exchange system [fitted with an empty and closed 3 x 2 cm cuvette (Li-Cor 6400-02B)] that was plumbed into the air-stream exiting the leaf chamber. T_{max} was identified as the high leaf T where rates of respiratory CO_2 release were maximal (O'Sullivan *et al.*, 2013). Post-measurement, each leaf was oven dried at ~60°C for a minimum of two days and weighed. To enable calculation of specific leaf area (SLA, ratio of leaf area to leaf dry mass with units of m^2 kg^{-1}), we measured the leaf area [using either a LI-3100C leaf area meter (Li-Cor Inc.) or *Image J* (www://imagej.nih.gov/ij/) analyses of a scanned leaf image] and dry mass of an adjacent leaf. Where stated, total nitrogen (N) and phosphorus (P) concentrations were also determined using Kjeldahl digests (Allen et al., 1974) that were analysed using a LaChat Quikchem 8500 Series 2 Flow Injection Analysis System (Lachat Instruments, Milwaukee, WI, USA).

Meteorological data

Local meteorological station data (using the nearest meteorological station at a comparable altitude; Table S2) from 2001-2010 were used to examine trends associated with actual thermal conditions and recent heat-waves experienced at each site, rather than exclusively assessing trends based on changes with latitude as is often done in non-plant organisms (Deutsch *et al.*, 2008; Sunday *et al.*, 2011; Araújo *et al.*, 2013). Restricting meteorological station data to 2001-2010 enabled standardization of data across stations (which vary in the number of years available), while also providing a common review period for all sites. In the case of two sites (Toolik, Alaska and Andes, Peru) meteorological station data from nearby field stations were used (Trubuzi, 2005; K. Halladay and Y. Malhi personal communication). For other sites, meteorological station data were collected from publically available records accessed from national government websites (Miller, 1972; Tyree & Wilmot, 1990; Ishida *et al.*, 1999). In most cases, data were collected from a station within 100 km with an elevation difference of <100 m, with the exception of the sites in Costa Rica and Atherton, QLD (Table S2).

The use of local meteorological data allowed for the assessment of recent heat-wave events to calculate the thermal safety margin of HT_{tol} for T_{crit} and T_{max} referenced against air T. The thermal safety margin was defined as the difference between the HT_{tol} of each species at each site and the observed heat-wave extremes, with latter being the mean maximum air T of the warmest three-day period at each site from 2001-2010 (Table 1). (*Note*: other ways of representing maximum Ts alongside details of met stations for each site are shown in Table S2).

Current modelled climate data for each were collected from the *WorldClim* database (Hijmans *et al.*, 2005) at 2.5 arc-minutes resolution. Future modelled climate data were also collected from the *WorldClim* website using the Hadley Centre model (HadGEM2-ES). Future heat-wave events for 2050 were estimated assuming that the warmest 3-day heat-wave event for 2001-2010 at each site would increase in parallel with predicted increases in the mean maximum T of the warmest month by 2050 as predicted in the high-emission representative concentration pathway (RCP) 8.5 IPCC scenario, the scenario which most closely matches current emission estimates (Friedlingstein *et al.*, 2014). The RCPs are four emission scenarios that range from early mitigation (RCP2.6) to very high baseline emissions (RCP8.5) that provide high resolution spatial data on future climate conditions (corresponding to radiative forcing values from 2.6 to 8.5 W/m^2) (Vuuren *et al.*, 2011).

Data analysis

Bivariate regression was used to explore relationships between HT_{tol} values (T_{max} and T_{crit}) and latitude, and with various measures of the thermal environment. For the latter, the correlations with the highest r^2 were against mean maximum T of the warmest month (MTWM). To test if relationships between HT_{tol} and latitude/MTWM differed between sites in the northern and southern hemispheres, we analyzed the data sets using analysis of covariance (ANCOVA), with latitude or MTWM as the covariant (Fig. S2). Similarly, ANCOVA was used to test whether relationships between HT_{tol} and latitude and MTWM differed between evergreen and deciduous species (Fig. S3). These analyses were conducted using SPSS Statistics V22 (IBM Corp Armonk, NY, USA).

Given the observed scatter in T_{max} and T_{crit} value at any given latitude, and the clear role that altitude plays (e.g. Andes site in Peru), it may be that much of the variation in observed values could be accounted for by including altitude in a model, or more simply site temperature (which is influenced by latitude and altitude). Similarly, the variation in observed values might reflect the impact of environmental factors other than mean maximum T of the warmest month, such as drought, on T_{max} and T_{crit}. With these issues in mind, we used backward-stepwise regression to select best-fitting equations from a starting set of input climate variables, using site-mean values of T_{max} and T_{crit}. To explore relationships between HT_{tol} and associated leaf structural and chemical composition traits, we conducted additional backward-stepwise regressions to select best-fitting equations from a starting set of both input climate variables and the following leaf traits: specific leaf area (SLA), leaf [N] and [P];

for theses analyses, we used both site-mean and species-mean values of the above traits. In all of the above cases, parameters were chosen that exhibited variance inflation factors (VIF) <2.0 (i.e. minimal co-linearity); *F*-to-remove criteria were used to identify best-fitting parameters. Multiple regression was then used to estimate predictive equations for the chosen variables. The *PRESS* statistic (predicted residual error sum of squares) was used to provide a measure of how well each model predicted T_{max} and T_{crit} values; the *PRESS* statistic provides a measure of how well each regression model predicts the observations, with smaller *PRESS* indicating better predictive capability. Relative contributions of climate variables to each regression were gauged from their standardized partial regression coefficients. Stepwise and associated multiple linear regressions were conducted using Sigmaplot Statistics v12 (Systat Software Inc., San Jose, CA, USA).

In the absence of extensive data documenting acclimation of T_{crit} and T_{max} in diverse assemblages of species (but see Results section for seasonal acclimation data at two sites), we calculated T_{crit} and T_{max} in a future, warmer world (taking into account acclimation) assuming that: (1) the existing biogeographic patterns in T_{crit} and T_{max} mirror those of phenotypic adjustments in response to differences in growth *T* experienced at each site – in doing so, we adopted an approach similar to that taken for modelling acclimation responses of leaf respiration using a global respiration data set (Atkin *et al.*, 2015; Slot & Kitjima, 2015; Vanderwel *et al.*, 2015); (2) that all species would acclimate to future increases in *T* to the same extent; and (3), that there are no limits to the degree of high *T* acclimation.

Based on these assumptions, we calculated future T_{crit} values using the formula:

$$\mathrm{acc}T_{\mathrm{crit}} = T_{\mathrm{crit}} + (\Delta T \cdot m)$$

whereby $accT_{\mathrm{crit}}$ is the acclimation value for T_{crit}, ΔT is the difference between the mean maximum *T* of the warmest month under current and future RCP8.5 conditions for each site, and *m* is the slope of the relationship between T_{crit} and mean maximum *T* of the warmest month. The same formula was used to account for acclimation in T_{max} using the slope of the relationship between T_{max} and mean maximum *T* of the warmest month. Using this approach, we note that increases in HT_{tol} may not be linear with respect to future increases in growth *T*; rather HT_{tol} might be expected to decrease disproportionally as growth *T* increases above an optimum (Barua *et al.,* 2004, 2008; Wang *et al.,* 2008). While this may mean that our approach over-estimates acclimation of HT_{tol} to rising growth *Ts*, it also had the benefit of providing a conservative estimate of deleterious effects of future increases in growth *T* on the thermal safety margin of HT_{tol}.

Results

Current biogeographical patterns in HT_{tol} and thermal safety margins

HT_{tol}, as measured by T_{crit} and T_{max}, was greater at lower latitudes (Fig. 2a) and at warmer sites (mean maximum T of the warmest month of the year) (Fig. 2b), noting that growth T co-varies with latitude. Mean site-based T_{crit} was 10.5 ± 0.6 °C lower than mean T_{max}, but both followed similar biogeographic patterns. These significant and parallel linear relationships with geographic and climatic origins (Table 2) were maintained when considering the northern and southern hemisphere sites separately (Fig. S2). Similarly, an analysis of covariance (with latitude or mean maximum T of the warmest month of the year as co-variants) revealed there was no main effect of whether species were categorized as evergreen or deciduous, indicating that the above HT_{tol} patterns are unlikely to be dependent on leaf growth habit (Fig. S3). Although we focus on the high T of the warmest months, the positive relationship of HT_{tol} with air T is similar for a variety of metrics (e.g. mean annual T). Furthermore, in no case did accounting for site aridity significantly improve model fits over and above fits obtained using data on location or climate, when using site-mean values (Table 3). Similarly, accounting for leaf traits central to the 'leaf economic spectrum' (Wright *et al*., 2004) - that being specific leaf area (SLA), and mass-based concentrations of leaf nitrogen ([N]) and phosphorus ([P]) - did not improve model fits in analyses that used site-mean values (Table 3). When using species-mean data, accounting for the above leaf traits also did not improve model fits for T_{crit} (Table S5), suggesting that HT_{tol} of photosynthesis is not linked to leaf traits strongly linked to growth potential; by contrast both mean maximum temperature of the warmest month (MTWM) *and* leaf [P] were retained the preferred model for T_{max}, when using species-mean values (Table S5).

Both T_{crit} and T_{max} ranged ~8 °C from arctic to equatorial sites (Fig. 2). This 8 °C range in HT_{tol} is narrow when compared to the 20 °C range in mean maximum daily T of the warmest month from arctic to equatorial sites (Table 1), suggesting that thermal safety limits of T_{crit} and T_{max} are not constant across the globe. To assess whether this might be true, we quantified geographic variation in heat-wave extremes using the mean maximum air T of the warmest recorded three-day period of each site from 2001-2010 (Fig. 3a) because these represent extreme events that might result in thermal leaf damage. The current station data (Fig. 3a) show that highest heat-wave Ts do not occur in equatorial tropical forests where there is high potential for loss of latent heat during evaporation (Strahler & Strahler, 1989); rather, the highest heat-wave Ts occur at the relatively dry, inland sites at mid latitudes (20-40°) (Fig. 3a). Air Ts during these periods approach mean HT_{tol} (for T_{crit}) thresholds at each site (Fig. 3a) and exceed some individual species HT_{tol} thresholds (Table 3). If leaf Ts exceed air Ts (e.g. by +5 or +10 °C; Fig. 3a), as can sometimes occur (Table S1), HT_{tol} (for T_{crit}) thresholds are breached for greater numbers of species (Table 4), surpassing some site means (Fig. 3a), with species in mid latitude sites (20-50°) most at risk. Greater numbers of species are at risk under heat-wave scenarios predicted under RCP8.5 (Fig. 3b). The narrowest thermal safety margins (calculated using the mean maximum heat-wave air T recorded at each site) occur in species at mid-latitude (~20° to 50°) sites (Fig. 4a; Figs S3 & S4), mirroring global heat-wave patterns (Fig. 3a). Thus, although HT_{tol} increases with increasing mean maximum T of the warmest month (Fig. 2b), such increases in HT_{tol} are insufficient to maintain geographic homeostasis of the thermal safety margin at mid-latitude sites where the severity of heat-waves is most pronounced.

Future warming impacts on thermal safety margins

At two sites (GWW and Atherton), we assessed the extent of seasonal acclimation of T_{crit} and T_{max} in three species at each site. By measuring HT_{tol} in the same species in cool and warm seasons, we could quantify the extent to which T_{crit} and T_{max} varied in response to changes in mean daily T of the 30-day period prior to measurements. Table S6 shows that at GWW (temperate woodland), T_{crit} and T_{max} increased by 0.30 and 0.35 °C per 1.0 °C difference in growth T, respectively, when averaged across the three selected species; at Atherton, T_{crit} and T_{max} increased on average by 0.49 and 0.18 °C per 1.0 °C difference in growth T, respectively. Thus, while not a definitive measure of thermal acclimation, the data point to seasonal adjustments consistent with HT_{tol} increasing as growth T increases.

Because of limited knowledge of the extent of thermal acclimation of T_{crit} and T_{max} across our all species and the 19 sites, we used the relationships with current climate in Fig. 2b and Table 3 to simulate T_{crit} and T_{max} increases with climate warming (see Methods), noting that both traits exhibit evidence of seasonal acclimation in the species shown in Table S6. Here, we assumed that the geographic patterns in HT_{tol} under current climates mirror that of local thermal acclimation or adaptation to a warming climate, as done for modelling leaf respiration rates (Atkin *et al.*, 2015; Slot & Kitjima, 2015; Vanderwel *et al.*, 2015). The observed relationship with climate indicates a ~0.3 °C difference in HT_{tol} per 1.0 °C difference in growth T (Fig. 2b), which is consistent with empirical evidence of Ghouil *et al*. (2003) and is similar to the seasonal shifts in HT_{tol} shown in Table S6; given this, we used relationships shown in Fig. 2b and Table 3 as a first-order approximation of acclimation of HT_{tol} parameters to warmer future growing conditions. Thus, the slopes of the relationships of T_{crit} and T_{max} against mean maximum T of the warmest month (Fig. 2b) were used to predict T_{crit} and T_{max} values of each species under warmer climates projected for sites by 2050 under the IPCC high emission RCP8.5 scenario. While T_{crit} and T_{max} may not thermally acclimate in all species, predicting future T_{crit}

and T_{max} values in a warmer world in this way avoids the unlikely assumption that HT_{tol} will remain static.

Under the above described thermal acclimation scenario, HT_{tol} increases in all species with climate warming. Despite this, heat-wave *T* extremes are likely to breach T_{crit} across a broader range of latitudes than today under the RCP8.5 scenario (Fig. 3b), with a narrowing of thermal safety margins across all sites by 2050 (Fig. 4b, Table 2), due to the predicted increases in air *T* being greater than the predicted acclimation-dependent increases in T_{crit} (for full comparison of different RCP scenarios, see Table S3). When averaged across all sites and assuming leaf and air *T* are in equilibrium, 5% and 7% of species are likely to exceed their T_{crit} thermal safety margins in current and future climates, respectively. Importantly, the degree of narrowing of the thermal safety margin in 2050 would be even greater for species that do not acclimate much or at all. Thus, by assuming a uniform degree of acclimation for all species, our approach provides a conservative estimate of the possible effects of future warming on the thermal safety margin, noting that the deleterious effects of warming would be even more severe if HT_{tol} fails to increase proportionally as growth *T* increases above an optimum, as has been suggested (Barua *et al.*, 2004, 2008; Wang *et al.*, 2008).

While future thermal safety margins are influenced by metabolic acclimation, consideration of the extent to which leaf *T*s exceed air *T*s is also important. The above analyses assumed that leaf *T*s are in equilibrium with air *T*, with the latter being based on climate station *T*s. Although leaf *T*s may match ambient air *T*s in the shade, they can become markedly elevated during periods of full sun (Vogel, 2009; Leigh *et al*., 2012), dependent on several factors including the extent of air movement, degree of evaporative cooling and leaf shape. Leaf *T*s have been recorded to be 5-15 °C and 5-20 °C above ambient air *T* in temperate and tropical regions, respectively (Table S1) at times of full sun, low wind speed and reduced transpiration - conditions likely to co-occur during heat-waves. An elevated leaf *T* that is 5-10 °C above ambient air *T* across all sites would result in 18-43% of the sampled species exceeding their thermal safety margin of T_{crit} based on recent heat-wave events (2001-2010; Table 2 & Fig. S4a,c), with the proportion rising to 28-62% by 2050 under the RCP8.5 scenario (Table 2 & Fig. S4b,d). Thermal safety margins calculated from T_{max}, reflecting tissue damage and death, are much less likely to be breached than thermal safety margins calculated from T_{crit} under current and future conditions (Fig. S5 & Table S4). The majority of species exceeding their thermal safety margins for T_{crit} occur at mid-latitude sites that currently experience the warmest heat-waves (2001-2010) with 100% of measured species at several sites exceeding their thermal safety margin in some climate change scenarios (Table S3). The mid-latitudes are also most likely to experience the warmest extremes in the future and to have the greatest fraction of species at risk of damage from such events (Table 2).

Discussion

Biogeographic patterns and acclimation/adaptation

Our results show that site mean values of observed T_{crit} and T_{max} of upper canopy leaves increase as site maximum air T increases (Fig. 2), although by less than half as much as the change in air T (Table 1, Fig. S1), resulting in a narrowing of the thermal safety margin in the mid-latitudes compared to the equatorial and high-latitude regions (Fig. 4; Figs S4 & S5). Importantly, thermal safety margins shown in Figure 4 likely over-estimate the true safety margin of upper canopy leaves as they assume that leaf and air Ts are in equilibrium; during heat-waves, leaf Ts are increasingly likely to exceed air Ts, further reducing the safety margin (Figs S4 and S5). Thus, the extent to which upper canopy leaves exhibit Ts greater than that of the surrounding air is crucial in determining how current and future heat-waves events impact on leaf metabolic processes central to plant growth and survival. A further factor that may contribute to the thermal safety margins shown for the 'Future (RCP 8.5)' scenarios shown in Figures 4, S4 and S5 is the assumption that HT_{tol} increases linearly with increasing growth T without an upper acclimation limit. Limits to the extent to which HT_{tol} can acclimate (Barua *et al.*, 2003, 2004; Krause *et al.* 2013) would further limit thermal safety margins of leaf energy metabolism in a future, warmer world.

The greater HT_{tol} in hot, low-mid latitude regions might simply reflect phenotypic adjustments of individual plants to sustained exposure to high air T (i.e. thermal acclimation). Indeed, at two field sites, we have observed seasonal adjustments in several species consistent with thermal acclimation of HT_{tol} (Table S6). Acclimation to high air Ts is underpinned by: changes in lipid composition and/or accumulation of volatile organic compounds that increase membrane thermostability; increases in leaf osmotic potential and soluble sugar concentrations that help protect chloroplast and mitochondrial membranes; induction of heat shock proteins (HSPs) which act as molecular chaperones to protect proteins from denaturation; and, accumulation of antioxidant enzymes to limit formation of reactive oxygen species (Björkman *et al.*, 1980; Seemann *et al.*, 1986; Downs *et al.*, 1998; Heckathorn *et al.;* 1998; Sung *et al.*, 2003; Larkindale *et al.*, 2005; Sharkey, 2005; Hüve *et al.*, 2006, 2011; Velikova *et al.*, 2011; Kim *et al.*, 2012). Acclimation can allow the plant to cope, up to a point (that is yet to be quantified), with extreme heat-wave events in a manner analogous to the way acclimation induced by sustained chilling allows a plant to cope with a freezing event (Iba, 2002). Notwithstanding evidence of seasonal adjustments in HT_{tol} at two of our field sites (Table S6), the extent to which T_{crit} and T_{max} adjust to changes in growth T *per se* is not certain. For example, Krause *et al.* (2013) reported no change in HT_{tol} of photosynthesis in tropical trees grown at two contrasting Ts. Moreover, elevated growth T (3-5 °C) did not increase T_{max} of an Australian broadleaved tree, *Eucalyptus saligna* (Gauthier *et al*., 2014) or an arctic shrub, *Betula nana* (Heskel *et al.*, 2014); similarly, increases in growth T along an 800 m elevational gradient in the sub-alpine/alpine region of SE Australia had no effect on either T_{crit} or T_{max} of *Eucalyptus pauciflora* (O'Sullivan *et al*., 2013). By contrast, Ghouil *et al*. (2003) reported that when cork oak (*Quercus suber*) plants were acclimated to a wider range of growth Ts (10-40°C), T_{crit} increased by ~0.3 °C per 1.0°C increase in growth T. Acclimation of T_{crit} and related photosynthetic properties has also been reported in several studies (Downton *et al.,* 1984; Seemann *et al.*, 1986; Knight & Ackerly, 2002). Thus, while the extent to which T_{crit} and T_{max} acclimates remains unclear, the possibility remains that acclimation may contribute to the biogeographical patterns observed in our study, particularly given our observation of seasonal adjustments in T_{crit} and T_{max} (Table S6). While further work is needed to assess the extent to which T_{crit} and T_{max} acclimate to sustained changes in *T per se*, a conservative approach to predicting potential negative effects of future warming would assume that acclimation does occur – it is for this reason that we assumed a uniform level of acclimation for all our selected species when modelling the effect of future (RCP 8.5) warming on the thermal safety margin of HT_{tol} (Fig. 4b and Figs S5 & S5). For our analyses, we assumed that the slope of relationships linking T_{max} and T_{crit} to mean maximum T of the warmest month (Fig. 3b) could provide an estimate of acclimation potential, with the assumed acclimation equations allowing for 0.38°C and 0.26°C change in T_{crit} and T_{max} respectively per 1.0°C change in mean maximum T of the warmest month (see Table 3).

A second factor that may contribute to greater HT_{tol} in plants in hot regions is that heat tolerance is a consequence of evolutionary history, whereby adaptation to hot climates results in inherently greater HT_{tol} compared to plants adapted cooler regions. Notwithstanding evidence that both T_{crit} and T_{max} likely acclimate to changes in T of the growth environment (see above), it remains unknown the extent to which species from thermally contrasting environments differ in basal HT_{tol} and/or ability to increase HT_{tol} when challenged with rising growth Ts [but see related studies by Knight & Ackerly (2002) & Curtis *et al.* (2014)]. Comparison of a diverse range of plant taxa from thermally contrasting biomes, when grown under common garden conditions, are therefore needed to gain insights into the role acclimation versus adaptation processes play in controlling the observed site-to-site patterns in HT_{tol} observed in our study.

Upper limits to thermal tolerance

The likelihood that there is an upper limit in *HT*$_{tol}$ of photosynthetic and respiratory metabolism in terrestrial plants – as has been suggested previously by studies focussing on the induction of chloroplast and/or mitochondrial HSPs (Heckathorn *et al.;* 1998; Downs *et al.*, 1998; Shakeel *et al.*, 2011; Kim *et al.*, 2012) and associated *HT*$_{tol}$ of photosynthetic rates (Barua *et al.*, 2004, 2008; Krause *et al.*, 2013) - is reflected in the observation that maximum heat-wave air *T*s are not matched by concomitant increases in *HT*$_{tol}$ (hence the fact that the thermal safety margin of *HT*$_{tol}$ is not constant – Fig. 4a; Figs S4 & S5). While the above mentioned acclimation mechanisms are likely to lead to increased heat tolerance, it seems likely that there is limit in the extent to which they facilitate increases in *HT*$_{tol}$ of photosynthesis and respiration, which in turn reduces the thermal safety margins of these processes for plants growing at the warmest sites. Further work will be needed to quantify the upper limits of thermal tolerance of photosynthesis and respiration in wide range of plant taxa from thermally contrasting biomes, particularly in plants acclimated to high growth *T*s.

Thermal safety margin

A key factor that will influence changing thermal safety margins in plants is the extent to which other driving variables modify how much leaf *T*s exceed air *T*s during heat-waves. The elevation of leaf *T* above air *T* will be greatest under high irradiance and low soil moisture conditions that reduce stomatal conductance, transpirational water loss, and associated cooling (Ameye *et al.*, 2012; Bauweraerts *et al.*, 2013, 2014; Schymanski *et al.*, 2013). Although there are reports of drought-mediated increases of 6-10 °C in T_{max} (Gauthier *et al.*, 2014) and T_{crit} (Ghouil *et al.*, 2003), loss of transpirational cooling during drought events could result in even greater increases in leaf *T*s during heat-waves. Similarly, elevated atmospheric CO_2 concentrations may also modestly increase leaf *T* (e.g. 2-5 °C) in both C_3 (Barker *et al.*, 2005) and C_4 (Siebke *et al.*, 2002) plants via reduced stomatal conductance to water vapour and latent heat loss. Such increases in leaf *T* could further narrow future thermal safety margins (Fig. 4b; Figs S4 & S5), depending on the extent to which rising atmospheric CO_2 concentrations impact on high *T* tolerance (Ameye *et al.*, 2012). There is also the possibility that T_{crit} could rise in response to future increases atmospheric CO_2, as a review by Wang *et al.* (2012) found increased heat tolerance of photosynthesis in a range of C_3 species grown under elevated CO_2. However, past studies of heat stress and elevated CO_2 (e.g. Faria *et al.*, 1996; Taub *et al.*, 2000) have not quantified changes in T_{crit} *per se*. In the one study to assess the effects of elevated atmospheric CO_2 on T_{max} (Gauthier *et al.*, 2014), elevated CO_2 had no effect.

What would be the impacts of exceeding heat tolerance levels? For leaves transiently exposed (i.e. < 5 mins) to *T*s between T_{crit} and T_{max}, metabolic functioning continues at an impaired level

(O'Sullivan *et al.*, 2013). However, with more prolonged exposure to such leaf Ts during heat-waves, leaf tissues will be permanently damaged (O'Sullivan *et al.*, 2013; Schymanski *et al.*, 2013). Thus, tissue death could occur during multi-day heat-wave events, with the ultimate impact depending on the extent to which leaves increase HT_{tol} during those events, combined with the extent to which embolisms and associated water stress increase during heat-waves. Further factors that may influence the impact of such heat-wave events on plant growth and survival is whether leaf metabolism can rapidly recover from heat stress (Curtis *et al.*, 2014), the extent to which lower canopy leaves maintain functionality and/or the speed with which upper canopy leaves re-grow following each heat-wave.

Variability in heat tolerance among co-occurring species

As reported in previous studies assessing heat tolerance of photosynthesis of field-grown plants (Knight & Ackerly, 2002, 2003; Curtis *et al.*, 2014), we found that T_{crit} and T_{max} were highly variable among co-occurring species at each site (Fig. 3). Thus, while many species can tolerate high leaf Ts, other co-occurring species possess a smaller safety margin (i.e. lower T_{crit} and T_{max}) (Fig. 4 and Fig. S4). To gain insights into what factors might be responsible for this variability, we analysed whether including leaf structural and chemical composition traits that are ecologically important and linked to growth potential (SLA, [N] and [P] (Reich *et al.*, 1997; Wright *et al.*, 2004; Poorter *et al.*, 2009) improve our ability to predict variability in HT_{tol}. For T_{crit}, inclusion of SLA, [N] and [P] did not improve the predictive power of the models (Table S5), irrespective of whether site-mean or species-mean data were used in the analysis. While the results in Table 3 and S5 point to T_{crit} being linked to the thermal environment in which plants are grown (either as a result of acclimation and/or adaptive processes), they do not explain why co-existing species differed in T_{crit} values. Rather, other factors must be responsible for the observed variability in T_{crit} among species at each site, such as variability in the abundance of heat shock proteins (Heckathorn *et al.*, 1998; Knight & Ackerly, 2001, 2003; Barua *et al.*, 2003; 2008) and/or membrane composition traits (Raison *et al.* 1982; Larkindale & Huang, 2004; Los & Murata, 2004).

Interestingly, leaf [P] was retained in the regression model predicting variability in T_{max} of respiration when analyses were conducted using site-mean and species-mean values, with higher leaf [P] being associated with decreased T_{max} (Tables 3 and S5). Thus, while membrane properties and heat shock proteins (particularly proteins that accumulate in mitochondria; Downs *et al.*, 1998; Kim *et al.*, 2012) are likely to be important for species variability in T_{max}, it appears that processes linked to foliar phosphorus concentrations influence species-to-species variation in heat tolerance of mitochondrial respiration; further work is needed to explore the mechanistic basis via which this association occurs.

Taken together, our results indicate that upper canopy leaves of many plants in mid-latitudes operate close to their metabolic thermal limits during heat-wave events (Figs 2 and 4). Leaves in such areas are particularly at risk considering that air *T* extremes are highest in the mid-latitudes. Dry summers are characteristic of the climate in large portions of this latitudinal zone, and if coupled with reduced transpiration would further elevate leaf *T* above air *T*s. Moreover, heat-wave events will generally become more common in the future. As mean air *T* in the tropics and mid-latitudes is predicted to exceed current extreme *T* events by 2100 (Battisti & Naylor, 2009), vulnerability of upper canopy leaf metabolism to heat-wave events will increasingly become a reality (Fig. 2b and Table 2; Figs S4 & S5 and Tables S4 & S5). Under future climate warming scenarios, our findings suggest that upper canopy leaf metabolism may be at substantially increased risk during heat-wave events, particularly when those are combined with drought (IPCC, 2012). Although this finding is speculative, it is worth noting that native plants in mid-latitude regions may be particularly at risk. If loss of metabolic capacity impacts on rates of net carbon gain at the whole-plant level, there is a possibility that heat-waves may contribute to dieback of heat-sensitive species, with consequences for gross primary production (GPP) and global species distributions in a future, warmer world (Reyer *et al.*, 2013). Finally, when considering the extent to which heat-waves will impact on GPP and distribution of plant species, an assessment is needed not just of HT_{tol} of leaf metabolism, but also how heat affects other plant processes during vegetative growth (e.g. efficiency of water use) and reproduction, with the latter being particularly sensitive to heat-waves (Barnabas *et al*., 2008; Hall, 2010).

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Supporting Information

Figure S1. Comparison of *WorldClim* predictions and observed values of maximum temperatures.

Figure S2. Re-analysis of Figure 2 in main text, revealing hemisphere-specific responses of high *T* tolerance

Figure S3. Re-analysis of Figure 2 in the main text, revealing growth habit specific responses of high *T* tolerance

Figure S4. Thermal safety margin values of T_{crit} for scenarios where leaf *T* is +5 and +10°C > air *T*.

Figure S5. Thermal safety margin values of T_{max} for scenarios where leaf *T* is in equilibrium with air *T*, and where leaf *T* is +5 and +10°C > air *T*.

Table S1. Maximum increase in leaf *T* above air temperatures as reported in literature

Table S2. Details of nearest meteorological stations to each of the sites.

Table S3. Percentage of species where thermal safety margin (T_{crit}) was exceeded under different climate scenarios

Table S4. Percentage of species where thermal safety margin (T_{max}) was exceeded under different climate scenarios

Table S5. Regression equations expressing T_{max} and T_{crit} as a function of climate and other leaf traits, using species mean data on leaf traits.

Table S6 Seasonal changes in in high *T* tolerance T_{max} and T_{crit} at two field sites in Australia

Table S7. Source data (Excel file).

Table 1 Sample sites, data collection dates, mean maximum daily temperatures of warmest months (from *WorldClim*) and heat-wave conditions of the sites (as determined from nearby meteorological stations) at which T_{crit} and T_{max} values were measured. For each site, mean values of T_{crit} and T_{max} are shown (°C). Also shown are specific leaf area (SLA; ratio of leaf area to leaf mass – $m^2\ kg^{-1}$), and concentrations of leaf nitrogen (N, mg g^{-1}) and leaf phosphorus (P, mg g^{-1}). Note that no data are available for [N] and [P] at several sites (--). Sites are listed in order of highest to lowest absolute latitude (i.e. from poles to equator). Altitude (alt, metres above sea level), number of species (*n*) and biome classes at each site are also shown. Biome classes: BF, boreal forests; TeDF, temperate deciduous forest; TeRF, temperate rainforest; TeW, temperate woodland; TrRF_lw, lowland tropical rainforest (<1500 asl); TrRF_up, upland tropical rainforest (>1500 asl); Tu, tundra. Other abbreviations: HWRC, Hubecheck Wilderness Research Center; TAS, Tasmania; ACT, Australian Capital Territory; ANU, Australian National University; WA, Western Australia; NT, Northern Territory; QLD, Queensland. T_{crit} and T_{max} refer to the high *T* tolerance of photosynthesis and leaf respiration, as defined in the main text.

Site	Biome	Location		alt	*n*	Sampling date	Mean max daily *T* of warmest month	Mean max *T* of warmest 3-day period during 2001-2010	T_{crit}	T_{max}	*SLA*	*[N]*	*[P]*
		°N	°E	m	spp		°C	°C	°C	°C	$m^2\ kg^{-1}$	mg g^{-1}	mg g^{-1}
Toolik, Alaska	Tu	68.38	-149.36	720	20	Jun 2010	16.7	25.2	41.5	53.0	11.8	22.55	2.01
Umea, Sweden	BF	63.82	20.31	29	11	Aug 2013	20.5	29.0	46.4	54.0	10.0	16.45	1.28
HWRC, Minnesota	BF	47.96	-91.75	420	15	Jul 2013	25.6	34.1	44.9	54.9	11.1	17.44	1.38
Warra, TAS low altitude	TeRF	-43.10	146.72	88	2	Mar 2012	20.7	35.0	43.5	55.7	5.4	8.19	0.42
Warra, TAS high altitude	TeRF	-43.09	146.67	237	10	Mar 2012	20.4	34.6	43.7	58.3	7.3	14.44	0.60
Black Rock Forest, New York	TeDF	41.41	-74.01	335	10	Jun 2013	27.1	37.2	47.3	55.6	14.6	21.62	1.59
Acton, Canberra, ACT	TeW	-35.28	149.11	571	4	Mar 2012	28.2	39.9	-	55.9	15.3	-	-
Aranda, Canberra, ACT	TeW	-35.28	149.08	580	10	Oct 2011	27.9	39.9	-	51.0	5.4	-	-
Calperum, SA	TeW	-34.04	140.67	35	18	Mar 2013	32.7	44.8	49.5	60.6	3.9	13.20	0.61

Texas A&M, Texas	TeW	30.60	-96.40	103	2	Oct 2010	35.5	40.8	-	55.2	4.7	-	-
Great Western Woodlands, WA	TeW	-30.26	120.69	459	16	Apr 2013	34.3	43.9	49.7	58.2	5.7	17.77	0.66
Jurien Bay, WA	TeW	-30.22	115.04	9	11	Nov 2011	31.3	43.3	42.4	57.8	6.8	14.36	0.80
Alice Mulga, NT	TeW	-22.28	133.25	607	5	Feb 2013	36.6	43.0	50.1	60.0	3.8	12.58	0.73
Atherton QLD	TrRF_lw	-17.12	145.63	728	16	Aug 2012	29.1	37.0	46.2	55.7	9.3	16.27	1.16
Cape Tribulation, QLD	TrRF_lw	-16.28	145.48	90	12	Sep 2010	31.9	37.3	-	59.4	9.2	2.20	0.10
Andes, Peru	TrRF_up	-13.19	-71.59	3000	16	Sep 2011	22.4	20.8	42.9	53.8	6.6	17.77	1.78
Costa Rica	TrRF_lw	10.38	-84.62	479	6	Jul 2011	30.7	33.4	-	58.5	19.8	-	-
Paracou, French Guiana	TrRF_lw	5.27	-52.92	21	37	Oct 2010	31.3	34.5	50.4	58.5	10.1	15.44	0.62
Iquitos, Peru	TrRF_lw	-3.95	-73.43	114	19	Sep 2011	32.0	37.5	50.8	59.6	7.8	17.18	0.75

Table 2 Equations of linear relationships shown in Fig. 2

Linear relationship (y = mx + c)					
y	x	m	c	r^2	p
T_{crit}	|latitude|	-0.1061	50.4975	0.4356	0.0141
T_{max}	|latitude|	-0.0984	59.9727	0.4380	0.0028
T_{crit}	Mean max T (warmest month)	0.3805	36.1375	0.6603	0.0004
T_{max}	Mean max T (warmest month)	0.2643	49.1545	0.3807	0.0049

Table 3. Regression equations expressing T_{max} and T_{crit} as function of site climate and leaf traits, using site-mean values of leaf traits. n = number of sites. To select the best fitting equation from a group of input independent variables, data were explored using *Backwards-Stepwise Regression* – this revealed that chosen parameters exhibited *variance inflation factors* (VIF) less than 2.0 (i.e. minimal multi-collinearity); it also identified best-fit parameters (using F-to-remove criterion). Thereafter, multiple regression analyses were conducted to estimate predictive equations for the chosen variables. All selected variables were significant (P<0.001). The *PRESS* statistic (predicted residual error sum of squares) provides a measure of how well each regression model predicts the observations, with smaller *PRESS* indicating better predictive capability. Relative contributions of location and climate variables to each regression can be gauged from their standardized partial regression coefficients (β_1-β_2, depending on model equation). Abbreviations: aridity index (AI) calculated as the ratio of mean annual precipitation to mean annual potential evapotranspiration (UNEP, 1997, Zomer *et al.*, 2008); mean maximum temperature of the warmest month (MTWM); specific leaf area (SLA; ratio of leaf area to leaf mass – m^2 kg^{-1}); leaf nitrogen (N, mg g^{-1}); leaf phosphorus (P, mg g^{-1}). MTWM at each site were obtained using site information and the *WorldClim* data base (Hijmans *et al.*, 2005).

			Multiple linear regression parameters				
Dependent variable	Input: independent variables *(Backwards-Stepwise Regression)*	Output: selected equations *(Multiple Linear Regression)*	n	r^2	*PRESS* statistic	Standardized partial regression coefficients	
						β_1	β_2
T_{crit}	Latitude, altitude, aridity index (AI), SLA, N, P	T_{crit} = 50.912 - (0.100 * Latitude) - (0.00215 * Altitude)	14	0.494	99.1	-0.616	-0.504
	MTWM, aridity index (AI), SLA, N, P	T_{crit} = 36.137 + (0.38 * MTWM)	14	0.660	56.9	0.813	
T_{max}	Latitude, altitude, aridity index (AI), SLA, N, P	T_{max} = 60.494 - (0.0960 * Latitude) - (0.00182 * Altitude)	19	0.521	79.4	-0.648	-0.447
	MTWM, aridity index (AI), SLA, N, P	T_{max} = 49.155 + (0.264 * MTWM)	19	0.381	93.8	0.617	

Table 4 Percentage of species measured which exceed the T_{crit} thermal safety margin (i.e. $T_{\mathrm{leaf}} > T_{\mathrm{crit}}$) during heat-waves under current and future (RCP8.5 in 2050) climate scenarios. For both 'Current' and 'Future (RCP8.5 in 2050)' scenarios, the percentage of species exceeding T_{crit} are shown for events where the elevation of leaf temperature (T_{leaf}) over that of the surrounding air temperature is calculated at +0, +5 °C and +10 °C.

Site	*Biome*	*Location*		*Scenario*					
				Current			Future (RCP8.5 in 2050)		
				Elevation in T_{leaf} (°C)			*Elevation in* T_{leaf} (°C)		
				+0	+5	+10	+0	+5	+10
		°N	°E						
Toolik, Alaska	Tu	68.38	-149.36	0.0	0.0	10.0	0.0	0.0	15.0
Umea, Sweden	BF	63.82	20.31	0.0	0.0	0.0	0.0	0.0	0.0
HWRC, Minnesota	BF	47.96	-91.75	0.0	0.0	20.0	0.0	6.7	86.7
Warra, Tasmania – low altitude	TeRF	-43.10	146.72	0.0	0.0	50.0	0.0	0.0	50.0
Warra, Tasmania – high altitude	TeRF	-43.09	146.67	0.0	10.0	60.0	0.0	30.0	100.0
Black Rock Forest, New York	TeW	41.41	-74.01	0.0	0.0	55.6	0.0	11.1	100.0
Acton, Canberra, ACT	TeW	-35.28	149.11	-	-	-	-	-	-
Aranda, Canberra, ACT	TeW	-35.28	149.08	-	-	-	-	-	-
Calperum, South Australia	TeW	-34.04	140.67	5.6	66.7	100.0	5.6	88.9	100.0
Texas A&M, Texas	TeW	30.60	-96.40	-	-	-	-	-	-
Great Western Woodlands, Western Australia	TeW	-30.26	120.69	6.3	18.8	87.5	6.3	62.5	100.0
Jurien Bay, Western Australia	TeW	-30.22	115.04	54.5	100.0	100.0	72.7	100.0	100.0
Alice Munga, Northern Territory	TeW	-22.28	133.25	0.0	40.0	80.0	0.0	40.0	100.0
Atherton, Queensland	TrRF_lw	-17.12	145.63	0.0	7.1	42.9	7.1	14.3	85.7
Cape Tribulation, Queensland	TrRF_lw	-16.28	145.48	-	-	-	-	-	-
Andes, Peru	TrRF_up	-13.19	-71.59	0.0	0.0	0.0	0.0	0.0	0.0
Costa Rica	TrRF_lw	10.38	-84.62	-	-	-	-	-	-
Paracou, French Guiana	TrRF_lw	5.27	-52.92	0.0	0.0	4.8	0.0	4.8	14.3
Iquitos, Peru	TrRF_lw	-3.95	-73.43	0.0	7.7	38.5	7.7	15.4	61.5
			Mean of all sites	**4.5**	**17.5**	**42.9**	**6.8**	**27.7**	**61.6**

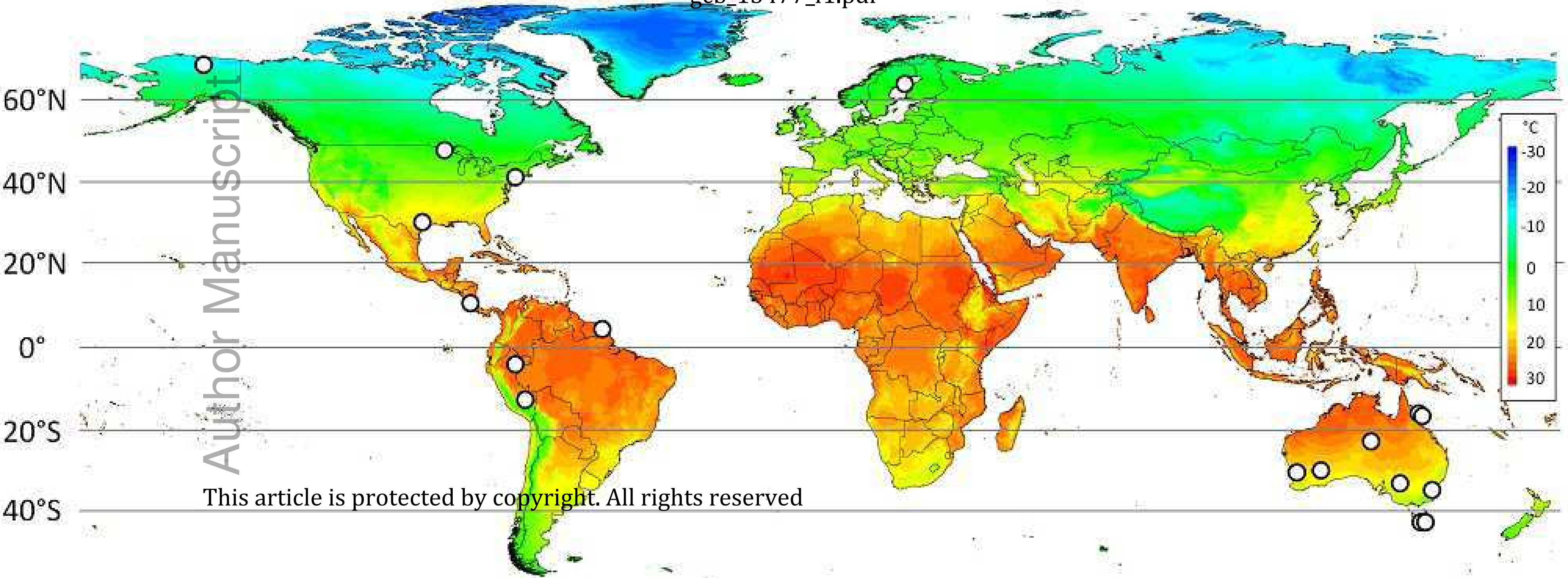
Figures

Fig. 1 Current mean annual temperature (°C) estimates from *WorldClim* data (Hijmans *et al*. 2005) with circles indicating site locations. See Table 1 for details on site locations.

Fig. 2 Global patterns in high temperature (T) tolerance in plants for 171 (T_{crit}) and 218 (T_{max}) different species. Linear regressions show (**a**) absolute latitude and (**b**) mean daily maximum T of the warmest month (MTWM) are significantly correlated ($p < 0.01$) with of T_{crit} (dashed lines) and T_{max} (solid lines). Equations for linear regressions through site mean data: Fig. 2a, $T_{\text{crit}} = 50.498 - 0.1061\text{*latitude}$, $T_{\text{max}} = 59.973 - 0.0984\text{*latitude}$; Fig. 2b, $T_{\text{crit}} = 36.138 – 0.3805\text{*MTWM}$, $T_{\text{max}} = 49.1545 – 0.2643\text{*MTWM}$ (see Table 2 for further details). Larger points indicate site means ± SE with species mean data indicated in smaller points. In (a), values for a tropical high altitude site in Peru excluded from latitude regression analysis are indicated in light (T_{crit}) and dark (T_{max}) grey symbols. Details on each site MTWM are shown in Table 1 & Figure S6.

Fig. 3 Current and future heat-wave temperature (T) extremes and relation to global patterns in T_{crit}. Mean maximum air T of the warmest three-day period (**a**) recorded at each site and (**b**) predicted for 2050 under RCP 8.5, with +5 °C and +10 °C above ambient included to illustrate scenarios in which leaf T further exceeds air T. Curved lines show relationship of site heat-wave Ts with latitude; bold dotted line (········) shows the mean T_{crit} change with latitude derived from Fig. 2a. Broken dash-dot lines (–··–) indicate latitudes where leaf Ts exceed T_{crit} (e.g. when predicted leaf T is +10 °C greater than air T). Excluded from the figure are data from the tropical, high-altitude site in Peru.

Fig. 4 Thermal safety margins (TSM) of T_{crit}, assuming leaf temperature (T) equals air T, both now and in the future. TSMs determined using: (**a**) observed values of T_{crit} (Fig. 2a) and mean maximum daily temperature (T) over warmest consecutive 3-day period from 2001-2010 (Fig. 3a); and, (**b**) predicted future values of T_{crit} (accounting for potential thermal acclimation) and estimated future mean maximum 3-day heat-wave temperatures under RCP 8.5 (Fig. 3b) for each measured species at each site. Grey shading indicates 95% CI of TSM across latitudes. Red hatched box when TSM < 0 and so corresponds to the leaf injury zone at which T_{crit} has been exceeded when no elevation in leaf T has been assumed. White/open symbols indicate site means ± SE. Green/closed circles indicate individual species mean TSM values at each site. Site-mean values for a high altitude site in the Peruvian Andes (excluded from regression analysis) are shown with an open, grey circle. Note: see Supplementary Fig. 4 for equivalent T_{crit} TSM values assuming that leaf T exceeds air T by +5 and +10 °C, and Supplementary Fig. 5 for equivalent T_{max} TSM values assuming that leaf T = air T, and exceeds air T


60°N
40°N
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°C
-30
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10
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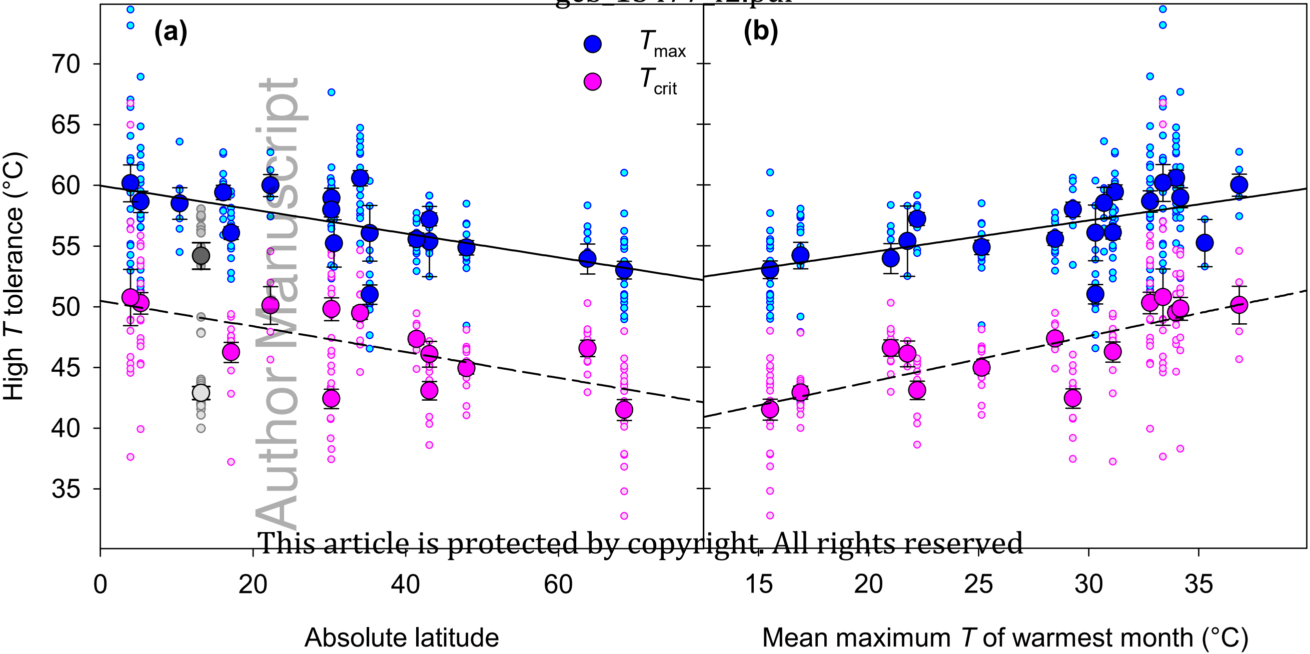
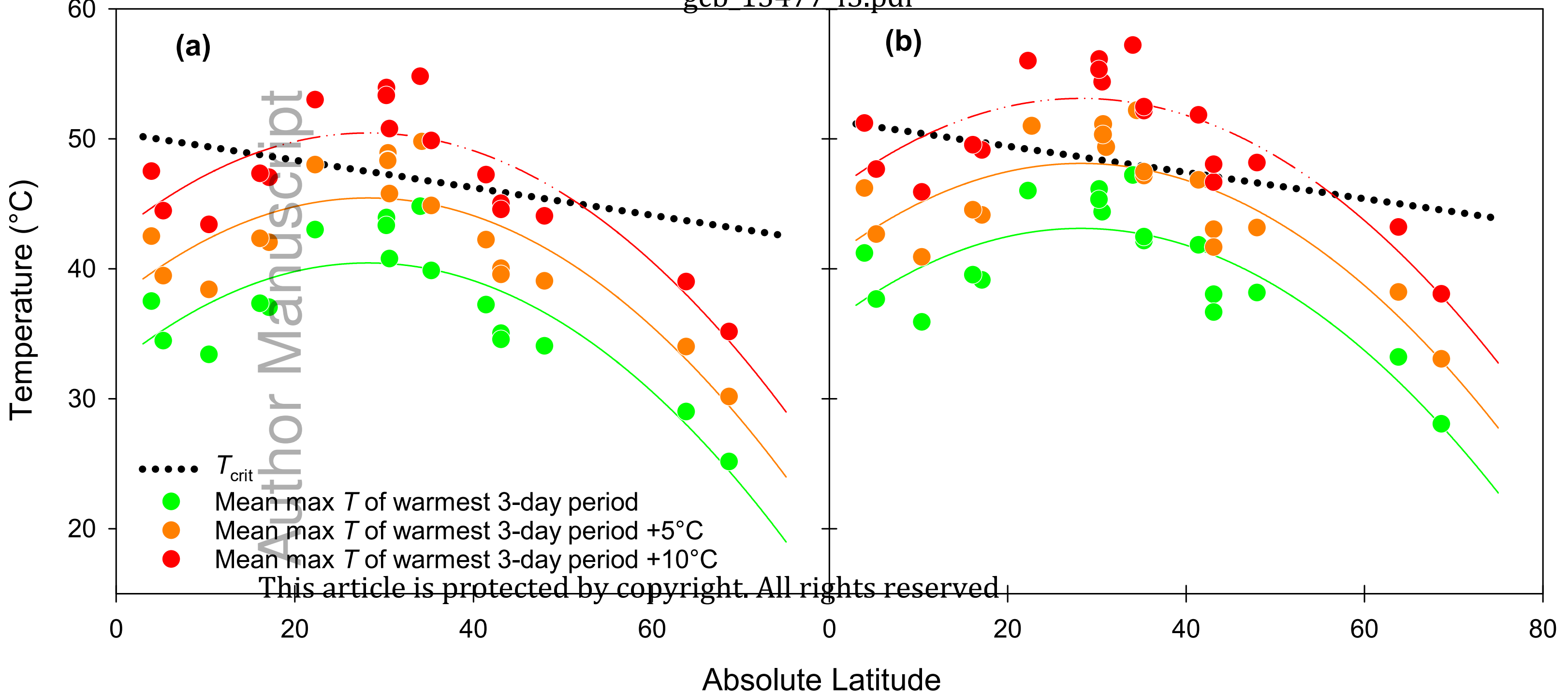
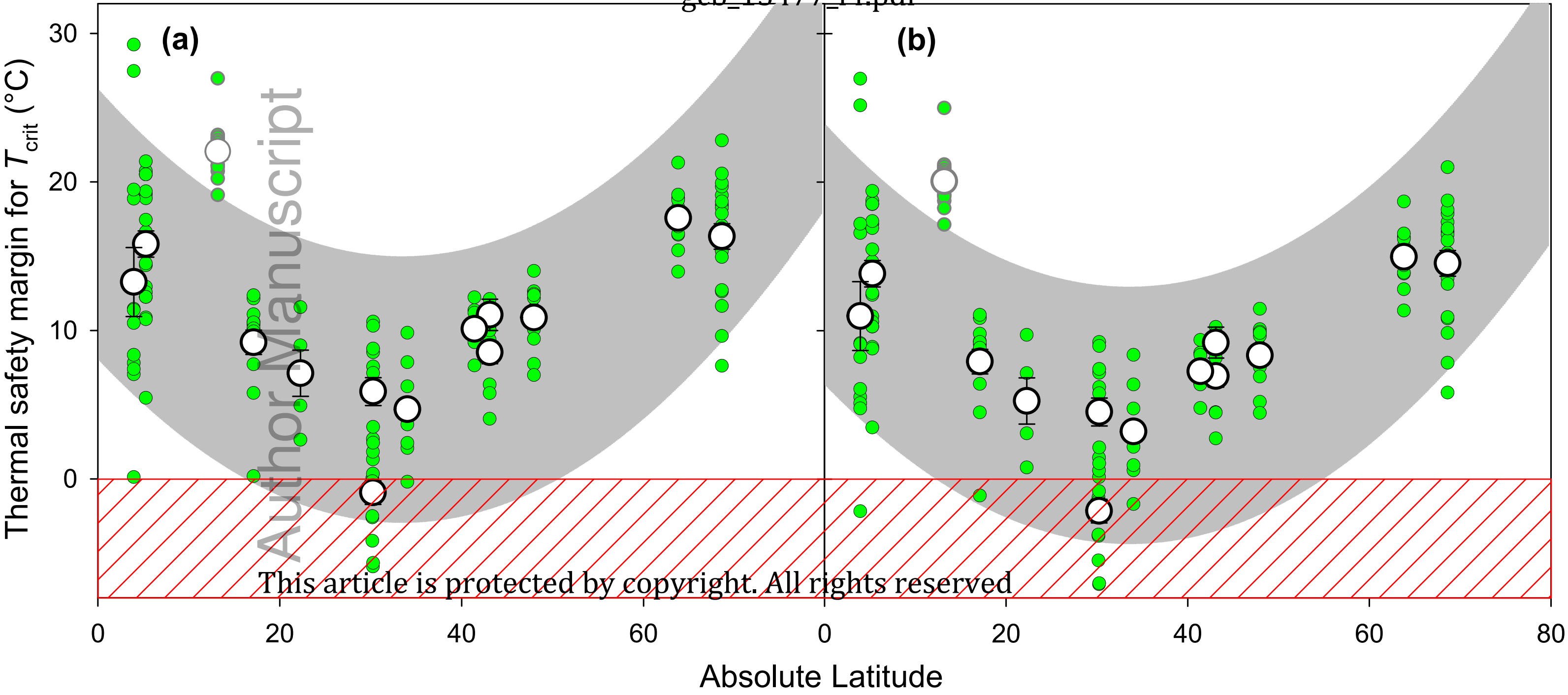

(a)
(b)
T_{max}
T_{crit}
High T tolerance (°C)
70
65
60
55
50
45
40
35
0
20
40
60
Absolute latitude
15
20
25
30
35
Mean maximum T of warmest month (°C)


(a)
(b)
Temperature (°C)
60
50
40
30
20
0
20
40
60
0
20
40
60
80
Absolute Latitude
T_{crit}
Mean max T of warmest 3-day period
Mean max T of warmest 3-day period +5°C
Mean max T of warmest 3-day period +10°C


(a)
(b)
Thermal safety margin for T_{crit} (°C)
30
20
10
0
0
20
40
60
0
20
40
60
80
Absolute Latitude