Repeated extreme heatwaves result in higher leaf thermal tolerances and greater safety margins

Running title: Adaptive capacity to heatwaves

Collin W Ahrens[1], Anthea Challis[1], Margaret Byrne[2], Andrea Leigh[3], Adrienne B. Nicotra[4], David Tissue[1], Paul Rymer[1]

[1] Hawkesbury Institute for the Environment, Western Sydney University, Richmond NSW, 2753, Australia; [2] Biodiversity and Conservation Science, Department of Biodiversity, Conservation and Attractions, Locked Bag 104, Bentley Delivery Centre, WA, 6983, Australia; [3] University of Technology Sydney, School of Life Sciences, PO Box 123, Broadway, NSW, 2007, Australia; [4] Research School of Biology, Australian National University, Canberra, 2601, Australia

Orcid identifiers:
Collin Ahrens: https://orcid.org/0000-0002-0614-9928
Anthea Challis: https://orcid.org/0000-0002-3814-9850
Margaret Byrne: https://orcid.org/0000-0002-7197-5409
Andrea Leigh: https://orcid.org/0000-0003-3568-2606
Adrienne Nicotra: https://orcid.org/0000-0001-6578-369X
David Tissue: https://orcid.org/0000-0002-8497-2047
Paul Rymer: https://orcid.org/0000-0003-0988-4351

Corresponding author:
Collin Ahrens
Email: collinwahrens@gmail.com

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Summary

- The frequency and severity of heatwave events are increasing, exposing species to conditions beyond their physiological limits. Species respond to heatwaves in different ways, however it remains unclear if plants have the adaptive capacity to successfully respond to hotter and more frequent heatwaves.

- We exposed eight tree populations from two climate regions grown under cool and warm temperatures to repeated heatwave events of moderate (40 ºC) and extreme (46 ºC) severity to assess adaptive capacity to heatwaves.

- Leaf damage and maximum quantum efficiency of PSII (F_v/F_m) were significantly impacted by heatwave severity and growth temperatures, respectively; populations from a warm-origin avoided damage under moderate heatwaves compared to those from a cool-origin, indicating a degree of local adaptation. We found that plasticity to heatwave severity and repeated heatwaves contributed to enhanced thermal tolerance and lower leaf temperatures, leading to greater thermal safety margins (thermal tolerance minus leaf temperature) in a second heatwave.

- Notably, while we show that adaptation and physiological plasticity are important factors affecting plant adaptive capacity to thermal stress, plasticity of thermal tolerances and thermal safety margins provides the opportunity for trees to persist among fluctuating heatwave exposures.

Introduction

Global temperatures have increased by an average of 1.0 ºC in the past century (IPCC, 2014) and are predicted to continue to increase due to greenhouse gas emissions. Changes in climate patterns result in increased frequency, duration, and severity of heatwaves (Cowan et al., 2014; Perkins-Kirkpatrick & Lewis, 2020). Heatwaves can be defined as the daily maximum temperature of more than five consecutive days exceeding the average maximum temperature by 5 °C (Frich et al., 2002). Recent global models from the fifth model inter-comparison project (CMIP5) predict that **heatwave severity** (bold terms are defined in glossary, Box 1) will increase by up to 4.8 °C under the RCP8.5 ('business as normal' CO_2 emission) Scenario (2081-2100 vs.1950-2005; Cowan et al., 2014). They also predict increases in heatwave frequency from, on average, 1.7 per decade (2006-2016) to 13.0 per decade (2090-2100). The extreme temperatures during heatwaves contribute to dry atmospheric conditions with high vapour pressure deficits (Teskey et al., 2015), and are well known to directly affect plant physiology. Short-term heatwaves have been shown to change the expression profile of heat shock proteins (Aspinwall et al., 2019), reduce photosynthetic rates (Duarte et al., 2016; Loik et al., 2017), impact photosystem II (PSII; Guha et al., 2018; Pšidová et al., 2018), and change carbon allocation (Werner et al., 2020). This can culminate in major ecosystem effects such as forest dieback events, loss of primary production, and alteration of important ecosystem functions (Allen et al., 2015; Ciais et al., 2005; van Gorsel et al., 2016; Reichstein et al., 2013; Teskey et al., 2015). Therefore, it is important to determine whether plant species have the **adaptive capacity** to respond to novel heatwave conditions under a rapidly changing climate.

There are different ways plants demonstrate adaptative differentiation of **thermal tolerance**, such as differences in structural tissues, proteins, and physiology (Aspinwall et al., 2019; Maher et al., 2019). While there are substantial gaps in knowledge in our understanding of plant thermal tolerance (Geange et al., 2020), common garden experiments controlling for environment have confirmed that traits providing tolerance to heatwaves are genetically determined across species (Aspinwall et al., 2019; Qu et al., 2020; Ruthrof et al., 2018). Among examples of interspecific genotypic differentiation is a global study measuring 218 species across seven biomes, which found that thermal tolerance (measured as the maximum temperature for PSII) was highly variable among co-occurring species (O'Sullivan et al., 2017). While interspecific trait variation is commonly measured, it is less clear whether similar intraspecific patterns of adaptations are present. For example, one study found that there were significant differences in thermal tolerances among **genotypes** of species *Fagus sylvatica* (Pšidová et al., 2018), while another study found no genotypic differences in thermal tolerance among populations of *Eucalyptus tereticornis* (Drake et al., 2017). If genotypic effects are present, they are potentially indicative of **local adaptation**, and thus may be critical for persistence under climate change (Hoffmann & Sgrò, 2011).

Plants can respond to varying temperatures by dynamically changing their physiology to heatwaves depending on 1) previous growth temperatures, 2) heatwave severity, and 3) frequency of heatwave exposure. While these three factors all elicit **physiological plasticity**, it is important to differentiate among them as they each operate at different temporal scales and are critical for long-lived trees to respond to their thermal environments. Growth temperatures have been known to affect a myriad of plant responses. For example, some studies found that trees growing under a warmed environment exhibited less stress when exposed to extended heatwaves compared to those from a cooled environment (Colombo & Timmer, 1992; Daas et al., 2008; Ghouil et al., 2003; Niinemets, 2010). In addition, dynamic responses to heatwaves reveal immediate effects of heatwave severity. This was shown in a study where white spruce trees showed an immediate and greater decline in maximum quantum yield of photosystem II (F_v/F_m) as temperatures increased (Bigras 2000). Lastly, prior heatwave events may alter plant thermal stress responses (Bruce et al., 2007; Walter et al., 2013). Here, we use the term **ecological stress memory** to denote this effect. Examples quantifying the effects of multiple heatwaves are generally lacking (Walter et al., 2013); however one study applied two heatwaves to two seagrass species and showed that plants performed better during the second heatwave (Nguyen et al., 2020).

Thermal safety margin (TSM) is the difference between leaf temperature (T_{LEAF}) and the temperature at which loss of function occurs (thermal tolerance). TSMs are a useful way to understand how leaf **thermoregulation** and thermal tolerances might change within and among genotypes with heatwave conditions. Indeed, transpirational cooling (thermoregulation) can contribute to larger TSMs, when soil water is available, to reduce T_{LEAF} and avoid leaf damage during heat stress (Ameye et al., 2012; Drake et al., 2018; Urban et al., 2017). Transpirational cooling is partly genetically determined through structural variation such as xylem vessel diameter and stomatal density (Doheny-Adams et al., 2012; Manzoni et al., 2013; Mencuccini et al., 2015). Thermal tolerance is also critical in estimating TSM, because a higher thermal tolerance will increase the TSM, decreasing leaf vulnerability to high temperatures. While TSMs have been estimated for many species, the degree of plasticity in TSM remains to be investigated.

Leaf tissue damage from high temperatures often occurs in conjunction with loss of physiological function (Karadar et al., 2018). The impact of extreme temperature events is frequently observed at the sites of photochemical reactions, including photosystem II (PSII; Chen et al., 2008; Lazár, 2006; Li et al., 2009; Tóth et al., 2005; Wise et al., 2004). PSII is a protein that uses light to excite electrons, which are transferred to PSI to create reducing agents (NADPH). Heat stress oxidises the PSII proteins, effectively stopping the electron excitation process, resulting in heat-induced damage to PSII and decreasing maximum efficiency of quantum yield (F_v/F_m); F_v is the maximum variable florescence and F_m is the maximum Chlα flouresence, and this ratio reveals the amount of electron excitation from PSII. In general, F_v/F_m provides a reasonable estimate of the impact on the photosynthetic pathway and carbon assimilation. F_v/F_m is often used as a proxy for the estimation of plant thermal stress (García-Plazaola et al., 2008; Sharma et al., 2015; Stirbet et al., 2018), with a commonly observed threshold of 50% decline of F_v/F_m (T_{50}) (Curtis et al., 2014). We expect that intraspecific variation in T_{50} thresholds would manifest as differences between genotypes from warm and cool climates. However, relatively few studies have investigated intraspecific variation in T_{50} in naturally occurring plants (Geange et al., 2020), highlighting knowledge gaps in understanding the adaptive capacity of T_{50} with heatwave dynamics.

We quantify the adaptive capacity of trees to heatwaves using a widespread Western Australian tree species as our model system. *Corymbia calophylla* ((Lindl.) K.D.Hill & L.A.S.Johnson; *Eucalyptus* sensu lato; family Myrtaceae) is known to have adaptive variation (Ahrens, Byrne, et al., 2019) and heritable traits (Ahrens et al., 2020; Ahrens, Mazanec, et al., 2019) associated with temperature. Here, we assess the main and interactive effects of adaptation (region), plasticity to growth temperature, heatwave severity, and repeated heatwaves to test the following: (1) patterns of regional adaptation hypothesis – populations from northern (warm) climate regions will have greater thermal tolerance and less heatwave damage compared to southern (cool) regions; (2) physiological plasticity to growth temperature hypothesis – warmer growth temperatures will increase tolerance to heatwaves; (3) physiological plasticity to heatwave severity hypothesis – more severe heatwaves will increase thermal tolerance compared to moderate heatwaves; and (4) physiological plasticity to repeated heatwave events hypothesis – a first heatwave event will increase plant tolerance to a second heatwave event (i.e. ecological stress memory). Overall, our objective was to assess the adaptive capacity of trees by quantifying the physiological limits to heatwaves, independent of water limitation.

Materials & Methods

Species, seed collection and seedling conditions

Corymbia calophylla is a southwestern Western Australia broad-leaf, evergreen tree, which spans orthogonal precipitation and temperature gradients. Our experimental design incorporates eight populations with contrasting climate origins (Fig. 1a, Supporting Information Table S1) and genetic variation associated with temperature (estimated with ~10 000 single nucleotide polymorphisms, Ahrens, Byrne, et al., 2019). Each population had seed collected from eight mother trees separated by >100 m to maximise independence of seed families. Seed was collected between 1991 and 1992, and two populations collected in 2013, then dried and stored in a cool room to maintain seed viability (~80%).

To test our hypothesis of genetic adaptation to heatwaves, we compared regions from contrasting climate origins. We grouped eight populations into two thermal regions (north ~ warm and south ~ cool; Fig. 1a). The northern populations experience higher maximum temperatures (range 30.4°C to 32.2°C), compared to the southern populations (range 25.6°C to 27.7°C; Supporting Information Table S1).

Glasshouse, growth, and treatment conditions

Seeds were germinated directly in tube stock at the Western Sydney University nursery on the Hawkesbury Campus, Richmond, NSW. At eight weeks, seedlings were transferred from tube stock directly to cylindrical experimental pots (40 cm deep and 15 cm diameter; 7 L). The pots contained a 2 cm base layer of crushed granite and approximately 9 kg of locally sourced sandy loam soil (approximately 80 % sand). While growing plants in pots is essential to control environmental variables, there are potential limitations to this approach (e.g., limited growth as the roots become pot bound). We limited the pot effect in two ways: 1) by applying the treatment during the logarithmic growth phase when plants were young and not root bound, and 2) using purpose-built, deep pots for tree species with tap roots (Ghannoum et al 2010). Pots from each population were randomly allocated along two raised benches within replicate bays with even spacing (~20cm apart) (Supporting Information Fig. S1).

To test our hypothesis of acclimation at different growth temperatures, plants were placed into one of two growth bays within a glasshouse with maximum daytime temperatures of 26 °C (*cool*) and 32 °C (*warm*). The *cool* growth bay had temperatures of 12 °C (10 pm - 4 am), 14 °C (4-6 am), 16 °C (6-8 am), 20 °C (8-10 am), 23 °C (10 am-12 pm), 26 °C (12-2 pm) and stepped down increments of 23 °C (2-4 pm), 20 °C (4-6 pm), 16 °C (6-8 pm), 14 °C (8-10 pm) (Supporting Information Fig. S2). The *warm* growth bay was 6 °C warmer at each time interval listed above. These temperatures simulated the mean temperatures of the respective regions of *C. calophylla's* distribution (Aspinwall et al. 2017; Supporting Information Notes S1).

To test the heatwave severity hypothesis, we administered two different heatwave treatments (Fig. 2 & S1): (1) *moderate* heatwave, reaching a day-time high of 40 °C and a night-time low of 22 °C and (2) *extreme* heatwave, reaching a day-time high of 46 °C and a night-time low of 26 °C, simulating temperatures in the northern and southern regions (supporting information; Fig. S3). Each heatwave treatment was applied for five consecutive days (based on Frich et al.,'s (2002) definition of a heatwave), and then plants were returned to their growth temperatures.

To test our hypothesis of ecological stress memory, a second, identical heatwave was applied after two weeks of **recovery** from the first heatwave. Following the second heatwave event, plants were returned to their growth temperatures for a two-week recovery period. The recovery period is deemed to be long enough for dynamic physiological and cellular processes to return to normal function, unless PSII was irreversibly damaged (Ruehr et al., 2019).

Replicates from each population were exposed to one of six treatments (combination of growth temperature and heatwave severity; Fig. 1b&c): (1) plants grown in *cool* bay (26 °C), no heatwave (control); (2) plants grown in *warm* bay (32 °C), no heatwave (control); (3) plants grown in the *cool* bay (26 °C), then exposed to the *moderate* heatwave (40 °C); (4) plants grown in the *cool* bay (26 °C), then exposed to the *extreme* heatwave (46 °C); (5) plants grown in the *warm* bay (32 °C), then exposed to the *moderate* heatwave (40 °C); and (6) plants grown in the *warm* bay (32 °C), then exposed to the *extreme* heatwave (46 °C). Following a 14-day recovery period in the growth temperatures (REC1), plants were exposed to the same six temperature treatments as above for the second heatwave event (HW2), followed by an additional 14-day recovery (REC2).

Measurements could not be completed on all 240 plants in a single day, so we created six randomised blocks that consisted of 1-2 replicates of each of the six temperature treatment combinations (40 plants each block; Supporting Information Notes S1).

Leaf temperature, chlorophyll fluorescence, and leaf damage

Leaf temperature was measured using fine-wire thermocouples inserted beneath the epidermis on the abaxial surface of one fully expanded, mid-canopy, sunlit leaf per sapling for the duration of the heatwave treatments (0.13 mm diameter, Model 5SRT; Omega Engineering, Norwalk, CT, USA). We inserted three thermocouples during HW1 in three populations subjected to the *extreme* heatwave (south region / *cool* bay [n = 2]; north/*cool* [n = 1]). During HW2, we inserted 23 thermocouples in both the *moderate* (south/*cool* [n = 4]; north/*cool* [n = 2]; south/*warm* [n = 2]; north/*warm* [n = 3]) and *extreme* heatwave (south/*cool* [n = 3]; north/*cool* [n = 3]; south/*warm* [n = 2]; north/*warm* [n = 2]). Leaf temperature (T_{LEAF}) data were logged every minute for the 5-day heatwave treatments. Data was extracted for the hottest glasshouse temperature set point of each day (12:15 – 13:45) and T_{LEAF} was estimated as the average leaf temperature logged during that same time period. We estimated leaf cooling capacity as the difference between daily air temperature and leaf temperature (T_{AIR}-T_{LEAF}), quantification of thermoregulation.

A visual assessment of leaf tissue damage was recorded to quantify physical leaf damage of plants from heatwaves. Leaf damage was visualised as burnt patches on the leaf surface associated with a change in colour from green to light brown with dark brown edges. Other forms of leaf damage were negligible, but could be differentiated and excluded from the leaf damage records. All plants were assessed for leaf damage on the day immediately after each heatwave event (Fig. 1d). Leaf damage from HW2 could be assessed separately from HW1 as the difference between new and old damage was obvious. Leaf damage was assessed through a damage score between 0-4: 0 was no damage; 1 was 1-25% of the leaves burnt; 2 was 26-50% of the leaves burnt; 3 was 51-75% of the leaves burnt; and 4 was 76-100% of the leaves burnt.

A portable chlorophyll fluorometer (PAM-2500 Heinz Walz GmbH, Effeltrich, Germany) was used to record F_v/F_m and dynamically measure the effect of heatwaves on all plants. All F_v/F_m measurements occurred after being dark-adapted for 60 minutes, with a saturation intensity of 1,800 µmol m^{-2} s^{-1} (indicative of a full sunlit day). We measured F_v/F_m at time points H0, H1, H3, H5, R1, R4, R8, R14 (H is for heatwave and R is for recovery; the number refers to the day of heatwave or recovery; see Fig. 1c&d for details). Measurements at day H0 (linear day 0) preceded the heatwave, and days H1 (1), H3 (3), and H5 (5) were measured during the heatwave, to assess the short-term response. We also took measurements during the first recovery period (R1 (linear day 6), R4 (9), R8 (13), and R14 (19)) to investigate if there were longer-term effects of heatwaves on PSII (Fig. 1c). These measurements were repeated for HW2 during days H0 (linear day 20), H1 (21), H3 (23), H5 (25), R1 (26), R4 (29), R8 (33), and R14 (39) (Supporting Information Fig. S4).

Thermal tolerance (T_{50})

We used an experimental protocol to estimate thermal tolerance (T_{50}), the temperature associated with 50% decline in PSII function. It broadly equates to the onset of irreversible damage to PSII and strongly correlates with field based T_{CRIT} (the temperature at which initial damage occurs) measurements from other studies (e.g., Knight & Ackerly, 2002; O'Sullivan et al., 2017; Zhu et al., 2018). The T_{50} protocol used here is described in detail in Curtis et al. (2014). We set up seven water baths: two controls were set to 24˚C, and the experimental water baths were set to 48 ˚C, 50 ˚C, 52 ˚C, 54 ˚C and 56 ˚C with lights (Supporting Information Notes S1). Leaf samples were collected at predawn and set up for experimental tests (Supporting Information Notes S1). Samples were dark adapted for 30 minutes, and initial F_v/F_m measurements were logged. Thereafter, the samples were placed into 24˚C bath for 15 minutes, then the temperature treatment bath for 15 minutes, then the 24˚C bath for 90 minutes, then dark adapted for 30 minutes, F_v/F_m was recorded, and then F_v/F_m was finally recorded after a dark adapted overnight period, to capture irreversible damage. T_{50} was calculated by plotting the overnight F_v/F_m against all treatment temperatures for each individual plant – three replicates across the five temperature points (48 - 56 ˚C). We then fitted a straight line through the treatment temperatures bracketing the 50% decline in F_v/F_m. We solved the linear equation for x ($x = \frac{y-b}{m}$) to find T_{50}, rounding to a tenth of a degree.

Finally, we calculated the physiological thermal "safety margin" as the difference between T_{50} and T_{LEAF}, as in Perez and Feeley (2020), providing a good prediction of leaf vulnerability. When T_{LEAF} is equal to T_{50}, the leaf temperature threshold has been reached. The thermal safety margin was estimated for plants from different climate regions subjected to different growth temperatures and heatwaves.

Statistical Analysis

All analyses were performed in R (R development team 2019). Plotting means and standard error were performed using *ggplot2* (Wickham 2016) and differences between treatments and genotypes were determined using linear mixed-effects models with the *lmer* function in the *lme4* package, and a linear model using the *lm* function. Full models explored how T_{LEAF}, leaf damage, F_v/F_m, T_{50}, and TSM were affected by regions, growth temperatures, heatwave severity, and heatwave events. Fixed factors for region (north, south), growth temperature (26 ºC, 32 ºC), heatwave treatment (40 ºC, 46 ºC), and heatwave event (HW1, HW2) along with two-factor interactions were included in the full model (Fig. 1; Supporting Information Table S1). Population and sampling block were considered random factors for all mixed-effects models. In the full model, F_v/F_m was included for H5 in both heatwave events. For F_v/F_m measurements, we created daily models (statistical models that used factors for regions, growth temperatures, and heatwave severity for each day separately) to identify significant differences on specific days. Analysis of variance was performed using Satterthwaite's method. Post-hoc tests were performed on mixed effects models using the *glht* function in the *multcomp* library (Hothorn et al., 2016), applying the Tukey method, correcting for multiple comparisons. To identify significant genotype-environment interactions (GEI) within contrasts, we calculated linear models and compared those linear models using an *anova* function in R. We explicitly quantified and tested differences in mean plasticity among regions by calculating the relative distance plasticity index (RDPI; Valladares et al., 2006) in R using the *rdpi* function in the Plasticity package (Ameztegui, 2021). Given as

$$\text{RDPI} = \sum^{\frac{d_{ij}\rightarrow i'j'}{x_{i'j'}+x_{ij}}} \Big/ n$$

Where n is sample size, j is the individual, i is the temperature, $d_{ij}\rightarrow i'j'$ is the distance between trait values for all pairs of individuals where i is different than i', and $x_{i'j'} + x_{ij}$ is the sum. The numerator is the relative difference for all pairs of individuals given temperature. This index ranges from 0 to 1 and allows for statistical comparisons between regions in trait plasticity. For plasticity calculations, region was the independent variable and heatwave severity was the environmental factor, and this was calculated separately for each growth temperature nested in heatwave event (four total comparisons within each dependent variable). To quantify the variance associated with the different factors for each response variable, we divided a factor's variance by the total trait variance (i.e., sum of all variance components). Finally, we calculated the intersection between the linear models for T_{50} and T_{LEAF} as a prediction of 0 TSM, a point at which thermoregulation no longer occurs. To calculate the intersection between the two linear models, we used the *solve* function in R. We interpret the intersection cautiously because we do not know if the relationship remains linear across greater temperatures, but we still find this measurement informative. All data and code provided in Supporting Information (Data S1-S5 & Notes S2).

Results

Thermoregulation differed substantially depending on heatwave severity, but T_{LEAF} was always lower than T_{AIR}, indicating that plants were cooling their leaf surface through transpiration. Calculated vapor pressure deficit (VPD) also changed depending on growth temperature and heatwave severity (Supporting Information Notes S1). T_{LEAF} was *ca.* 29.0 °C in the *moderate* heatwave conditions, while in the *extreme* heatwave T_{LEAF} was *ca.* 42.4 °C in HW1 and 41.0 °C in HW2. The leaf thermoregulation (T_{AIR} - T_{LEAF}) was significantly greater in *moderate* compared to *extreme* heatwave treatments ($F_{1,69} = 781.5$, $p < 0.001$; 10.9°C and 5.6°C, respectively) (Table S2). There was also a significant difference between the two heatwave events in the southern populations exposed to the *extreme* heatwave treatment, where the HW2 elicited lower T_{LEAF} than HW1 ($F_{1,46} = 2.34$, $p = 0.02$).

There was a strong effect for heatwave severity on leaf damage (Table 1) with significantly greater leaf damage under the *extreme* compared to the *moderate* treatment (Fig. 2; Table 1); no leaf damage was recorded in control plants. Leaf damage had a significant GEI between climate region and heatwave severity (Table 1), an effect that was more pronounced in HW1 (Fig. 2a; $F = 5.10$; p

= 0.006). If we consider heatwave events separately, the northern region had lower levels of damage following the *moderate* heatwave compared to the *extreme* heatwave (z-value = -3.64, p = 0.01), but the southern region plants exhibited similar responses to the *moderate* and *extreme* heatwaves (z-value = 0.08, p = 1.00). In HW2, there was a significant difference between the climate regions, with greater leaf damage on the southern origin plants compared to plants of northern origin, regardless of heatwave treatment (Supporting Information Fig. 2b; F = 7.89, p = 0.03); southern regions had high levels of leaf damage following all heatwaves suggesting that 40 ºC has a similar effect as 46 ºC temperatures. A significant environment-environment interaction (EEI; an interaction effect between environmental factors [growth temperature, heatwave severity, heatwave event] without region as a factor) revealed lower leaf damage under extreme treatment in HW2 but not in HW1 (Treat x HWE p < 0.01; Table 1). Further, significant differences among regions in plastic response were expressed as variation in leaf damage (p < 0.01; Table 2), and the RDPI score showed leaf damage as having the highest plastic response compared to the other metrics.

The maximum quantum efficiency of PSII (F_v/F_m) significantly declined for all treatments from the baseline (H0) to the first day (H1) of HW1 ($F_{1,470}$ = 13.51; p = 0.0003). F_v/F_m increased on subsequent days during HW1. Growth temperature had the strongest significant effects for the duration of the experiment (Table 1; Fig. 3). There were also significant GEI effects between region and heatwave severity during both recovery periods (Fig. 3), and between region and growth temperature during HW2 and REC2. Direct F_v/F_m treatment measures were highly variable, yet it was possible to discern the importance of growth temperature throughout the study and the significance one interaction effect, region and heatwave severity, during both recovery periods. Lastly, during REC2, we reveal all main effects are significant on at least one day, and an EEI effect between growth temperature and heatwave severity and a GEI effect between region and growth temperature, indicating the importance of adaptive and plastic effects for recovery to thermal stress (Fig. 3).

Heatwave severity had a significant effect on thermal tolerance to heat stress (T_{50}), increasing with heatwave severity (Table 1; Fig. 4). Control plants (no heatwave) always had the lowest thermal tolerance (47.8 ºC ± 0.10), followed by plants subjected to *moderate* heatwaves (49.3 ºC ± 0.09), and plants subjected to *extreme* heatwaves (50.4 ºC ± 0.10) for HW1. There was a significant EEI interaction between heatwave severity and heatwave event (Table 1). While the control and *moderate* heatwave treatments had similar thermal tolerance in HW1 and HW2, trees in the *extreme* heatwave treatment significantly increased thermal tolerance in HW2 (50.9 ºC ± 0.12) compared to HW1 (50.4 ºC ± 0.10; $F = 10.1$; $p = 0.002$), indicative of ecological stress memory. Likewise, there were significant differences between regions for physiological plasticity in HW2, but not HW1 (Table 2).

There were signals of genetic adaptation detected within HW1, where different genotypes exhibited significantly different T_{50} responses to the *moderate* heatwave treatment ($F = 3.50$; $p = 0.004$; Fig. 4a, denoted with *). We also uncovered two significant GEI effects on thermal tolerance during HW1, where the southern region plants were more responsive to temperature changes than plants from the north. The first GEI was within the *moderate* heatwave among regions and growth temperatures, where the southern region had decreasing thermal tolerance as growth temperatures increased, while no change was detected between growth temperatures for the northern region (indicative of differential acclimation) ($F = 3.50$; $p = 0.015$; Fig. 4a, denoted with **). The second GEI was within the *cool* growth temperature in HW1, where southern regions had a greater change in thermal tolerance between the *moderate* heatwave (40ºC) and control (no heatwave) plants, compared to those from the northern regions ($F = 23.86$; $p = 0.025$; Fig. 4a, denoted with †). For HW2, T_{50} likewise differed significantly in response to different heatwave treatments; however, no G or GEI effects were detected (Fig. 4b).

We found that the TSM (T_{50} - T_{LEAF}) changed based on heatwave treatment and heatwave event (Fig. 5; Table 1). There were no significant differences among climate regions or growth temperatures, but there were strong differences for heatwave severity (Table 1). However, plastic TSM responses were high compared to other metrics but regional differences were dependent on growth temperature (Table 2). Nevertheless, under *moderate* heatwave conditions, plants had a substantially higher safety margin (T_{50} - T_{LEAF} = 10.88ºC) compared to the *extreme* heatwave (T_{50} - T_{LEAF} = 5.64ºC; Fig. 5; Table 1). There was also a significant difference in the safety margin between the two heatwave events in the *extreme* treatment (3.92 ºC HW1 versus 5.64 ºC HW2; $F_{1,46} = 2.34$, $p = 0.02$), with the T_{50} significantly increasing (0.45 ºC; $F_{1,50} = 28.62$; $p < 0.001$) and T_{LEAF} significantly decreasing from HW1 to HW2 (1.83 ºC). We estimated that the T_{50} and T_{LEAF}

linear models would intersect at 53 ºC, providing a crude estimate for the reduction of TSM to zero.

When partitioning the variance across the full models for each trait, we found that heatwave severity was the main determinant for all traits (i.e., T_{LEAF}, leaf damage, T_{50} and TSM), except for F_v/F_m (Fig. 6). The large proportion of error rate found within leaf damage and F_v/F_m measurements are indicative of highly variable traits. Aside from the large proportion of unknown variation in the model (error), F_v/F_m showed growth temperature as the major driver (17.5%). The proportion of variance for T_{50}, T_{LEAF}, and TSM all had greater than 50% of the variation driven by plasticity to heatwave severity. The greatest signal of genetic (regional) adaptation was found in leaf damage (4.4%). The greatest proportion of variance attributed to ecological stress memory was found in TSM and leaf damage (17.6% and 2.2%, respectively). GEI effects contributed the most to visual scores of leaf damage (GEI: 6.39%), and those without region as a factor were greatest for T_{50} (EEI: 2.62%).

Discussion

In this study, plants showed high levels of physiological plasticity in response to variable heatwave severity through enhanced capacity for thermal tolerance. Under extreme heatwaves, leaf thermoregulation was lower (i.e., small T_{AIR} - T_{LEAF}), thereby reducing thermal safety margins. The effect of ecological stress memory was low overall, but did enhance thermal thresholds, leading to greater safety margins and reduced leaf damage in those trees that originated from hotter climates. While we found genotypic signals in the T_{50} response in the *moderate* heatwave, all genotypes were capable of adjusting to climate shifts in the near term.

Patterns of regional adaptation (hypothesis 1)

Adaptation, measured here as genotypic differences among regions in controlled environmental conditions, can be the result of selection pressure from contrasting climates. While intraspecific variation is essential for adaptation to climate change (Ahrens et al., 2021), few studies have investigated variation in physiological thermal tolerance within naturally occurring plant species. Of these few studies, findings show significant differences in thermal tolerance among populations of *Chenopodium album* (Barua et al., 2008), local adaptation among *Wahlenbergia ceracea* populations from different elevations (Nicotra et al., 2015), and local adaptation among eucalypt populations across extreme temperature treatments (Hancock & Hughes, 2014). In this study, we found plants sourced from warm (north) climate origin had significantly less leaf damage induced by the heatwaves. We also found significant differential responses among genotypes from the two regions to heatwaves, suggesting that thermal tolerance is under selection from contrasting climate origins. However, while we found some evidence of regional differentiation in thermal tolerance during our first heatwave, this was only in the *moderate* heatwave treatment. Local adaptation advantages were lost when the heatwave was more severe or more frequent. Nonetheless, the signatures of adaptation might be important for first exposure to heatwave events, effectively increasing likelihood of survival in seedlings. In addition, small amounts of variation in photoinhibition and leaf damage could be explained by genotypic differences, but these were only observed during the second heatwave. There were also multiple significantly different plastic responses among regions, where different regional genotypes respond differently to the same environmental stress. These plastic differences could provide adaptive advantages across regions as the climate continues to shift. Overall, finding few genotypic (regional) effects in this study was somewhat surprising, because previous studies on *C. calophylla* have documented adaptive leaf-level patterns across northern/southern regions for photosynthesis and maximum carboxylation rate (Aspinwall et al. 2017). Hence, temperature effects on PSII appear to be more plastic than anticipated, due to the capacity to thermoregulate leaf temperatures and/or limited capacity to shift thermal tolerance.

Physiological plasticity to growth temperature (hypothesis 2)

Trees optimise growth under different temperature regimes through acclimation of physiological processes. We expected F_v/F_m to be down-regulated during exposure to severe thermal stress, as has been shown in other experiments (e.g., Marias et al., 2016). Counterintuitively, we demonstrate a small but significant upregulation of F_v/F_m during heatwaves and downregulation during recovery periods in plants grown in *cool* bays. Notably, our findings suggest that the F_v/F_m response is dependent on sustained growth temperatures (Fig. 3 & 6). In similar ways, acclimation to five different growth temperatures significantly affected an oak (*Quercus canariensis*) response to thermal stress events (Daas et al., 2008). In eucalypt species, trees grown in warmer temperatures were more susceptible to heatwave damage (including to PSII) than trees grown in cooler temperatures (Aspinwall et al. 2019). The intraspecific differences in plastic responses of

F_v/F_m that we observed among regions likely occur in other species but are often not investigated. Although there was some variation in the immediate response to heatwaves in the *cool* and *warm* growth temperatures, all plants recovered to pre-experiment levels, as found elsewhere (French et al., 2019). Further, F_v/F_m was never below operating levels (0.7-0.84; Ritchie 2005), suggesting that even *extreme* heatwaves did not generate long-term negative effects on PSII. Ultimately, acclimation to growth temperature was likely less important for heatwave tolerance than the plastic responses to heatwave severity.

Physiological plasticity to heatwave severity (hypothesis 3)

Response to heatwave severity was significantly plastic, particularly for leaf damage and TSM (Table 2). Evenso, we found small, but significant differences in F_v/F_m due to heatwave severity (main effect; Fig.3), but only in the second recovery period. A similar situation was observed in a study of populations of a European oak species (*Q. ilex*) that was able to tolerate heatwave conditions through physiological plasticity (Gimeno et al., 2009). In contrast to the subtle changes in F_v/F_m, thermal tolerance (T_{50}) significantly increased with heatwave severity. Plasticity in thermal tolerance has been found in many organisms, including *Drosophila* (MacLean et al., 2019), fish (Comte & Olden, 2017), and ectotherms (Gunderson & Stillman, 2015), and is a critical mechanism for survival in local environments. In plants, photosynthetic thermal tolerance is shown to be plastic (Feeley et al., 2020; Lancaster & Humphreys, 2020), and has been illustrated in a study of contrasting populations of four congeneric species, where photosynthetic thermal tolerance was highly plastic for most populations grown in a common environment compared to *in situ* measurements with little indication of adaptation (Knight & Ackerly, 2003). In many ways, the consistent plastic response of thermal tolerance is indicative of requirements for plants to tolerate fluctuations of temperature in the natural environment, particularly heatwaves. This idea is supported by a multi-generational study on a beetle (*Callosobruchus maculatus*), where genetic variation decreased after exposure to fluctuating temperatures and suggests that plasticity is subject to selection when environmental variability increases (Hallsson & Bjorklund, 2012). Indeed, theory and quantitative models indicate that plasticity increases in proportion with environmental fluctuations (Lande, 2009), while more recent evidence also suggests that species from more variable environments have higher levels of plasticity (Chevin & Hoffmann, 2017).

Thermal safety margins shifted between the two heatwave treatments, including a large change in T_{LEAF} between the *moderate* and *extreme* heatwaves. Plastic TSM response is a phenomenon that has gained little attention, but there is some evidence that TSM acclimates to growth temperatures in a study that includes 62 species across five biomes (Zhu et al., 2018). In the current study, the TSM was determined by the difference between T_{50} and T_{LEAF}, and while both were plastic, T_{LEAF} was most affected by heatwave severity, shifting by 11.9 ºC compared to only 1.7 ºC for T_{50}, indicating that leaf-level thermoregulation decreases as the temperature increases above 40ºC. Under moderate heatwave conditions, woody species with access to water may be able to cool their leaves close to optimal operating temperatures (Crous et al., 2018; Cook et al., 2021). While eucalypt trees under field conditions have been found to tolerate heatwaves with sustained transpirational cooling (Drake et al., 2018), T_{LEAF} approximated T_{AIR} over longer periods ($T_{LEAF} \approx T_{AIR}$; Drake et al., 2020). In water-limited field conditions, T_{LEAF} may not thermoregulate to the same degree found here, suggesting that trees may be more vulnerable to heatwaves (Cook et al., 2021). Under extreme heatwave temperatures, the demand for cooling may also be limited by the structural tissues (e.g., xylem vessels, stomata) and hydraulic processes limiting maximum transpiration rates (Peters et al., 2010; Bush et al., 2008). Hence, leaf cooling capacity may diminish when temperatures are greater than applied here, resulting in leaf temperatures approaching critical thermal limits (heatwave severity; Fig. 5). While we found that physiological plasticity enhanced thermal tolerance (T_{50}) with increasing heatwave severity (control 47 ºC; moderate 49 ºC; extreme 51 ºC), it is likely that increased thermal tolerance will be biologically limited. Indeed, we estimate that the safety margin may disappear at 53 ºC ($T_{LEAF} \approx T_{50}$), because the plasticity in both T_{50} and T_{LEAF} will be limited at higher temperatures. While this currently exceeds recorded maximum temperatures (48.8 ºC within the natural distribution of *C. calophylla*; 50.5 ºC within Western Australia), given the rapid rate of climate change, this extreme heatwave scenario may become a reality sooner than anticipated.

Physiological plasticity to repeated heatwave events (hypothesis 4)

Repeated heatwaves elicited responses for several key traits. Ecological stress memory was identified as important for leaf damage, where genotypes from the northern region showed reduced leaf damage in response to HW2 in the *extreme* heatwave condition, while the southern region had similar responses to both heatwave events. Leaf damage has been shown to have major ecosystem impacts, such as in recent European heatwave events, where multiple evergreen species exhibited significant crown dieback (Pollastrini et al., 2019), and could also represent significant selection pressures on living individuals. Ecological stress memory was not observed between heatwave events for F_v/F_m measurements, indicative of the high variability for F_v/F_m. However, there were differences between the two recovery periods (Fig. 3). Ecological stress memory has been found in other species, particularly under drought stress. For example, one study found that recurrent mild drought events increased drought resistance in plant communities (Backhaus et al., 2014). Another found that ecological stress memory increased drought resistance over a decadal scale (Itter et al., 2019). Although rarely quantified in the literature (but see Bruce et al., 2007; Walter et al., 2013), we postulate that ecological stress memory to heatwaves may be common among populations and species. This has potentially significant implications for the long-term persistence of natural populations, biodiversity, and ecosystem function.

Ecological stress memory effects also enhanced TSM under extreme heatwaves. We found a relationship with leaf temperature decreasing and thermal tolerance (T_{50}) increasing on second exposure to extreme temperatures, leading to a slight but significantly larger TSM. This effect was likely not affected by a change in total leaf area, because leaf drop was negligible. We were unable to identify any studies that attributed ecological stress memory to driving thermal safety margins. Ours therefore is a significant finding, particularly because TSM estimates based on thermal niches suggest that 47% of the Australian vegetation is at risk of decline from increases in mean annual temperature by 2070 (Gallagher et al., 2019). These estimates rely on several assumptions (no plasticity, no adaptation, and no physiological tolerance) that our findings indicate may not be justified. Instead, we show that TSMs are dynamic over multiple heatwave events, and models incorporating antecedent stress events can explain an extra 20% of the variation in heatwave processes (similar to the 17% of variation explained by heatwave event for TSM in our study) compared to models without these events (Ogle et al., 2014). The mechanistic drivers of these changes are enticing directions for further research.

Conclusions

The response of trees to sequential *moderate* and *severe* heatwave events may be enhanced through a combination of genetic adaptation, physiological plasticity, and the combination of the two. These mechanisms independently provide different relative contributions to explain the variability of each leaf trait and, combined, have important implications for the persistence and function of future forests. Together, these factors indicate that adaptive capacity to tolerate heatwaves is mostly governed by plasticity and the ability to withstand extreme thermal events. However, if future climates do exceed the limits of this adaptive capacity, assisted gene migration strategies should be deployed (Aitken & Bemmels, 2016). Even so, we found that as exposure to heatwaves increases through severity and frequency, thermal sensitivity also increases (measured as smaller thermal safety margins), indicating an increasing vulnerability of forest trees to heatwaves as the climate warms. While severe heatwaves in the current climate may not be enough, by themselves, to limit the capacity of plants to persist, future vulnerability may constrain capacity to respond to the rapid rate of global warming with novel growth temperatures, extreme thermal conditions, and more frequent heatwaves.

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Author contribution

CWA, PR, AC, and DT designed the experiment. CWA, PR, and AC collected data. CWA analysed the data and wrote the first draft. CWA, AC, MB, ABN, AL, DT, and PR contributed to interpretations and revisions of the manuscript.

Data availability

Data and R code available as supporting information [.zip].

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Supporting Information

Additional supporting information may be found in the online version of this article.

Notes S1 Additional details on growing conditions, T_{50} methods, and vapor pressure deficit (VPD).
Notes S2 R code to perform all analyses
Table S1 Population information for each seed collection.
Table S2 Leaf temperature and air temperature measurements summary.
Table S3 Statistical differences between F_v/F_m measurements.
Fig. S1 Photograph of the glasshouse with plants.
Fig. S2 Daily temperature changes for the two growth bays and two heatwave treatments.
Fig. S3 Daily maximum temperature for two regions.
Fig. S4 Experimental treatment and study design in a linear layout.
Data S1 Leaf damage
Data S2 Fv/Fm

Data S3 T50

Data S4 Thermal Safety Margin

Data S5 Proportion of variance

Box 1. Glossary

Adaptive capacity	Capacity of a species to cope with or adjust to changing climatic conditions through a combination of plastic and genetic attributes.
Ecological stress memory	The capacity of past states abiotic or biotic factors to influence present or future responses of a species.
Genotype	When use genotype to reference the genetic similarities between individuals. In this study, we use genotype to refer to region.
Heatwave severity	Heatwave severity refers to the maximum temperature of a heatwave. Here, we use this term to refer to the two heatwave treatments applied to the plants. Moderate = 40 ºC and extreme = 46 ºC.
Local adaptation	Local individuals outperform non-local individuals. In this study, better tolerance of heat in the northern region populations can be interpreted as indicative of potential local adaptation.
Phenotype	The appearance or characteristics of an organism resulting from both genetic and environmental influences.
Physiological plasticity	The range of phenotypes expressed by a single genotype as a function of its environment.
Recovery	The period following a stress event, when an organism returns to normal performance. In this study, recovery is the 14 day period following five day heat events.
Thermal safety margin (TSM)	The difference between thermal tolerance (T_{50}) and T_{LEAF}, we interpret this as being the plant leaf temperatures optimal for photosynthesis.
Thermal tolerance	The temperature at which we measure a 50% decline in

	photosystem II function (T_{50}).
Thermoregulation	The difference between T_{AIR} and T_{LEAF}, reflecting the capacity of a leaf to maintain temperatures for normal leaf-level functions.

Table 1 Analysis of variance of genotype (Region), growth temperature (GT), heatwave event (HWE), and heatwave severity (Treat) effects on *Corymbia calophylla*'s leaf-level physiological traits: LD = leaf damage; F_v/F_m = maximum efficiency of quantum yield; T_{50} = thermal tolerance; TSM = thermal safety margin.

	LD	F_v/F_m	T_{50}	TSM
Region	0.32	0.51	0.07	0.43
GT	2.38	**66.11*****	0.68	-0.97
Treat	**19.42*****	0.00	**17.48*****	**19.00*****
HWE	**15.03*****	0.03	1.91	**2.52***
Region x GT	1.42	0.52	0.99	–
Region x Treat	**7.22****	0.06	1.18	–
Region x HWE	0.64	0.02	0.07	–
GT x Treat	0.69	0.32	**3.29***	–
GT x HWE	2.85.	0.00	0.97	–
Treat x HWE	**5.07****	1.18	**5.69****	–

Only two-way interactions were kept in the full model to minimise uninterpretable interactions. All models are mixed effects linear models with block and populations as random effects. The F_v/F_m model used the final values in each heatwave (H5; day five) to represent the independent variable heatwave event. TSM measures did not have enough replication for interaction effects, therefore we just provide the main effects. *F*-values are provided with level of significance, denoted with bold and "***", "**", and "*" at $p < 0.001$, $p < 0.01$, and $p \leq 0.05$, respectively.

Table 2 Relative distance plasticity index (RDPI) differences between regions (South and North) of *Corymbia calophylla* based on environmental distance of heatwave severity (40 ºC and 46 ºC).

	Heatwave1						Heatwave2					
GT	26 ºC			32 ºC			26 ºC			32 ºC		
	South	North	t-value	South	North	t-value	South	North	t-value	South	North	t-value
LD	0.532	0.618	2.78**	0.542	0.742	6.44***	0.471	0.555	2.60**	0.362	0.508	4.98***
F_v/F_m	0.010	0.010	-0.23	0.018	0.017	-0.27	0.010	0.013	4.56***	0.013	0.016	2.50*
T_{50}	0.012	0.015	3.76***	0.012	0.010	-2.82**	0.024	0.021	-2.94**	0.020	0.018	-2.67**
TSM	–	–	–	–	–	–	0.242	0.284	3.89***	0.307	0.298	-0.69

Means within region are provided, all standard errors are below 0.001. GT = growth temperature; LD = leaf damage; F_v/F_m = maximum quantum efficiency of photosystem II; T_{50} = thermal tolerance; TSM = thermal safety margin (T_{50} - T_{LEAF}); $p < 0.001$ ***, 0.01 **, 0.05*.

Fig. 1 Representation of the experimental design testing adaptive capacity of *Corymbia calophylla* to heatwaves with six experimental treatments (lines – dashed lines indicate exposure to moderate heatwave temperatures, solid lines indicate exposure to extreme heatwave temperatures, and dotted lines indicate control plants) with regions (symbols) grown under cool/warm temperatures (symbol colour – blue/red) exposed to control (C)/*moderate* (M)/*extreme* (E) heatwaves (symbol fill type – light/striped/dark). (a) Northern/southern populations represented by squares (north) and circles (south) to test hypothesis about adaptation with maximum temperature of the warmest month in the background (T_{MAX}; ºC); (b) cool/warm growth temperatures to test the hypothesis about acclimation; (c) the first heatwave treatment (HW1) to test the hypothesis about **physiological plasticity**; a second heatwave treatment (HW2) is represented by the large loops from recovery to heatwave to test the hypothesis about ecological stress memory; and (d) a 14 day recovery period for both heatwave events (REC1 and REC2). H = heatwave; R = recovery; and numbers refer to the day of period.

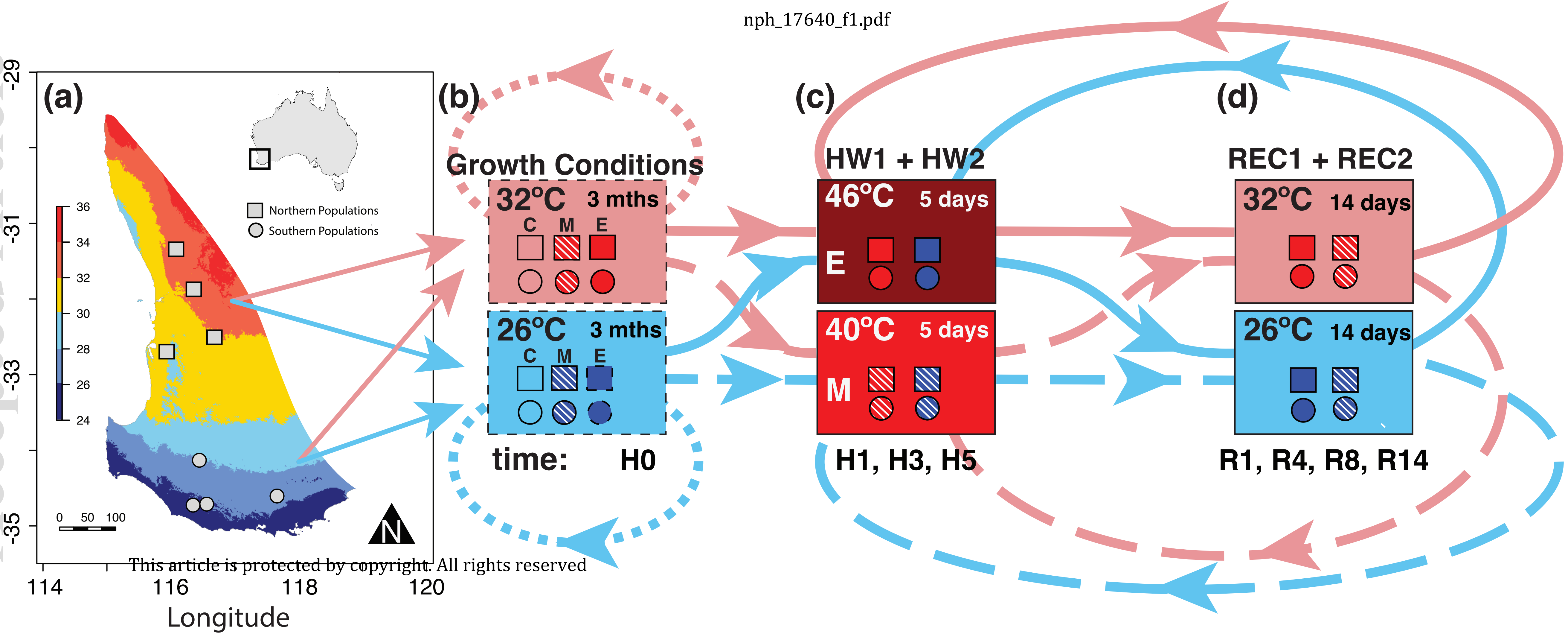
Fig. 2 Mean leaf damage (percentage of leaves with visible patches of damage) between climate regions and heatwave treatments for *Corymbia calophylla* in response to two consecutive heatwave events: (a) HW1 and (b) HW2. E represents a significant main effect due to heatwave treatment (difference between *moderate* and *extreme*); G represents a significant main effect among regions (difference between north and south). GEI represents a genotype-environment interaction between region and heatwave treatment in HW1. Error bars are standard error of the mean. * = $p < 0.05$; ** = $p < 0.01$.

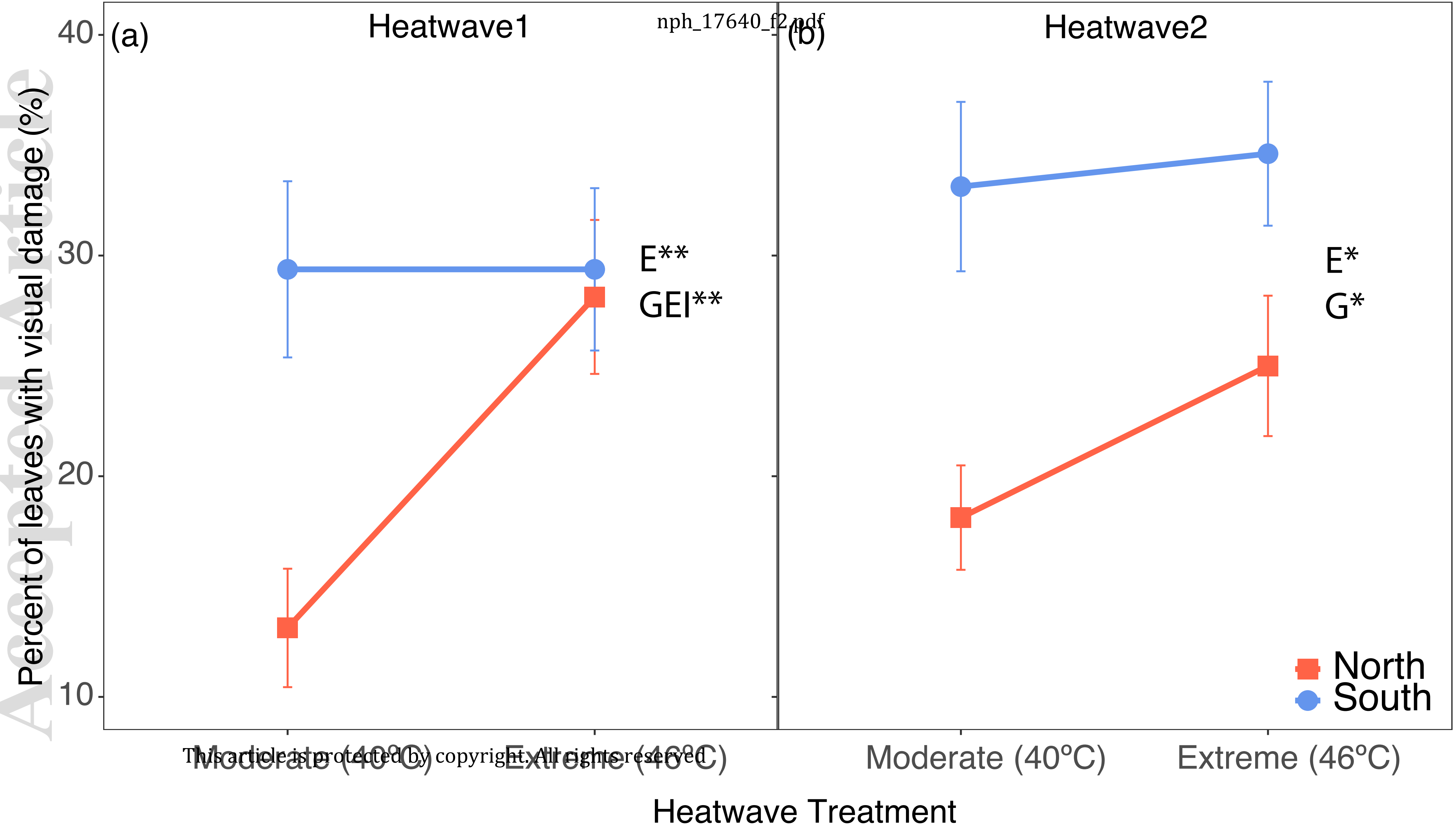
Fig. 3 Maximum quantum efficiency of photosystem II (F_v/F_m) for *Corymbia calophylla* plants from the two regions in the different growth and heatwave temperatures during the study period. Y-axis spans F_v/F_m 0.71-0.81. Shaded areas represent the five-day heatwave periods. Statistical differences between F_v/F_m among genotypes (Region), growth temperature (GT), and heatwave severity (Treat) are denoted with "***", $p < 0.001$, "**", $p < 0.01$ and "*" $p \leq 0.05$ (corresponding degree of freedom, *F*-values, and *p*-values are provided in Table S3). H = heatwave; R = recovery; and numbers refer to the day of period. HW temperature = heatwave temperature.

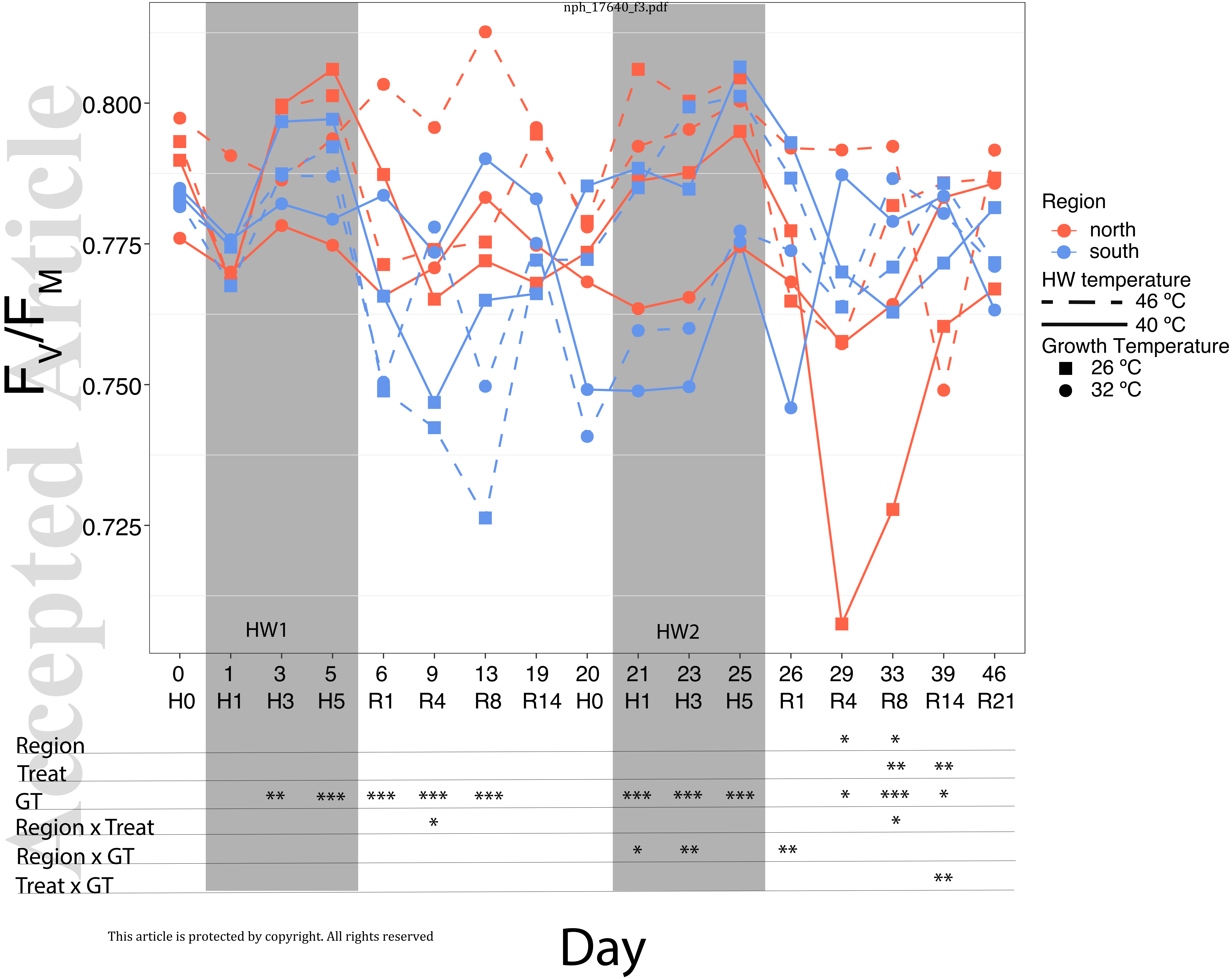
Fig. 4 Thermal tolerance (T_{50}) of *Corymbia calophylla* plants from northern and southern regions growing under cool and warm temperatures following exposure to moderate and extreme heatwave conditions with multiple events (a) heatwave 1 and (b) heatwave 2. Error bars are standard error of the mean. The letters indicate significant differences among heatwave treatments. The results of linear models within each heatwave treatment for genotype (G), environment (E) and genotype-environment interaction (GEI) effects shown with significance within the *moderate* heatwave treatment (**<0.01; *<0.05). † symbol denotes a significant interaction between region (north vs south) and heatwave treatment (*moderate* vs *control*) within the *cool* growth temperature.

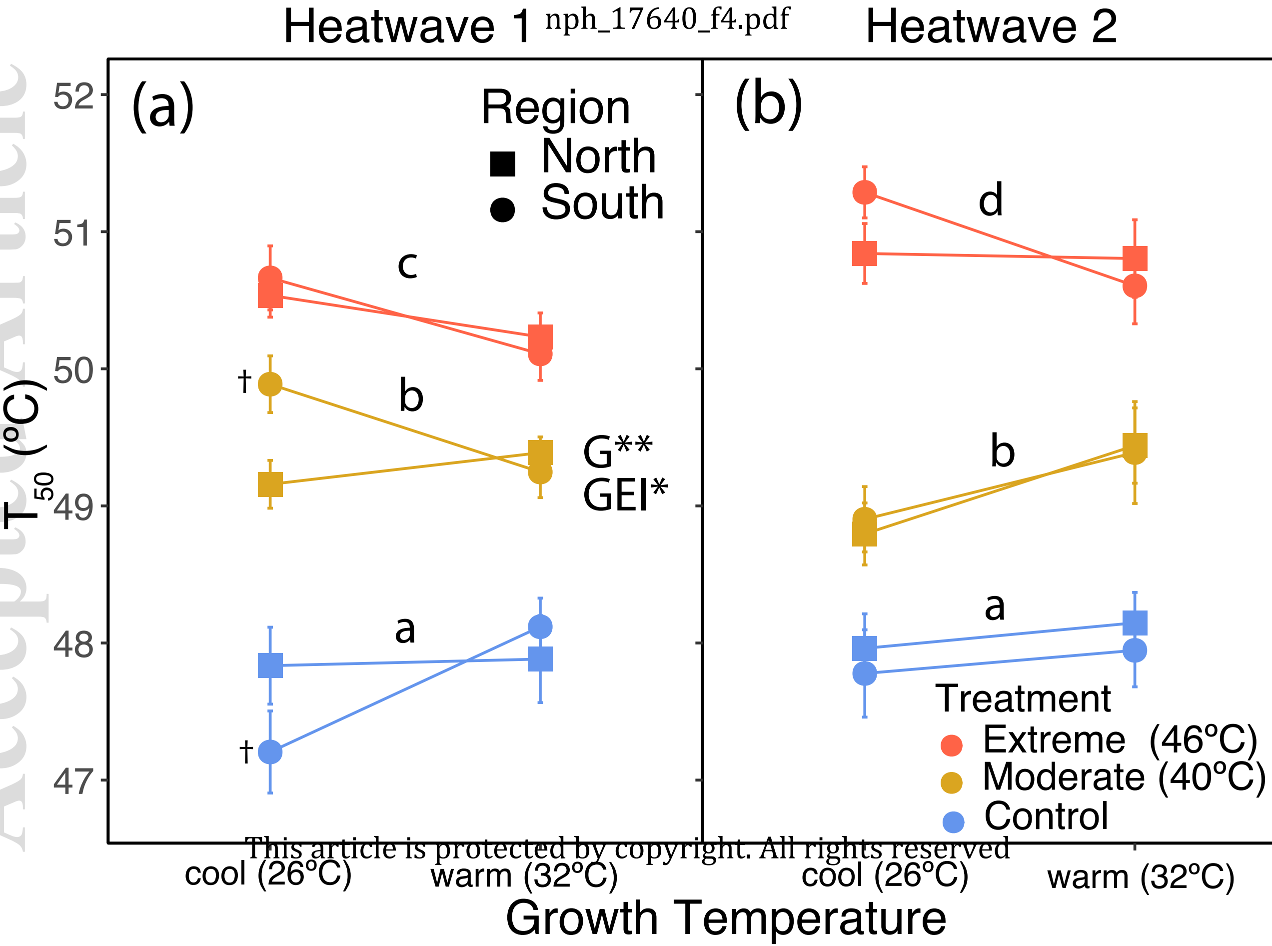
Fig. 5 Estimates of the thermal safety margin (TSM) for *Corymbia calophylla* to different treatment parameters and is measured as the difference between T_{50} (thermal tolerance, square symbols) and T_{LEAF} (leaf temperature, round symbols). Here shown for both heatwave treatments (colours) in HW2 (solid symbols) and for the *extreme* heatwave in both HW1 (open symbols) and HW2. Dashed lines indicate air temperature (T_{AIR}). Error bars are the standard error of the mean. HW1 = heatwave1; HW2 = heatwave2.

Fig. 6 Partitioning the sources of leaf trait variation for each *Corymbia calophylla* trait measured. Interactions are split between genotype-environment interactions (GEI; any interaction effect with region as a factor) and environment-environment interactions (EEI; any interaction effect without region as a factor). The F_v/F_m model used the final values in each heatwave (H5; fifth day of the heatwave) to represent the independent variable heatwave event. T_{LEAF} = leaf temperature; Leaf Damage = percentage of leaves with visible patches of damage; F_v/F_m = maximum quantum efficiency of photosystem II; T_{50} = 50% loss of leaf function or thermal tolerance; Safety Margin = thermal safety margin is the difference between T_{50} and T_{LEAF}; heatwave severity = temperature of heatwave (40 ºC or 46 ºC); heatwave event = first or second heatwave; growth temperature = temperature of growing conditions (26 ºC or 32 ºC); Region = origin of collections (northern or southern distribution); Error = unknown source of variation.

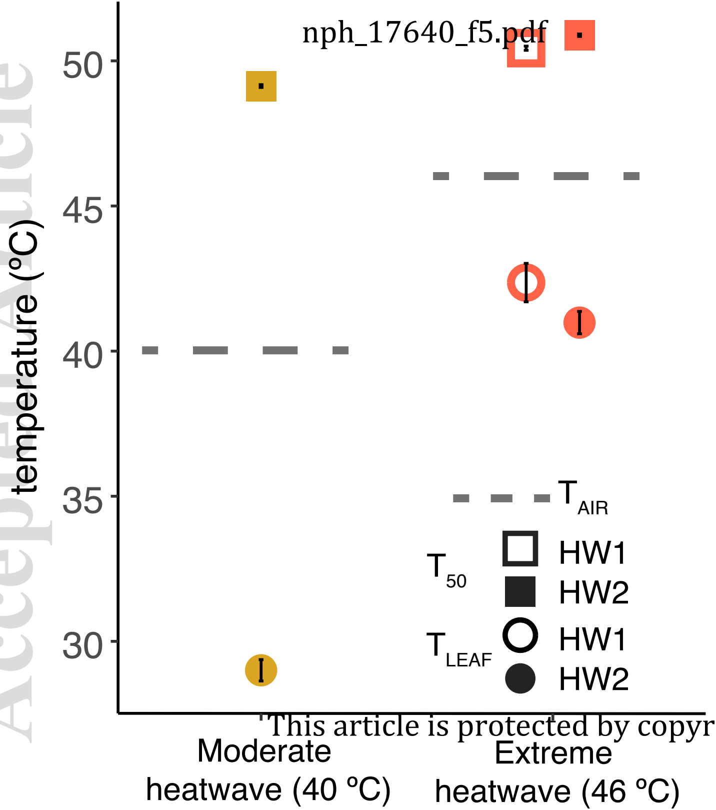

(a)
Latitude
-29
-31
-33
-35
Longitude
114
116
118
120
36
34
32
30
28
26
24
Northern Populations
Southern Populations
0 50 100
N
(b)
Growth Conditions
32°C 3 mths
C M E
26°C 3 mths
C M E
time: H0
(c)
HW1 + HW2
46°C 5 days
E
40°C 5 days
M
H1, H3, H5
(d)
REC1 + REC2
32°C 14 days
26°C 14 days
R1, R4, R8, R14

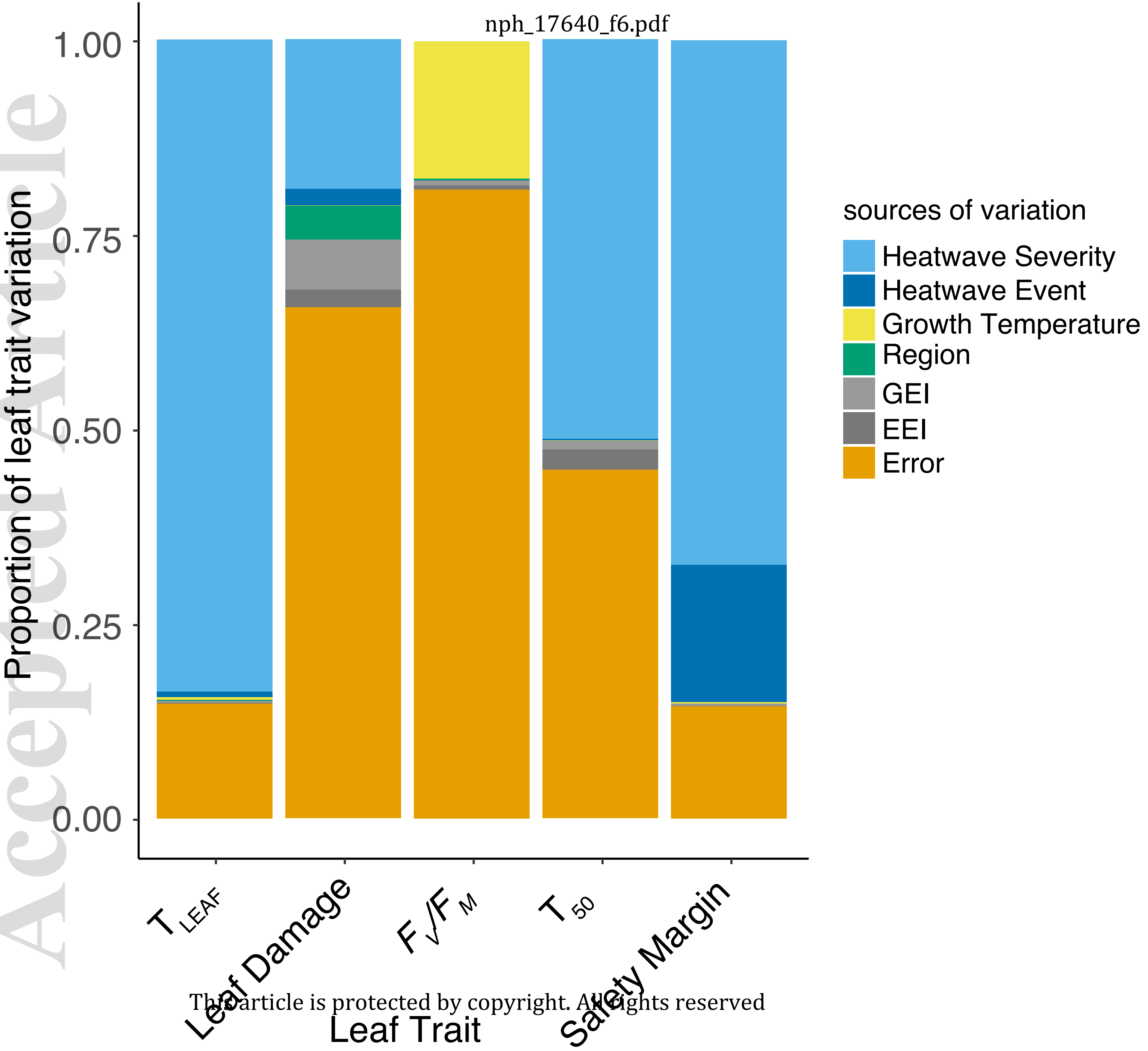

(a)
Heatwave1
(b)
Heatwave2
Percent of leaves with visual damage (%)
40
30
20
10
E**
GEI**
E*
G*
North
South
Moderate (40ºC)
Extreme (46ºC)
Moderate (40ºC)
Extreme (46ºC)
Heatwave Treatment


F_V/F_M
0.800
0.775
0.750
0.725
HW1
HW2
0
H0
1
H1
3
H3
5
H5
6
R1
9
R4
13
R8
19
R14
20
H0
21
H1
23
H3
25
H5
26
R1
29
R4
33
R8
39
R14
46
R21
Region
north
south
HW temperature
46 °C
40 °C
Growth Temperature
26 °C
32 °C
Region * *
Treat ** **
GT ** *** *** *** *** *** *** *** * *** *
Region x Treat * *
Region x GT * ** **
Treat x GT **
Day


Heatwave 1
Heatwave 2
(a)
(b)
Region
■ North
● South
Treatment
● Extreme (46°C)
● Moderate (40°C)
● Control
c
b
a
d
b
a
†
†
G**
GEI*
T_{50} (°C)
52
51
50
49
48
47
cool (26°C)
warm (32°C)
cool (26°C)
warm (32°C)
Growth Temperature

nph_17640_f5.pdf




1.00
0.75
0.50
0.25
0.00
Proportion of leaf trait variation
T_{LEAF}
Leaf Damage
F_V/F_M
T_{50}
Safety Margin
Leaf Trait
sources of variation
Heatwave Severity
Heatwave Event
Growth Temperature
Region
GEI
EEI
Error