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# Ecotypic Variation in Leaf Thermoregulation and Heat Tolerance but Not Thermal Safety Margins in Tropical Trees

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## ABSTRACT

To avoid reaching lethal temperatures during periods of heat stress, plants may acclimate either their biochemical thermal tolerance or leaf morphological and physiological characteristics to reduce leaf temperature ( $T_{\text{leaf}}$ ). While plants from warmer environments may have a greater capacity to regulate  $T_{\text{leaf}}$ , the extent of intraspecific variation and contribution of provenance is relatively unexplored. We tested whether upland and lowland provenances of four tropical tree species grown in a common garden differed in their thermal safety margins by measuring leaf thermal traits, midday leaf-to-air temperature differences ( $\Delta T_{\text{leaf}}$ ) and critical leaf temperatures defined by chlorophyll fluorescence ( $T_{\text{crit}}$ ). Provenance variation was species- and trait-specific. Higher  $\Delta T_{\text{leaf}}$  and  $T_{\text{crit}}$  were observed in the lowland provenance for *Terminalia microcarpa*, and in the upland provenance for *Castanospermum australe*, with no provenance effects in the other two species. Within-species covariation of  $T_{\text{crit}}$  and  $\Delta T_{\text{leaf}}$  led to a convergence of thermal safety margins across provenances. While future studies should expand the number of provenances and species investigated, our findings suggest that lowland and upland provenances may not differ substantially in their vulnerability to heat stress, as determined by thermal safety margins, despite differences in operating temperatures and  $T_{\text{crit}}$ .

## 1 | Introduction

The conservation of existing forests and active reforestation are pivotal to mitigating the worst impacts of climate change (Girardin et al. 2021; Griscom et al. 2017). However, periodic heat stress induced by rapid climate change (IPCC 2022) threatens forest function and thereby the future success of these conservation efforts (Jordan, Harrison, and Breed 2024). Increased frequency of hot, dry conditions has driven declines

in forest tree populations (Hammond et al. 2022) and associated carbon accumulation in biomass (Andregg et al. 2016; Brien et al. 2015). Tropical rainforests may be particularly susceptible, given their canopies already experience temperatures in excess of their thermal limits for maximum photosynthesis (Mau et al. 2018). With the frequency of lethal temperatures predicted to increase with future global warming (Doughty et al. 2023), it is essential to understand the capacity of species to tolerate or avoid heat stress.

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The leaf thermal safety margin (TSM) describes the difference between observed maximum leaf temperatures and thermal tolerance of photosynthesis, and as such is a useful proxy to determine vulnerability of ecosystems, species or populations to climate warming. Photosynthetic heat tolerance is often determined by assessing damage to leaf photosystem II using chlorophyll fluorescence (Maxwell and Johnson 2000). Two commonly used metrics are  $T_{crit}$  and  $T_{50}$ , representing the temperatures at which there is a 5% and 50% decline in photosystem II functioning. Higher photosynthetic heat tolerance is a common adaptation to warmer leaf temperatures (Perez and Feeley 2020), with higher values generally found in leaves exposed to higher ambient temperatures (Geange et al. 2021; O'sullivan et al. 2017), lower soil moisture (Cook et al. 2021), and higher radiation (Slot et al. 2019). While species originating from warmer habitats exhibit higher  $T_{crit}$ , the increase observed across an increase in growth temperature is modest, ranging from 0.24°C to 0.60°C increase per 1°C increase in mean annual temperature (O'sullivan et al. 2017; Slot et al. 2021; Zhu et al. 2018). Consequently, higher observed leaf temperatures in warmer climates such as lowland tropical forests result in them having a narrower TSM, which has been observed across species in contrasting biomes (Kitudom et al. 2022; Perez and Feeley 2020) and within species (Kullberg et al. 2023).

Forest canopy leaf temperatures can be substantially different from ambient air temperatures (Blonder and Michaletz 2018; Crous et al. 2023; Doughty et al. 2023; Mau et al. 2018; Rey-Sánchez et al. 2016; Song et al. 2017; Still et al. 2022). Leaf temperature is a result of the balance of sensible and latent heat fluxes, along with net incoming radiation, and is impacted by a broad suite of morphological and physiological traits that interact with the microclimate of the leaf (Campbell and Norman 1998; Jones 2013). Fully illuminated sunlit leaves are typically warmer than ambient air temperatures (Zhou et al. 2023), with the magnitude of this offset ( $\Delta T$ ) varying due to differences in leaf thermal traits (Blonder and Michaletz 2018), such as effective leaf width, solar absorptance profile, inclination angle and orientation, and dynamic stomatal conductance ( $g_s$ ) (Fauset et al. 2018; Guo et al. 2022; Perez and Feeley 2020). Trait variation resulting in enhanced leaf cooling is an important strategy to avoid heat stress (Deva et al. 2020; Drake et al. 2018). Indeed, there is growing evidence that communities of plant species grown under warmer conditions preferentially express leaf trait combinations that decrease leaf warming compared to those grown under cooler conditions (Kitudom et al. 2022; Kullberg et al. 2023; Leigh et al. 2012; Middleby, Cheesman, and Cernusak 2024; Posch et al. 2022; Wright et al. 2017), providing some support to the idea of plant thermoregulation and limited homeothermy (Blonder and Michaletz 2018; Michaletz et al. 2016).

However, it is unclear to what extent intraspecific genetic variation may also show similar patterns of limited homeothermy. A recent study in sage-brush reported lower canopy temperatures of warm origin populations, attributing this to differences in plant height altering canopy microclimate (Olsoy et al. 2023). Similarly, warm origin genotypes of *Populus fremontii* exhibited higher transpiration rates and consequently lower leaf temperatures compared to cool origin genotypes (Hultine et al. 2020). In addition, enhanced leaf cooling has been associated with more heat-tolerant genotypes

in *Phaseolus vulgaris* (Deva et al. 2020). On the other hand, evidence for increased thermal tolerance of warm-origin genotypes is mixed (Chen et al. 2016; Coast et al. 2022; Gimeno et al. 2008; Marias et al. 2017), with some studies suggesting a physiological limit to the high temperature acclimation of thermal tolerance (Neri et al. 2024; Slot et al. 2021; Tiwari et al. 2020). If tropical trees do exhibit a simultaneous increase in thermal tolerance and decrease in leaf temperatures of warm origin provenances, there is potential for warm origin genotypes to have greater TSMs when planted under common conditions, such as in restoration plantings. However, if  $T_{crit}$  acclimates to  $T_{leaf}$  across populations as it does across species and biomes (Perez and Feeley 2020), this may ultimately lead to a convergence of TSMs. Therefore, understanding how these two variables covary across provenances is important for the conservation and management of tropical rainforests.

Here we tested whether upland and lowland provenances of four tropical tree species differed in their TSMs when grown in a lowland common garden. This was achieved by measuring photosynthetic heat tolerance, leaf and canopy temperatures, as well as leaf thermal traits which were then used to parameterise a leaf energy balance model. We hypothesised that provenances from the warmer lowlands would have a lower  $\Delta T$  compared to provenances from the cooler uplands (H1), and that this provenance variation could be explained by variation in both leaf thermal traits, and differences in microclimate in plants of differing canopy heights (H2). We also expected thermal tolerance to acclimate to  $T_{leaf}$ , such that  $T_{crit}$  and  $T_{50}$  would show similar patterns of provenance-differentiation as  $\Delta T$ , leading to a convergence of TSMs across provenances (H3).

## 2 | Materials and Methods

### 2.1 | Study Site

This study was located in the Wet Tropics of Queensland, Australia, declared a UNESCO World Heritage Area in 1988 (UNESCO World Heritage Centre 1988), due to its high diversity, endemism and relicts of Gondwanan plant lineages. The region covers just 8900 km<sup>2</sup>, but due to its mountainous terrain experiences steep environmental gradients across relatively short geographic distances. The lowland tropical rainforest community of this region was recently declared 'Endangered' due to a history of clearing resulting in substantial fragmentation leaving it susceptible to pressures exacerbated by current climate change (Threatened Species Scientific Committee 2021).

Our study site was a 1.5-year-old provenance trial established in March 2022 at James Cook University's Daintree Rainforest Observatory (DRO) in Cape Tribulation (−16.10449°S, 145.4511°E), Queensland, Australia. The provenance trial site has an area of 2251 m<sup>2</sup> and is surrounded by mature secondary forest regrowth. The plot used in this study is one of three set up across the Australian Wet Tropics bioregion as part of the TropAdapt project (<https://tropadapt.org>) to determine differences in growth and survival across upland and lowland provenances in 16 rainforest tree species. At each site, 6- to 12-month-old saplings of 16 species and provenance (upland

and lowland for each species) were planted in a randomised block design ( $n = 32$  plants per block  $\times$  10 blocks at each site) at  $3 \times 3$  m spacing. The DRO has a mean annual temperature (MAT) of  $24.1^\circ\text{C}$  and a mean annual precipitation of 3516 mm (Karger et al. 2017). The soils are acidic, dystrophic, brown dermosol, formed in the colluvium from the metamorphic and granitic mountains to the west (Murtha 1989).

## 2.2 | Plant Material

To ensure our findings are useful both for understanding natural systems, and for informing restoration management, we chose tree species that are characteristic of rainforest communities in the Wet Tropics of Queensland (Goosem and Tucker 1995; Threatened Species Scientific Committee 2021), and among the most frequently used in regional rainforest tree plantings (Engert et al. 2020). Given how speciose the tropics are, and to gain insight into how generalisable potential patterns are across diverse species types, we include species that differ in plant families, life history strategies and deciduousness. From the original 16 species planted, we therefore selected four species as the focus for this work (Table 1), including *Castanospermum australe* A.Cunn. ex Mudie (Fabaceae), *Homalanthus novo-guineensis* (Warb.) Lauterb. & K.Schum. (Euphorbiaceae), *Melicope elleryana* (F.Muell.) T.G.Hartley (Rutaceae) and *Terminalia microcarpa* Decne. (Combretaceae). All four species have a wide elevational distribution, are relatively abundant in the Wet Tropics of Queensland and naturally occur throughout the tropical and subtropical Australian and Indo-Malesian floristic regions. Three of the species, *C. australe*, *H. novo-guineensis* and *M. elleryana*, are evergreen, whilst *T. microcarpa* is considered semi-deciduous with a period of leaflessness during the dry season (September or October) in mature trees. *H. novo-guineensis* is a fast-growing, shade-intolerant pioneer species, while the other three are late secondary, shade-tolerant species (Goosem and Tucker 1995). Of the four species, *C. australe* has the slowest growth rates and highest wood density and is also a nitrogen fixer. While all four species are present in well-developed rainforest, *M. elleryana* and *C. australe* are characteristic upper canopy species, and *T. microcarpa* is often present as an emergent species in the canopy of mature forests in the region (Threatened Species Scientific Committee 2021).

Saplings were sourced from local nurseries (Tablelands Regional Council, Rainforest Rescue and Douglas Shire Council), with the coordinates of seed source used to determine the climate of origin for the source populations (Table 1 and Figures S1 and S2). Saplings of upland origin were grown from seeds collected in the Atherton tablelands, while those of lowland origin were sourced from the Daintree Rainforest around Cape Tribulation and Diwan. The mean geographic distance between upland and lowland collection sites was 121 km (range 97–150 km) (Table 1). The lowland collection site for all species is close both geographically and climatically to the DRO common garden site (Figure S1). While multiple nurseries were used to obtain seedlings, propagation and seedling hardening techniques followed standard practice developed by local reforestation practitioners (Goosem and Tucker 1995). It should be noted that while provenance seed collection location information was verified, nurseries did not record the number of individual trees that seeds were collected from. Nursery guidelines recommend a seed-sourcing strategy of collecting from multiple individuals (Commander 2021); however, given the potential for asynchronous phenology in species with only low native density in natural plantings, we cannot confirm whether seeds from a particular provenance originated from one or more individuals from that locality.

## 2.3 | Experimental Design

To determine if leaf thermal traits, leaf temperatures and thermal tolerance differed between the lowland and upland provenances of the four selected species, we identified six replicate individuals per group ( $n = 6$  individuals  $\times$  2 provenances per species) that had healthy and fully sun-exposed canopies on which to conduct all measurements. Plant heights ranged from 0.7 to 9.0 m (mean 4.46 m). Leaf morphological traits and thermal tolerance, which requires destructive leaf sampling, were measured on leaves sampled from the top of the canopy using a pole pruner. Leaf temperature, stomatal conductance and leaf angle were measured in situ, with the aid of a step-ladder. As a result, these measurements were conducted at canopy heights of  $c. 2.5$  m, except in smaller stature trees which were measured at the top of their canopy.

**TABLE 1** | Provenance information for target species in this study.

Species	Provenance	Lat	Long	Elev	MAT	MTWM	MAP
<i>Castanospermum australe</i>	Upland	-17.34	145.50	763	20.3	27.7	1898
	Lowland	-16.47	145.36	83	24.3	29.2	2298
<i>Homalanthus novo-guineensis</i>	Upland	-17.52	145.57	1074	18.6	25.9	2874
	Lowland	-16.18	145.41	19	24.4	28.6	3334
<i>Melicope elleryana</i>	Upland	-17.30	145.46	782	19.9	27.3	1665
	Lowland	-16.18	145.41	19	24.4	28.6	3334
<i>Terminalia microcarpa</i>	Upland	-17.26	145.48	767	20.4	27.8	1604
	Lowland	-16.26	145.33	5	24.3	29.3	2354

Note: Climate data obtained from CHELSA V.2.1 1981-2010 at 1 km resolution.

Abbreviations: Elev, Elevation, m a.s.l.; MAP, mean annual precipitation (BIO12), mm; MAT, mean annual temperature (BIO1),  $^\circ\text{C}$ ; MTWM, max temperature of the warmest month (BIO5),  $^\circ\text{C}$ .

Three of the species, *C. australe*, *H. novo-guineensis*, and *T. microcarpa* had provenance-level differences in plant growth, with trees from the lowland-origin provenance taller than trees from the upland-origin provenance. In contrast, plant height did not differ between provenances in *M. elleryana* (Figure S3). To determine if potential provenance differences in leaf morphological traits and thermal tolerance (measured at the top of the canopy) were due to the greater plant heights of lowland provenances, we chose a subset of individuals ( $n = 5$  per species) from *C. australe* and *H. novo-guineensis* to measure leaves from mid-canopy (but still fully sun-exposed) positions, at canopy heights similar to the upland provenance trees.

In situ measurements of leaf temperatures took place daily from 19 until 25 October 2023, and then again from 6 to 8 November 2023. Stomatal conductance was measured over 3 days, combined with leaf temperature measurements on 25 October 2023 and 7 and 8 November 2023. Destructive leaf sampling for thermal tolerance assays and leaf morphological traits began on 19 October 2023 and ended on 25 October 2023. All leaves measured and sampled represented healthy, mature, fully sun-exposed leaf material of similar positioning and aspect, on the North side of the canopy. It rained frequently in the lead-up to our measurement campaign, so plants were not expected to be moisture-limited.

## 2.4 | Leaf and Canopy Temperatures

We measured observed  $T_{\text{leaf}}$  and thereby observed leaf-to-air temperature differences ( $\Delta T$ ) using two different methods: first by tracking individual  $T_{\text{leaf}}$  with point measurements taken over 10 days using an infrared thermometer, and subsequently by determining whole canopy temperature ( $T_{\text{can}}$ ) using UAV-based thermal imagery. Individual leaf temperatures were recorded using a dual laser infrared thermometer (MS6530, Mastech, Pittsburgh, Pennsylvania, USA), with a spectral response of 8–14  $\mu\text{m}$ , a distance to spot size ratio of 12:1, and an assumed emissivity at 0.95. Measurement campaigns for  $T_{\text{leaf}}$  took place between the times of 10:45 and 14:45 during cloud-free periods with low windspeed. Measurements were standardised across trees by taking measurements on 10 sun-exposed leaves on branches that were perpendicular to the solar angle to ensure maximum incoming radiation. This was repeated over multiple days and in a randomised order. The total number of leaves measured on each plant ranged from 30 to 50 ( $n = 1990$ ). The difference between leaf and air temperatures ( $\Delta T_{\text{leaf}}$ ) for each plant was calculated by subtracting 10-min average air temperature.

For  $T_{\text{can}}$ , thermal images of whole tree canopies were taken using a drone (DJI Mavic 2 Enterprise Advanced, DJI Technology Co. Ltd, Shenzhen, China) fitted with a FLIR M2ED thermal camera (Teledyne Flir, Wilsonville, OR, USA) with a thermal spectral band ranging from 8 to 14  $\mu\text{m}$ , an uncooled Vox microbolometer thermal sensor (with automated calibration), sensor resolution of  $640 \times 512$  pixels, approx. 9 mm lens with a  $57^\circ$  horizontal field of view and 30 Hz frame rate. The flight was completed on 11 December 2023 at 12:07 PM, with a 15 min flight time. Both thermal (IR) and true colour (RGB)

images were taken simultaneously and flown with 50% overlap at  $1.3 \text{ m s}^{-1}$ . With a flight altitude of 27 m above the ground the pixel resolution was  $4.2 \text{ cm pixel}^{-1}$ . RGB images were captured at the same times and locations as the thermal images, with an image size of  $5472 \times 3648$  pixels, a 35 mm format equivalent lens length of 24 mm and a horizontal field of view of  $84^\circ$ . An RGB orthophoto mosaic of the trial site with an average ground sample distance of 2.18 cm was created using Pix4Dmapper (Pix4D S.A., Prilly, Switzerland).

Thermal images were converted to TIFF, with radiometric data converted to temperatures using the mean air temperature ( $29^\circ\text{C}$ ) and relative humidity (38%) measured on the crane weather station during the flight, and an object distance of 27 m minus the target tree height, resulting in object distances ranging from 18.00 to 26.34 m (mean 22.54). Images were analysed using imageJ, with target tree canopies determined manually using polygons, and background temperatures of the ground excluded using threshold filtering. For each tree, calculations of the mean, standard deviation, median, minimum, maximum and skewness of canopy temperatures were recorded. We used the mean of each individual canopy for analysis of  $T_{\text{can}}$ .

## 2.5 | Stomatal Conductance

Stomatal conductance ( $g_s$ ) was measured on abaxial leaf sides using a leaf porometer (SC-1 Leaf Porometer, Decagon Devices Inc., Pullman, United States) with a measurement range of 0–1000  $\text{mmol m}^{-2} \text{s}^{-1}$  and a stated accuracy of  $\pm 10\%$  of measurement from 0 to 500  $\text{mmol m}^{-2} \text{s}^{-1}$ . Porometer measurements took place over 3 days, with one leaf per plant measured each day ( $n = 3$  measurements per plant), in conjunction with  $T_{\text{leaf}}$ . For analysis of provenance differentiation, we used the plant-level average of the measurements per plant.

## 2.6 | Leaf Angle

Leaf inclination angle ( $^\circ$ )—the angle of the leaf relative to the horizontal plane—was measured in situ on 10 leaves per plant using an electronic protractor using the plumb measuring mode from the ‘Protractor’ app (EXA Tools, Bielsko-Biala, Poland) within a cell phone (OPPO A74 5G Android smartphone, OPPO, Dongguan, China). This technique has demonstrated similar accuracy to traditional manual and digitiser methods for measuring leaf angle (Escribano-Rocafort et al. 2014).

## 2.7 | Photosynthetic Heat Tolerance

Photosynthetic heat tolerance was measured using a chlorophyll fluorometer (PAM-2000, Walz) following a protocol (Leon-Garcia and Lasso 2019; Slot et al. 2021) modified from Krause et al. (2010). We sampled leaf material early in the morning, from north facing branches within 1 m of the top of the canopy. For a subset of individuals from the lowland

provenance of *C. australe* and *H. novo-guineensis*, we also sampled leaf material from the lower in canopy. We randomised the species and individuals that were measured each day, making sure to always include at least one replicate from each provenance of each species.

To ensure assessment at steady state with respect to the photosynthetic system, fresh leaf material was dark adapted for 30 min, and then the ratio of  $F_v/F_m$  was recorded. Subsequently a cork corer was used to cut four to six discs per leaf, which were pooled per tree. Leaf discs had a diameter of 8 mm for smaller leaved species (*C. australe* and *T. microcarpa*) and 10 mm for larger leaved species (*H. novo-guineensis* and *M. elleryana*). From this pool, six leaf discs were randomly assigned to each temperature treatment. Leaf discs were heated in a water bath for 15 min at temperatures of 34, 38, 40, 42, 44, 46, 48, 50, 52, 54, or 58°C. Control discs were kept in a water bath at ambient lab temperature of 25°C. Treatment duration times of 15 min were selected as is common for other studies of thermal tolerance (Perez and Feeley 2020; Slot et al. 2021; Tiwari et al. 2020). After the heat treatment, leaf discs were stored in the dark in Petri dishes with their abaxial side face down on a moist paper towel. After 24 h,  $F_v/F_m$  was measured on dark-adapted leaf discs to determine irreversible damage to photosystem II. The temperature response of  $F_v/F_m$  was fitted using the following equation:

$$F_v/F_m = \frac{F_v/F_{m,\max}}{1 + e^{b(T_{\text{leaf}} - T_{50})}},$$

where  $F_v/F_{m,\max}$  is the upper horizontal asymptote that represents the  $F_v/F_m$  of non-stressed, healthy leaves and  $T_{\text{leaf}}$  is the temperature treatment of the water bath. The 'nls\_multstart' function in the 'nls\_multstart' package was used to fit curves and obtain estimates of  $T_{50}$  for each plant, whereas  $T_{\text{crit}}$  was defined as the temperature where the horizontal line indicating  $F_v/F_{m,\max}$  intersects with the line of the slope of the  $F_v/F_m$  decline at  $T_{50}$ .

## 2.8 | Leaf Morphological Traits

Leaf morphological traits were measured according to standard protocols (Pérez-Harguindeguy et al. 2013) and included leaf fresh weight (g), leaf area (cm<sup>2</sup>), leaf width (m) and leaf dry mass following 3 days of drying at 60°C. From these we calculated leaf mass per area, LMA (kg m<sup>-2</sup>) and leaf dry matter content, LDMC (g g<sup>-1</sup>). Leaf morphological traits were measured on 3–10 leaves (average 9) per plant.

The thermal time constant,  $\tau$  (s), influences the time taken for leaves to heat up or cool down following a change in microclimate (Michaletz et al. 2015). We calculated this using the leaf traits LMA, LDMC and leaf width and by assuming an air temperature of 28°C, an air pressure of 1012 hPa and a wind speed of 2.3 m s<sup>-1</sup>, as these were the average values observed throughout our measurement campaign, and we wanted to isolate the impact of leaf traits:

$$\tau = \varphi \times LMA \times \left( \frac{c_{pw}}{LDMC \times H} + \frac{c_{pd} - c_{pw}}{H} \right),$$

where  $\varphi$  is the ratio of the projected to total leaf area, which is 0.5 for flat leaves,  $c_{pw}$  is the specific heat capacity of water (4180 J kg<sup>-1</sup> K<sup>-1</sup>),  $c_{pd}$  is the specific heat capacity of dry leaf matter (J kg<sup>-1</sup> K<sup>-1</sup>), which varies across species so we used a value of 2814 J kg<sup>-1</sup> K<sup>-1</sup> which was the mean of seven tropical tree species in (Jayalakshmy and Philip 2010) and has been previously used in other studies based in the tropics (Fauset et al. 2018; Slot et al. 2021). The heat transfer coefficient,  $H$  (W m<sup>-2</sup> K<sup>-1</sup>), was calculated using the formula:

$$H = \rho_a \times c_{p,a} \times g_h,$$

where  $\rho_a$  is air density (1.170685 kg m<sup>-3</sup>),  $c_{p,a}$  is the specific heat capacity of air at a constant pressure (1004.78 J kg<sup>-1</sup> K<sup>-1</sup>) and  $g_h$  (m s<sup>-1</sup>) is the heat conductance which was calculated for a flat plate under laminar forced convection conditions as per Jones (2013):

$$g_h = 1.5 \times 0.00662 \times \sqrt{\frac{U}{w}},$$

where outdoor turbulence is accounted for by a factor of 1.5,  $U$  is the wind speed (m s<sup>-1</sup>) and  $w$  is the leaf width (m). We did not include the effects of radiation or transpiration, instead using it to explore the impacts of leaf traits on  $\tau$ . The thermal time constant was not incorporated into steady-state leaf energy balance modelling but was analysed as a response variable independently.

## 2.9 | Meteorological Data during Campaign

Meteorological variables including air temperature ( $T_{\text{air}}$ ), photosynthetic active radiation, wind speed and relative humidity were recorded at nearby weather stations during the measurement campaign (Figures S4 and S5). Air temperature and wind speed at 1.3 m height were recorded 200 m away from the plot in a clearing using a permanently mounted weather station (WeatherMate 3000, Environdata, Warwick, QLD, Australia). In addition, a research grade weather station located on the jib of a 47 m height canopy access crane recorded air temperature and relative humidity (HMP60, Vaisalla, Vantaa, Uusimaa, Finland), wind speed (YOUNG Model 05103 Wind Monitor, R.M.Young, Traverse City, MI, USA), incoming photosynthetic active radiation (SQ-521 full-spectrum quantum sensor, Apogee, Logan, UT, USA) and precipitation with a tipping bucket rain gauge (TB7, HyQuest Solutions, Warwick Farm, NSW, Australia) with data recorded every minute via a networked datalogger (CR310, Campbell Scientific, Logan, UT, USA). Gap filling of wind speed data from the clearing was carried out by calibration of extant data against the crane meteorological station. We found multiplying wind speed measured at the top of the canopy crane by a factor of 0.6 was a better

predictor of wind speed in the clearing than using a logarithmic wind profile.

## 2.10 | Leaf Energy Balance Model

To determine whether there were provenance-level differences in how leaf thermal traits covaried, we modelled  $T_{\text{leaf}}$  using a leaf energy balance equation implemented using the `FindTleaf` function in the ‘`plantecophys`’ package in R (Duursma 2015). The leaf trait inputs used were individual plant-averages of leaf width (m) measured at the canopy top, along with stomatal conductance ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and leaf inclination angle ( $^{\circ}$ ) measured at *c.* 2.5 m height. Microclimate inputs were set to their average conditions during the measurement campaign, with wind speed set to  $2.3 \text{ m s}^{-1}$ ,  $T_{\text{air}}$  to  $28.0^{\circ}\text{C}$  and VPD to  $1.7 \text{ kPa}$ . While PPFD averaged  $1545 \mu\text{mol m}^{-2} \text{s}^{-1}$  during the campaign, we modified this value to account for variation in the interception of incoming radiation with the measured mean leaf inclination angle of each plant using the ‘`insolation`’ package in R (Corripio 2021).

## 2.11 | Data Analysis

To test whether provenances from the warmer lowlands have a lower  $\Delta T_{\text{leaf}}$  compared to the cooler upland provenances (H1) we used a linear mixed effects model including fixed effects for ‘provenance’ and random effects for measurement days ‘doy’ as well as individual leaves nested within individual plants ‘`unique_id/leaf`’. The underlying data were based on individual spot measurements of  $T_{\text{leaf}}$  minus measured  $T_{\text{air}}$  at that time. Models were fitted to each species separately using ‘`lme4`’ (Bates et al. 2015) and pairwise comparisons between provenances were assessed using ‘`emmeans`’ (Lenth 2022).

We conducted several analyses to assess whether  $\Delta T_{\text{leaf}}$  could be explained by leaf thermal traits and plant canopy heights (H2). We tested for provenance differentiation in leaf traits (width, angle, conductance, thermal time constant) and leaf-air temperature differences based on leaf energy balance modelling ( $\Delta T_{\text{mod}}$ ) and canopy temperatures ( $\Delta T_{\text{can}}$ ). For this we performed a two-way unpaired Student’s *t*-test on each species separately. We used plant averages as a single observation so that all tests had balanced designs with  $n = 6$  plants for each group. Homogeneity of variance was tested using the Levene test and normality was tested using the Shapiro test. To determine whether provenance differentiation in traits was a result of systematic differences in plant height between provenances, we tested for differences in leaf traits and thermal tolerance between leaves sampled in the upper and lower canopy of the lowland provenance of *C. australe* and *H. novo-guineensis*. To do so we performed paired *t*-tests on five replicate plants per group. The leaf trait, LMA, did not meet assumptions of normality so data were log transformed for analysis. In addition, we examined the relationship between modelled and observed  $T_{\text{leaf}}$ , and plant height using linear regression with either  $T_{\text{leaf}}$ , or  $T_{\text{mod}}$  as the response variable, and plant height as an explanatory

variable. We considered these analyses in combination when interpreting how well variation in leaf traits and canopy height explained observed variation in  $T_{\text{leaf}}$ .

To assess the impact of provenance differentiation on thermal tolerance and the consequences of this on TSMs (H3), we first tested for provenance differences in  $T_{\text{crit}}$  and  $T_{50}$ , as well as the TSMs, as per the other traits (using two-way unpaired Student’s *t*-tests). The TSM of each plant was calculated with either  $T_{\text{crit}}$  or  $T_{50}$  as the upper threshold. From this value, we subtracted the maximum air temperature observed during the study period,  $32^{\circ}\text{C}$ , and then subtracted either the mean observed leaf-air temperature difference measured with the handheld IR sensor ( $\Delta T_{\text{leaf}}$ ), the mean observed canopy-air temperature difference measured with UAV thermal imagery ( $\Delta T_{\text{can}}$ ), or the modelled leaf-air temperature difference calculated under common conditions from the traits leaf width, stomatal conductance and leaf inclination angle ( $\Delta T_{\text{mod}}$ ). We then assessed correlations between  $T_{\text{leaf}}$  and  $T_{\text{crit}}$  using linear regression. All analyses were performed using R version 4.2.2. Throughout the manuscript, provenance differences are reported as arithmetic means  $\pm 1 \text{ SD}$ .

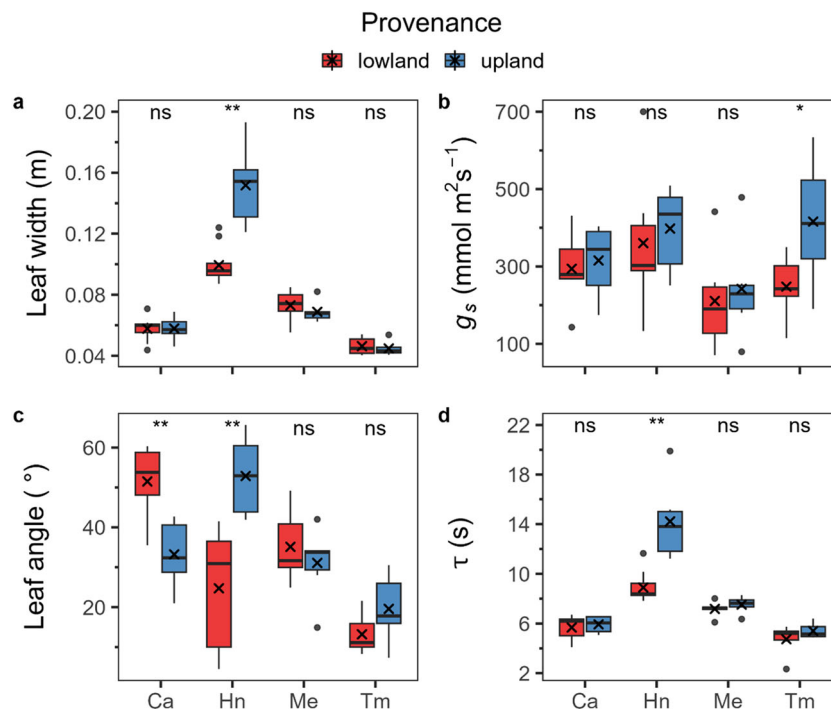
## 3 | Results

### 3.1 | Provenance Level Differentiation in Leaf Traits and Stomatal Conductance

Provenance effects on leaf width differed across species. For *H. novo-guineensis*, there was strong evidence that provenances differed in leaf width ( $t_{(10)} = -4.27$ ,  $p = 0.00163$ ), with leaves from the lowland provenance substantially narrower than leaves from the upland provenance, averaging  $10.0 \pm 1.3$  and  $15.2 \pm 2.7$ , respectively (Figure 1a). There was no evidence of a provenance effect in leaf width for *C. australe* ( $t_{(10)} = -0.16$ ,  $p = 0.87$ , Figure 1a), *M. elleryana* ( $t_{(10)} = 0.81$ ,  $p = 0.44$ ) or *T. microcarpa* ( $t_{(10)} = 0.49$ ,  $p = 0.64$ , Figure 1a).

The mean stomatal conductance ( $g_s$ ) measured around midday was  $311 \text{ mmol m}^{-2} \text{s}^{-1}$  and ranged from 71 to  $700 \text{ mmol m}^{-2} \text{s}^{-1}$  across all species and provenances (Figure 1b). For all species, mean  $g_s$  was lower in the lowland provenance than the upland provenance; however, moderate evidence for a provenance effect on  $g_s$  was found only in *T. microcarpa* ( $t_{(10)} = -2.25$ ,  $p = 0.0479$ ), in which  $g_s$  averaged  $248 \pm 83 \text{ mmol m}^{-2} \text{s}^{-1}$  in the lowland provenance and  $416 \pm 163 \text{ mmol m}^{-2} \text{s}^{-1}$  for the upland provenance (Figure 1b).

Mean leaf inclination angle was  $32.1^{\circ}$  and ranged from  $4.5^{\circ}$  to  $65.7^{\circ}$  across all species and provenances (Figure 1c). For *C. australe*, we found strong evidence that leaves from the lowland provenance had steeper leaf angles than the upland provenance ( $t_{(10)} = 3.64$ ,  $p = 0.00454$ ), averaging  $51.7^{\circ} \pm 9.1^{\circ}$  for the lowland provenance and  $33.2^{\circ} \pm 8.5^{\circ}$  for the upland provenance (Figure 1c). In *H. novo-guineensis*, we found strong evidence for the opposite pattern ( $t_{(10)} = -3.83$ ,  $p = 0.00333$ ), with the lowland provenance having shallower leaf angles than the upland provenance, averaging  $24.8^{\circ} \pm 14.7^{\circ}$  and  $52.9^{\circ} \pm 10.3^{\circ}$  respectively (Figure 1c). We found no evidence for a provenance effect



**FIGURE 1** | Provenance differences in leaf functional traits of four tropical tree species grown under common environmental conditions. Panels show (a) leaf width, (b) stomatal conductance,  $g_s$ , (c) leaf inclination angle and (d) thermal time constant,  $\tau$ . Species include Ca, *Castanospermum australe*; Hn, *Homalanthus novo-guineensis*; Me, *Melicope elleryana*, Tm, *Terminalia microcarpa*. Data represent tree averages ( $n = 6$ ) presented as box and whisker plots showing median and interquartile range, with the mean marked by  $\times$ . Results of intraspecific  $t$ -test of provenance differences indicated by subscript ns = not significant, \* $p < 0.05$  and \*\* $p < 0.001$ .

on leaf inclination angle for *T. microcarpa* or *M. elleryana* ( $p > 0.1$ , Figure 1c).

The mean thermal time constant was 7.5 s and ranged from 2.3 to 19.9 s. For all species, the thermal time constant of the lowland population was lower on average than the upland provenance (Figure 1d). However, we found strong evidence of a provenance effect only for *H. novo-guineensis* ( $t_{(10)} = -3.45$ ,  $p = 0.00619$ ) which had a thermal time constant of  $9.3 \text{ s} \pm 1.4$  for the lowland provenance and  $14.2 \text{ s} \pm 3.2$  for the upland provenance.

### 3.2 | Provenance Level Differentiation in Leaf and Canopy Temperatures

Temperatures of individual leaves recorded with handheld IR sensors ranged from  $18.0^\circ\text{C}$  to  $45.9^\circ\text{C}$  (Figure S6). When averaged to the plant level, mean  $T_{\text{leaf}}$  was  $32.2^\circ\text{C}$  and ranged from  $27.2^\circ\text{C}$  to  $36.4^\circ\text{C}$  across all individuals. The mean leaf-to-air temperature difference,  $\Delta T_{\text{leaf}}$ , was  $4.1^\circ\text{C}$  and ranged from  $-0.7^\circ\text{C}$  to  $8.1^\circ\text{C}$  (Figure 2a). For most plants mean  $\Delta T_{\text{leaf}}$  was positive, except for *H. novo-guineensis*, which had multiple occurrences of  $T_{\text{leaf}}$  cooler than  $T_{\text{air}}$  (Figure 2b).

The mixed effects models found very strong evidence for provenance differences in  $\Delta T_{\text{leaf}}$  for both *T. microcarpa* and *C. australe*. In *T. microcarpa*,  $\Delta T_{\text{leaf}}$  was higher in the lowland provenance than the upland provenance ( $t_{(124)} = 6.196$ ,  $p < 0.0001$ ), with a mean of  $5.2 \pm 1.19^\circ\text{C}$  and  $3.4 \pm 1.35^\circ\text{C}$ , respectively (Figure 2b). The opposite was observed for

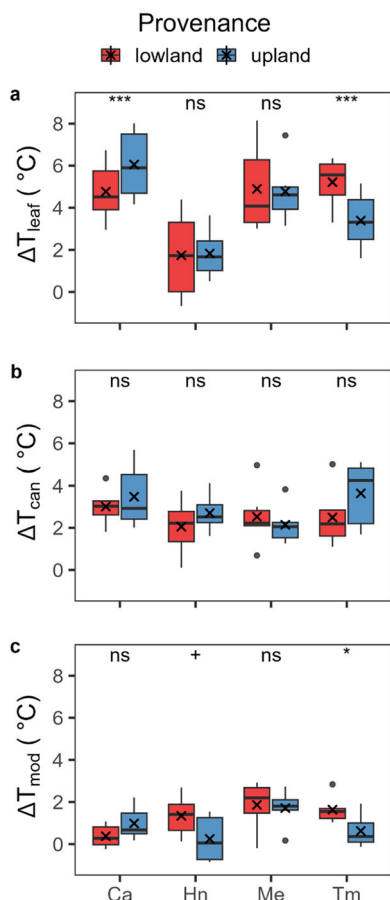
*C. australe*, in which  $\Delta T_{\text{leaf}}$  in the lowland provenance was lower than the upland provenance ( $t_{(121)} = -3.788$ ,  $p = 0.0002$ ), with a mean of  $4.8 \pm 1.42^\circ\text{C}$  and  $6.0 \pm 1.68^\circ\text{C}$  respectively (Figure 2b). We found no evidence for provenance-differentiation in  $\Delta T_{\text{leaf}}$  for *M. elleryana* or *H. novo-guineensis* ( $p > 0.1$ ; Figure 2b).

For  $\Delta T_{\text{can}}$ , we found no evidence of provenance effects in any of the four species (Figure 2c).

Modelled leaf-to-air temperature differences ( $\Delta T_{\text{mod}}$ ) represent the variation in potential leaf temperatures as resulting from the covariation between leaf width, leaf angle and stomatal conductance. Across all individuals, mean  $T_{\text{mod}}$  was  $29.1^\circ\text{C}$  and ranged from  $27.1^\circ\text{C}$  to  $31.0^\circ\text{C}$ . For *T. microcarpa*, we found moderate evidence for a provenance effect in  $\Delta T_{\text{mod}}$  ( $t_{(10)} = 2.39$ ,  $p = 0.0381$ ), with  $\Delta T_{\text{mod}}$  higher in the lowland provenance than the upland provenance averaging  $1.6 \pm 0.69$  and  $0.6 \pm 0.78$ , respectively. For *H. novo-guineensis*, we found weak evidence for provenance differences ( $t_{(10)} = 1.81$ ,  $p = 0.0996$ ), with the lowland provenance also having a higher average  $\Delta T_{\text{mod}}$  than the upland provenance, averaging  $1.4 \pm 1.0$  and  $0.3 \pm 1.09$ , respectively. For the other two species, the data showed no evidence of provenance-differentiation in  $\Delta T_{\text{mod}}$ .

### 3.3 | Correlation Between Modelled and Observed Leaf and Canopy Temperatures

Observed variation in  $\Delta T_{\text{leaf}}$  was well predicted by the trait-based energy balance model ( $\Delta T_{\text{mod}}$ ), with an RMSE of 3.517



**FIGURE 2** | Provenance differences in leaf-to-air temperature differences ( $\Delta T$ ) of four tropical tree species grown under common environmental conditions. Panels show  $\Delta T$  calculated using (a) observed leaf temperatures  $\Delta T_{\text{leaf}}$ , (b) observed canopy temperatures  $\Delta T_{\text{can}}$ , (c) and trait modelling  $\Delta T_{\text{mod}}$ . Ca, *Castanospermum australe*; Hn, *Homalanthus novo-guineensis*; Me, *Melicope elleryana*; Tm, *Terminalia microcarpa*. Data are of tree averages ( $n=6$ ) presented as a box and whisker plots showing median and interquartile range, with the mean marked by  $\times$ . Results of intraspecific provenance differences indicated by subscript ns = not significant, + is marginally significant at  $p < 0.1$ , \* $p < 0.05$ , and \*\*\* $p < 0.0001$ . Note that for panels (a) and (c), statistical analysis was based on unpaired  $t$ -tests on the plant-level means, whereas for panel (b) statistical analysis was based on mixed effects model including random effects of day and individual plant, conducted on the individual leaf temperatures. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

and MAE of 3.11 (Figure S7a). However,  $\Delta T_{\text{mod}}$  tended to underestimate  $\Delta T_{\text{leaf}}$  for *C. australe*, and overestimate  $T_{\text{leaf}}$  for *H. novo-guineensis*. This was likely due to errors associated with the differences in  $T_{\text{air}}$  at the canopy height of each plant, versus from the clearing where  $T_{\text{air}}$  was measured at 2.5 m height. This is reflected in Figure S7b, where the difference between observed and modelled  $\Delta T$  is correlated with plant height ( $R^2 = 0.35$ ,  $p < 0.0001$ ). This trend is driven by a decrease in measured  $\Delta T_{\text{leaf}}$  with plant height (decreasing by  $0.66^\circ\text{C}$  every 1 m increase in plant height), and not by  $\Delta T_{\text{mod}}$  (Figure S7c,d). The correlation between  $\Delta T_{\text{leaf}}$  and  $\Delta T_{\text{mod}}$  was improved when modifying air temperature and wind speed vertically through the canopy, however as this did not alter results of provenance-differentiation it is not included.

### 3.4 | Provenance Level Differentiation in Thermal Tolerance

At the common garden experimental site, mean  $T_{\text{crit}}$  was  $46.0^\circ\text{C}$  and ranged from  $42.0^\circ\text{C}$  to  $51.1^\circ\text{C}$  (Figure 3a). For *C. australe*, we found moderate evidence of a provenance effect on  $T_{\text{crit}}$  ( $t_{(10)} = -2.46$ ,  $p = 0.0336$ ), with the lowland provenance having a lower  $T_{\text{crit}}$  than the upland provenance, averaging  $46.6 \pm 2.81^\circ\text{C}$  and  $49.6 \pm 1.05^\circ\text{C}$  respectively (Figure 3a). For *T. microcarpa*, we found weak evidence for a provenance effect ( $t_{(10)} = 1.85$ ,  $p = 0.0947$ ), with  $T_{\text{crit}}$  averaging  $47.7 \pm 2.07^\circ\text{C}$  and  $45.8 \pm 1.41^\circ\text{C}$ , for the lowland and upland provenance respectively (Figure 3a). We found no evidence for a provenance effect on  $T_{\text{crit}}$  for *H. novo-guineensis* or *M. elleryana* ( $p > 0.1$ ).

The mean thermal tolerance metric  $T_{50}$  was  $49.7^\circ\text{C}$  overall and ranged from  $46.6^\circ\text{C}$  to  $54.7^\circ\text{C}$  (Figure 3b). We found no evidence for provenance-differentiation in  $T_{50}$  in any of the species ( $p > 0.1$ ; Figure 3b).

### 3.5 | Within-Canopy Variation in Leaf Traits and Thermal Tolerance

To test whether provenance differences in thermal tolerance and leaf traits were due to differences in tree height between provenances, a subset of individuals from the lowland provenance of both *C. australe* and *H. novo-guineensis* were sampled at a mid-canopy position. There were no substantial differences in any leaf traits, or thermal tolerance metrics due to vertical canopy position (Figure S8).

### 3.6 | Provenance Level Differentiation in TSMs

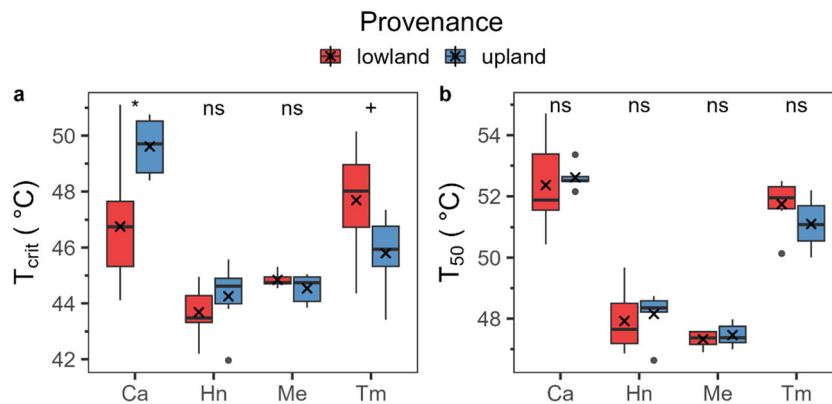
The mean TSM calculated using  $T_{\text{crit}} - T_{\text{leaf}}$  ( $\text{TSM}_{\text{crit-leaf}}$ ) was  $9.8^\circ\text{C}$  and ranged from  $4.4^\circ\text{C}$  to  $14.2^\circ\text{C}$  across all species and provenances (Figure 4a). We found no evidence of a provenance effect in any of the species ( $p > 0.1$ , Figure 4a). However, it should be noted that averages do follow the same general trend as for  $\text{TSM}_{\text{crit-mod}}$  (Figure 4c).

For TSMs calculated using  $T_{\text{crit}} - T_{\text{can}}$  ( $\text{TSM}_{\text{crit-can}}$ ), we found weak evidence to support a provenance effect in *T. microcarpa* ( $t_{(10)} = 2.23$ ,  $p = 0.05$ ) (Figure 4c).

The mean TSM calculated using  $T_{\text{crit}} - T_{\text{mod}}$  ( $\text{TSM}_{\text{crit-mod}}$ ) was  $12.8^\circ\text{C}$  and ranged from  $8.2^\circ\text{C}$  to  $18.2^\circ\text{C}$  across all species and provenances (Figure 4c). In *C. australe*, we found weak evidence for a provenance effect ( $t_{(10)} = -1.90$ ,  $p = 0.0861$ ), with  $\text{TSM}_{\text{crit-mod}}$  of the lowland provenance lower than the upland provenance and averaging  $14.2 \pm 2.76^\circ\text{C}$  and  $16.6 \pm 1.40^\circ\text{C}$ , respectively (Figure 4c). We found no evidence for a provenance effect in  $\text{TSM}_{\text{crit-mod}}$  for the other three species ( $p > 0.1$ ; Figure 4c).

No evidence of a provenance effect was observed in TSMs calculated using  $T_{50}$  for any of the species ( $p > 0.1$ , Figure S9).





**FIGURE 3** | Provenance differences in leaf thermal tolerance of four tropical tree species grown under common environmental conditions. Panels show (a) values for  $T_{crit}$  and (b)  $T_{50}$ . Ca, *Castanospermum australe*; Hn, *Homalanthus novo-guineensis*; Me, *Melicope elleryana*; and Tm, *Terminalia microcarpa*. Data are of tree averages ( $n = 6$ ) presented as a box and whisker plots showing median and interquartile range, with the mean marked by  $\times$ . Results of intraspecific *t*-test of provenance differences indicated by subscript ns = not significant, + is marginally significant at  $p < 0.1$  and  $*p < 0.05$ . [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

### 3.7 | Contribution of $T_{crit}$ and $T_{leaf}$ to the TSM

Across all species and provenances, there was a significant, positive correlation between  $T_{crit}$  and  $T_{leaf}$  ( $R^2 = 0.11$ ,  $F_{1,46} = 6.90$ ,  $p = 0.012$ ,  $n = 48$ ) that represented a  $0.4^\circ\text{C}$  increase in  $T_{crit}$  per  $1^\circ\text{C}$  increase in  $T_{leaf}$  (Figure S10). However, within-species, we did not find evidence for a correlation between  $T_{crit}$  and  $T_{leaf}$  in any of the four species ( $p > 0.1$ , Figure S10). Species differed in the absolute magnitude that either  $T_{crit}$  or  $T_{leaf}$  varied, as a result different variables contributed more to within-species variation in the TSM depending on the species (Figure 5). In *C. australe* and *T. microcarpa*, the within-species range of  $T_{crit}$  was 1.3 and 1.4 times larger than the within-species range of  $T_{leaf}$  (respectively), and therefore contributed more to variation in the TSM for these species. In contrast, for *M. elleryana* and *H. novo-guineensis*, within-species variation in  $T_{crit}$  was the weaker driver of changes in TSMs, as  $T_{crit}$  variation was 0.3 and 0.7 that of  $T_{leaf}$ .

## 4 | Discussion

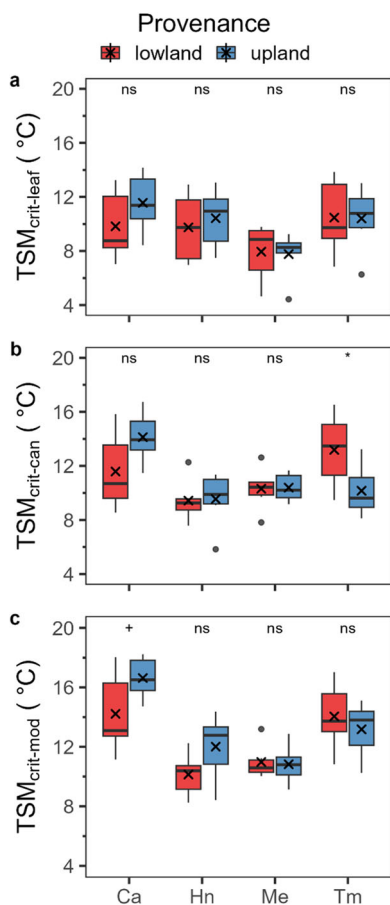
Provenance trials in tropical tree species are relatively rare, and as such our understanding of how tropical forests acclimate or adapt to environmental change is limited. Here we established and utilised a provenance trial of tropical rainforest species in Queensland, Australia to assess whether trees originating from the cooler uplands and warmer lowlands differed in their leaf thermoregulatory traits, or thermal tolerance, and as a result operate with different TSMs when grown under common environmental conditions.

With contrasting patterns of provenance-differentiation observed across the four species tested, we found only partial support for our first hypothesis that the lowland provenances would have cooler leaf temperatures than upland provenances when grown under common garden conditions (H1). Despite this, we did find provenance-based variation of leaf thermal traits and plant height was consistent with variation in  $T_{leaf}$ , supporting our second hypothesis (H2). Finally, although we found limited within-species association between  $T_{leaf}$  and  $T_{crit}$ ,

these variables covaried in such a way that TSMs converged between provenances, supporting our third hypothesis (H3). Together, these results suggest that despite variation in either  $T_{crit}$  or observed  $T_{leaf}$ , there was no provenance-level difference in vulnerability to thermal stress as defined by the TSM.

### 4.1 | Species-Specific Leaf Thermoregulation Provenance Effects

In our study, we observed provenance effects in at least one thermal trait (i.e., leaf width, leaf angle, or  $g_s$ ) in three of the four species, and provenance effects in measured  $T_{leaf}$  in two species. Assuming the expression of leaf thermal traits under common garden conditions would reflect underlying genetic adaptation associated with provenance, we expected higher  $T_{leaf}$  for cool-adapted, and lower  $T_{leaf}$  for warm-adapted provenances, which would be consistent with selective pressure to limit plant exposure to extreme leaf temperatures (Michaletz et al. 2016). However, patterns of provenance-differentiation in thermal traits and  $T_{leaf}$  followed expected trends (H1) only in one species, *C. australe*, with the opposite pattern observed for *T. microcarpa*, and no variation associated with provenance for the other two species. While this contrasts with other studies that have found warm-adapted plants to have a lower  $T_{leaf}$  compared to cool-adapted plants (Blasini et al. 2022; Kitudom et al. 2022; Kullberg et al. 2023), species-specific patterns in intraspecific variation of thermal traits have been reported previously (Kullberg et al. 2023; Kullberg and Feeley 2022; Manishimwe et al. 2022). This suggests that different species may have different selection pressures, genetic history, or trait trade-offs resulting in contrasting patterns of provenance-associated variation in leaf thermoregulation. It is also interesting to note that the species where we did observe the expected direction of provenance-differentiation in leaf thermoregulation, *C. australe*, was also the species that had the coolest realised thermal niche (Figure S1). As a result, the mean annual temperature of the lowland provenance collection site, and the common garden planting location used were at the warmer limit of the species distribution. This is similar to the provenance-trial set up and findings of Blasini et al. (2022), and



**FIGURE 4** | Provenance differences in thermal safety margin (TSM) of four tropical tree species grown under common environmental conditions. TSM calculated using  $T_{crit}$  as upper threshold in all panels, with operating temperature defined as  $32^{\circ}\text{C}$  (mean max air temperature of the site) plus  $\Delta T_{leaf}$  in (a),  $\Delta T_{can}$  in (b) and  $\Delta T_{mod}$  in (c). Ca, *Castanospermum australe*; Hn, *Homalanthus novo-guineensis*, Me, *Melicope elleryana*; and Tm, *Terminalia microcarpa*. Data are of tree averages ( $n = 6$ ) presented as a box and whisker plots showing median and interquartile range, with the mean marked by  $\times$ . Results of intraspecific  $t$ -test of provenance differences indicated by subscript ns = not significant, + is marginally significant at  $p < 0.1$  and  $*p < 0.05$ . [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

partly supports the theory that selection pressures leading to local adaptation are stronger at the climate extremes of a species distribution (Rehm et al. 2015; Zimmermann et al. 2009). However, further work assessing variation in thermoregulation in multiple provenances, across sites, and in a broader range of species would be needed to establish if this could explain the contrasting patterns observed across the four species tested here.

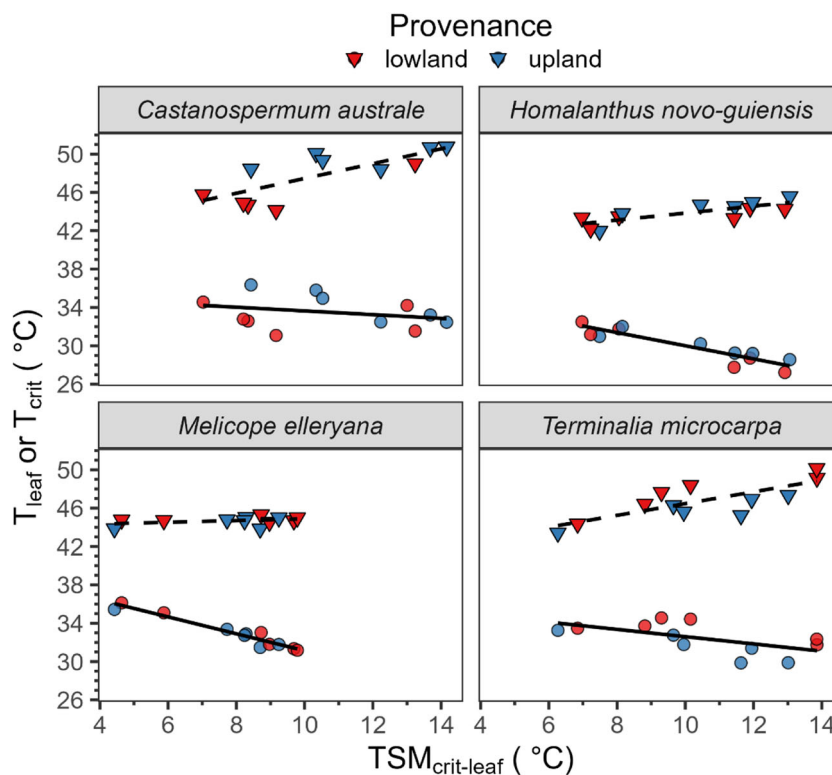
## 4.2 | Effects of Leaf Thermal Traits and Plant Height on Leaf Temperature

We expected that provenance differences in  $\Delta T_{leaf}$  could be explained by observed variation in leaf thermal traits, as well as the influence of plant height on microclimate. Leaf energy balance modelling proved a useful tool to explore the implications of coordinated shifts in multiple thermal traits, with

observed and modelled  $\Delta T_{leaf}$  showing close agreement, as in other studies (Fauset et al. 2018; Guo et al. 2022; Perez and Feeley 2020). Deviations in the leaf energy balance modelling can probably be attributed to the scale of microclimate inputs coming as they do from a clearing 200 m away. Our provenance trial microclimate is partly buffered by the surrounding forest, which likely reduces wind speed compared to that observed in the clearing (Davies-Colley, Payne, and van Elswijk 2000). As a result, our modelled  $\Delta T_{leaf}$  is generally lower than that observed. In addition, we show that deviations between modelled and observed  $\Delta T_{leaf}$  are associated with plant height. Despite these errors in absolute leaf temperature, the relative patterns of provenance differentiation in  $\Delta T_{mod}$  was unaffected; therefore, we believe leaf energy balance modelling can still be utilised to explore acclimation and adaptation of leaf thermoregulation to climate change.

### 4.2.1 | *C. australe*

Consistent with our expectations,  $T_{crit}$  and  $T_{leaf}$  were both higher in the upland provenance of *C. australe* compared to the lowland provenance. Leaves of the upland provenance had substantially shallower leaf angles than those from the lowland provenance. This likely resulted in an increased radiative load, resulting in warmer  $T_{leaf}$ , driving  $T_{crit}$  higher for the upland provenance. Leaf angle is often ignored in applications of leaf energy balance modelling, despite the strong effect it can have on modifying the net radiation term (Ponce De León and Bailey 2024; Woods, Saudreau, and Pincebourde 2018; Yang et al. 2023). This effect is partially reflected in the modelled leaf temperatures ( $T_{mod}$ ); however, covariation of other thermal traits dampened the influence of leaf angle on  $T_{mod}$  resulting in no statistically significant differences in  $T_{mod}$  between provenances. However,  $T_{crit}$  (measured on leaves sampled at the top of the canopy) still showed strong provenance differentiation, suggesting  $T_{leaf}$  at the top of the canopy in the upland provenance was indeed warmer than for the lowland provenance. While this could indicate that leaf angles at the top of the canopy still differed sufficiently between provenances to impact leaf energy balance, another explanation is that the provenance-differentiation observed in  $T_{leaf}$  was also driven by systematic differences in microclimate due to plant height. The upland population, being smaller in stature compared to the lowland population, had a hotter thermal environment due to its proximity to the exposed ground with higher sensible heat/longwave radiation, as well as lower turbulence associated with wind speed. During our experiment, with the  $3 \times 3$  m spacing between trees, the ground is exposed, and spot measurements revealed ground temperatures around  $50^{\circ}\text{C}$  to  $60^{\circ}\text{C}$ . In a provenance trial of sage-brush, Olsoy et al. (2023) also found that sub-species differences in  $T_{leaf}$  were associated with plant height, with shorter stature plants having higher  $T_{leaf}$ . With no compensatory changes in leaf traits with height within the canopy observed for *C. australe*, this could lead to higher  $T_{leaf}$  and  $T_{crit}$  in the smaller, upland provenance. This spatial pattern contrasts with what is observed in trees in a closed canopy where the lower canopy and ground are shaded and thus cooler than the upper canopy (Rey-Sánchez et al. 2016).



**FIGURE 5** | Contribution of  $T_{\text{leaf}}$  and  $T_{\text{crit}}$  to thermal safety margins ( $TSM_{\text{crit-leaf}}$ ) of four tropical tree species grown under common environmental conditions. Points represent plant-level averages of each variable and lines represent linear regressions. In each panel,  $T_{\text{leaf}}$  data are represented by circles for the individual points and solid lines for the regression, whereas  $T_{\text{crit}}$  data are represented by triangles for the points and a dashed line for the regression. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

#### 4.2.2 | *T. microcarpa*

In contrast, *T. microcarpa* had the opposite pattern to *C. australe*, with the lowland provenance having higher  $T_{\text{mod}}$ ,  $T_{\text{leaf}}$  and  $T_{\text{crit}}$  than the upland provenance. This was likely driven by substantially lower  $g_s$  rates of the lowland provenance compared to the upland provenance.

Although an assumption of local adaptation of plant thermoregulation led to an expectation of higher  $g_s$  in lowland provenances, it is not surprising that *T. microcarpa* showed the reverse pattern. This is due to the likely trade-offs in gas exchange with temperature and vapour pressure deficit, which covary. A recent glasshouse experiment (Middleby, Cheesman, and Cernusak 2024) compared acclimation of gas exchange (including the stomatal function parameter  $g_1$ ) under different temperature and VPD conditions, revealing tropical trees may decrease water use efficiency in response to increased air temperature but increase water use efficiency in response to increased VPD. Supporting this, a study assessing water use in a lowland and upland forest in this region showed the lowland forest with high VPD had lower canopy conductance and annual transpiration than the upland forest with low VPD (Binks et al. 2023). It is therefore possible the lower  $g_s$  of the lowland provenance in *T. microcarpa* is an adaptation to the higher evaporative demand of the seed source location, rather than temperature itself.

This pattern of provenance differences in  $T_{\text{leaf}}$  traits for *T. microcarpa* is particularly interesting considering, like *C. australe* and *H. novo-guineensis*, plants from the lowland provenance

were taller than the upland provenance (Figure S3) and thus experience a cooler microclimate to the upland plants. The close relationship between  $T_{\text{leaf}}$  and  $T_{\text{mod}}$  suggests the measured variation in leaf thermal traits was adequate to explain these patterns, although it is also possible that the variation in thermal tolerance and  $T_{\text{leaf}}$  was due to some unmeasured variable, as canopy architecture, including leaf clumping, can also play a large role in determining  $T_{\text{leaf}}$  (Gauthey et al. 2023; Leuzinger and Körner 2007; Woods, Saudreau, and Pincebourde 2018).

#### 4.2.3 | *H. novo-guineensis*

Two of the species had no provenance-differentiation in  $T_{\text{leaf}}$  or  $T_{\text{crit}}$ , but for differing reasons. For *H. novo-guineensis*, strong provenance-differentiation was observed in leaf width, leaf inclination angle and the thermal time constant, all of which were higher for the upland provenance than the lowland provenance. However, the net effect of variation in these traits—wider leaves would increase  $T_{\text{leaf}}$ , but steeper leaf angles would reduce  $T_{\text{leaf}}$ —led to a lack of provenance-differentiation in  $T_{\text{mod}}$  that was supported by measured  $T_{\text{leaf}}$ . This highlights the importance of considering the suite of leaf thermal traits in combination.

#### 4.2.4 | *M. elleryana*

*M. elleryana* was the only species with no provenance effect in  $T_{\text{leaf}}$ ,  $T_{\text{can}}$  or  $T_{\text{crit}}$ . Considering there was also no

provenance-differentiation for any leaf thermal traits, nor in  $T_{\text{mod}}$ , it is highly likely that trait variation was not sufficient to cause a difference in  $T_{\text{leaf}}$  between provenances. In addition, this species had no provenance-level difference in plant height (Figure S3), so there were also no expected indirect differences due to microclimate.

### 4.3 | Covariation Between $T_{\text{leaf}}$ and $T_{\text{crit}}$ Impacts TSM

Across all species we found a correlation between  $T_{\text{leaf}}$  and  $T_{\text{crit}}$ , supporting other studies that suggest thermal tolerance (either  $T_{\text{crit}}$  or  $T_{50}$ ) broadly acclimates to maximum  $T_{\text{leaf}}$  (Bison and Michaletz 2024; Geange et al. 2021; Perez and Feeley 2020; Tserj and Feeley 2021). This represented a +0.4°C increase in  $T_{\text{crit}}$  per 1°C increase in plant averaged  $T_{\text{leaf}}$  measured around midday, which is comparable to values reported with mean annual temperature in other studies (O'sullivan et al. 2017; Slot et al. 2021; Zhu et al. 2018). We expected that this trend would also be evident within species; however, we did not observe a significant positive correlation between  $T_{\text{leaf}}$  and  $T_{\text{crit}}$  for any of the measured species. This is similar to Kullberg and Feeley (2022) where only one of seven species had a significant correlation between  $T_{50}$  and max  $T_{\text{air}}$ , and none with  $T_{50}$  and modelled  $T_{\text{leaf}}$ . While this could reflect the importance of other variables besides  $T_{\text{leaf}}$  as drivers of variation in thermal tolerance (Lancaster and Humphreys 2020), it could also be a result of a relatively low sample size compared to the variation of these traits and may require further study across an even larger gradient of  $T_{\text{leaf}}$ .

The strength of within-species covariation between  $T_{\text{leaf}}$  and  $T_{\text{crit}}$  is important as it will determine patterns in the TSM, and thus how susceptible different provenances are to reaching critical leaf temperatures. While we observed differences across provenances in  $T_{\text{leaf}}$  and  $T_{\text{crit}}$ , no provenance-differentiation was observed when considering these traits together through their TSMs. This highlights that if attempting to consider vulnerability of provenances to heat stress for climate-matching of seed sources, an assessment based on either  $T_{\text{leaf}}$  or  $T_{\text{crit}}$  alone could misinform practitioners, resulting in a priority of one provenance over another. Although our findings suggest provenance-level variation in TSMs may not be a high priority for climate matching of seed sources within species, a provenance effect may be observed in other traits influencing survival and growth rates. Climate-matching for provenance selection should therefore consider suites of leaf and whole plant traits and their potential trade-offs, rather than TSMs alone.

### 4.4 | Future Recommendations

To achieve a comprehensive understanding of ecotypic variation, selected provenances should be representative of species-wide variation (Wadgyamar et al. 2022). In our study, we compared phenotypic variation between just two provenances (i.e., upland and lowland) due to practical constraints when working in highly diverse tropical systems. Fruiting phenology varies widely across species distributions due to both climate

and genetics (Abasolo et al. 2014; Duboscq-Carra et al. 2020; Stephens et al. 2022; Vogado et al. 2020), and gaining simultaneous access to a larger array of populations would require significant effort and/or focusing on a smaller cadre of species. Nevertheless, the two provenances we included (lowland sourced from the Daintree rainforest and upland sourced from the Atherton Tablelands) represent the two primary regions of seed collection and rainforest restoration efforts in the Australian Wet Tropics. Accordingly, our results are well-aligned with current seed sourcing practices of regional restoration practitioners. Future efforts should include coordination among researchers, national parks management, and restoration and forestry practitioners to allow for comparison amongst different planting programs, and to include the collection of and incorporation of information on population genetics.

## 5 | Conclusion

To avoid lethal leaf temperatures, plants may acclimate their thermal tolerance or leaf thermal traits in such a way to enhance leaf cooling. While emerging evidence suggests plants in warmer environments have greater capacity to regulate their leaf temperatures (Blasini et al. 2022; Kitudom et al. 2022), we only found evidence of this intraspecific adaptation across provenances in one of the four species studied here. To understand whether tropical forests may be vulnerable to climate warming and determine appropriate seed-sourcing strategies for tree-planting, we urgently need more research assessing within-species variation in thermoregulation across a larger range of species, populations and test sites. To achieve this, we encourage restoration and forestry practitioners and scientists to engage with each other to establish a greater network of research trials in the tropics.

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### Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## Supporting Information

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