Large differences in leaf cuticle conductance and its temperature response among 24 tropical tree species from across a rainfall gradient

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Summary

- More frequent droughts and rising temperatures pose serious threats to tropical forests. When stomata are closed under dry and hot conditions, plants lose water through leaf cuticles, but little is known about cuticle conductance \( g_{\text{c}} \) of tropical trees, how it varies among species and environments, and how it is affected by temperature.
- We determined \( g_{\text{c}} \) in relation to temperature for 24 tropical tree species across a steep rainfall gradient in Panama, by recording leaf drying curves at different temperatures in the laboratory.
- In contrast with our hypotheses, \( g_{\text{c}} \) did not differ systematically across the rainfall gradient; species differences did not reflect phylogenetic patterns; and in most species \( g_{\text{c}} \) did not significantly increase between 25 and 50°C. \( g_{\text{c}} \) was higher in deciduous than in evergreen species, in species with leaf trichomes than in species without, in sun leaves than in shade leaves, and tended to decrease with increasing leaf mass per area across species. There was no relationship between stomatal and cuticle conductance.
- Large species differences in \( g_{\text{c}} \) and its temperature response suggest that more frequent hot droughts may lead to differential survival among tropical tree species, regardless of species’ position on the rainfall gradient.

Introduction

Increasing frequency and intensity of extreme climate events threaten the continued contribution of forests to mitigating anthropogenic climate change through net carbon uptake and storage. Drought-induced tree mortality is already increasing across continents (Allen et al., 2010), and many areas of the world are predicted to get drier during the current century (Sheffield & Wood, 2008; Chadwick et al., 2016). Tropical forests are particularly important reservoirs of carbon and have profound influence on the global carbon cycle due to the large quantities of carbon flowing through the system. Droughts can have major impacts on tropical forests, especially in wet forests where species may lack the necessary adaptations to survive drought events (Corlett, 2016). Furthermore, droughts are increasingly occurring in combination with above-average temperatures, for example during El Niño events (Rifai et al., 2019), pushing plants in already hot environments further towards, or beyond, critical thresholds.

Plants are estimated to lose 62000 Pg water per year globally through transpiration (Jasechko et al., 2013). As temperature increases, the driving force of plant water loss, the leaf-to-air vapour pressure deficit, also increases. Species differ in how readily they close their stomata — the principal sites of leaf water loss — in response to increased vapour pressure deficit. Isohydric species minimise water loss and rapidly close their stomata to avoid high tension in the xylem, while anisohydric species keep their stomata open up until a much greater vapour pressure deficit is reached, to maximise carbon uptake at the cost of a strain on their xylem, with most species falling on a continuum between these extremes (Klein, 2014). While species at both extreme ends can co-occur (e.g. Lange, 1959; McDowell et al., 2008; Bueno et al., 2019), forests with relatively long dry seasons are expected to contain more isohydric species than aseasonal forests (Konings & Gentine, 2017). However, even when stomata are closed, water continues to be lost as a result of cuticular permeability (Boyer et al., 1997; Riederer & Schreiber, 2001; Burghardt & Riederer, 2006). Furthermore, stomatal closure may not be complete, with some water still escaping from ‘leaky stomata’ (e.g. Muchow & Sinclair, 1989; Machado et al., 2021).

At moderate temperatures (e.g. 20–25°C), the cuticular pathway of water loss is slow in healthy, undamaged leaves, as the cuticular waxes provide a powerful barrier to water movement (Boyer et al., 1997; Burghardt & Riederer, 2003; Riederer,
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Differences in cuticle conductance across environments (Duursma et al., 2019) or plant life forms (Schuster et al., 2017). However, in a pot experiment with tree saplings grown under similar conditions, cuticle conductance showed a strong, positive relationship with mean rainfall in the driest quarter in the native habitat of 42 tree species (Brodribb et al., 2014). To date, no study has directly examined whether cuticle conductance varies systematically across a rainfall gradient in the tropics.

In many species studied to date, a strong increase in the permeability of the cuticle has been observed at temperatures above c. 40°C, leading to a bi-phasic response across a broad temperature gradient (Schreiber, 2001; Riederer, 2006; Bueno et al., 2019; Billon et al., 2020). The bi-phasic response appears to be related to a change in the structure of either the polymer chains in the cutin matrix or the crystalline wax barrier (Schreiber & Schönherr, 1990; Schuster et al., 2016; Bueno et al., 2019), and has been observed in species from cold as well as hot climates. Nonetheless, species differ in the extent of high-temperature increase (Riederer, 2006; Billon et al., 2020), and some hot-adapted and drought-adapted species can maintain the integrity of the cuticular transpiration barrier up to temperatures of > 50°C (Bueno et al., 2019). The importance of cuticle conductance in drought survival is increasingly being recognised (Blackman et al., 2016; Martin-StPaul et al., 2017; Cochard, 2020; de Kauwe et al., 2020), and characteristics of the bi-phasic temperature response in particular have a strong impact on modelled tree mortality under heatwave conditions (Cochard, 2020). Even under current conditions, it is not uncommon for leaf temperatures of tropical forest trees to exceed 40°C (Doughty & Goulden, 2008; Krause et al., 2010; Rey-Sánchez et al., 2016; Slot et al., 2016) and rising temperatures and associated increases in vapour pressure deficit may lead to a strong and potentially lethal increase in cuticular water loss. Tropical forests are already seeing an increase in drought-related mortality (e.g. Brando et al., 2014; McDowell et al., 2018), but data on the temperature effect on cuticle conductance of tropical tree species is lacking.

We determined cuticle conductance and characterised its relationship to temperature for 24 tropical tree species growing along a steep rainfall gradient in the Republic of Panama. We tested the following hypotheses. First, cuticle conductance at a set temperature decreases with increasing dry-season intensity across the rainfall gradient. Second, species differences in cuticle conductance are associated with phylogeny, leaf habit (i.e. evergreen versus deciduous species) and leaf traits. Third, cuticle conductance exhibits a bi-phasic response to temperature, with stable, low rates at moderate temperatures, followed by rapid increases above c. 40°C, consistent with previous observations. In addition, for five species we determined whether cuticle conductance of shade leaves – that typically experience lower maximum temperatures – exhibits the same response to temperature as that of sun leaves. Finally, for a subset of the species we compared temperature responses of cuticle conductance with those of total leaf conductance measured in the field (stomatal + cuticular). Leaf conductance typically decreases in response to increasing temperature, but a sudden increase when temperatures exceed c. 40°C has also been reported for some laboratory-measured tropical tree seedlings (Slot et al., 2016; Slot & Winter, 2017c), and other studies have similarly observed high conductance under hot conditions (Urban et al., 2017; Aparecido et al., 2020; Sadok et al., 2021). It remains unclear whether the measured increase in water loss can be assigned to stomatal opening or increased permeability of the cuticles.

Materials and Methods

Study sites and species selection

In the rainy seasons of 2018 and 2019, we collected healthy, fully expanded sun-exposed leaves from mature, field-grown trees at three sites along a steep rainfall gradient across the isthmus of Panama. Panama City (PC) (8.9637°N, 79.5458°W), at the Pacific coast, is the driest site, with c. 1800 mm per year, of which > 90% falls during the rainy season from May to December. Parque Nacional Soberanía (PNS) (9.1240°N, 79.6965°W) receives intermediate rainfall of c. 2200 mm yr⁻¹. Parque Nacional San Lorenzo (PNSL) (9.3640°N, 79.9593°W), near the Atlantic coast, has a mean annual precipitation of 3300 mm, with a relatively short dry season from January to March with < 100 mm rainfall per month. Maximum vapour pressure deficit of the air tends to be highest in PC and lowest in PNSL (Grossiord et al., 2019). Mean annual temperature (and mean maximum temperature of the warmest month) is 27.0°C (31.6°C) in PC, 26.6°C (30.7°C) at PNS, and 26.4°C (30.1°C) at PNSL (data from WorldClim2; Fick & Hijmans, 2017). By 2050, both mean and maximum temperatures across the rainfall gradient are predicted to be c. 2.3°C higher than the 1980–2012 mean, as based on HADGEM2-ES model projections under the RCP 8.5 emission scenario (Anderson-Teixeira et al., 2015). At each site we selected at least five species, aiming for diverse representation across the phylogeny. In total, 24 species from 21 genera and 17 families were included in this study (Table 1). Branches of most species were collected with an extendable pole pruner in sun-exposed forest edges and road sides; at PNSL some species were accessed using a construction crane maintained by the Smithsonian Tropical Research Institute. We expect that most leaves developed in the early wet season, but cannot rule out that some of the evergreen species retained leaves that developed in the dry season preceding our measurements.
Table 1 Study sites, species studied, the family to which they belong (nomenclature following POWO, Kew’s Plants of the World Online), their leaf habit (E, evergreen, D, deciduous, FD, facultatively deciduous), the presence of trichomes and the side of the leaves on which they occur, and the method used to determine cuticle conductance (see text for details).

| Site                        | Species                                    | Family              | Leaf habit | Trichomes | Method               |
|-----------------------------|--------------------------------------------|---------------------|------------|-----------|----------------------|
| Panama City                 | *Calophyllum inophyllum* L.                     | Calophyllaceae      | E          | Whole leaf|                     |
|                             | *Glicidica sepium* (Jacq.) Kunth              | Fabaceae            | E          | Whole leaf|                     |
|                             | *Hymenaea courbari* L.                       | Fabaceae            | E          | Whole leaf|                     |
|                             | *Tectona grandis* L.f.                       | Lamiaceae           | D          | Abaxial   | Envelope            |
|                             | *Sweitienia macrophylla* King                | Meliaceae           | E          | Whole leaf|                     |
|                             | *Ficus benjamina* L.                         | Moraceae            | E          | Whole leaf|                     |
|                             | *Matayba scrobiculata* Radik.                | Sapindaceae         | E          | Whole leaf|                     |
|                             | *Citharexylum caudatum* L.                   | Verbenaceae         | E          | Whole leaf|                     |
| Parque Nacional Soberanía   | *Annona spraguei* Saff.                     | Annonaceae          | D          | Abaxial   | Envelope            |
|                             | *Calophyllum longifolium* Wild.              | Calophyllaceae      | E          | Whole leaf|                     |
|                             | *Clusia pratensis* Seem.                    | Clusiaceae          | E          | Whole leaf|                     |
|                             | *Clusia rosea* Jacq.                        | Clusiaceae          | E          | Both      |                     |
|                             | *Garcinia intermedia* (Pittier) Hammel       | Lyrhaceae           | E          | Envelope  |                     |
|                             | *Lagerstroemia speciosa* (L.) Pers.          | Lyrhaceae           | E          | Envelope  |                     |
|                             | *Luehea seemannii* Triana & Planch.          | Malvaceae           | E          | Abaxial   | Both                |
|                             | *Castilla elastica* Cerv.                   | Clusiaceae          | E          | Both      |                     |
|                             | *Ficus insipida* Wild.                      | Moraceae            | D          | Both      | Envelope            |
|                             | *Chrysophyllum cainito* L.                   | Moraceae            | E          | Both      |                     |
|                             | *Simarouba amara* Aubl.                     | Moraceae            | E          | Both      |                     |
| Parque Nacional San Lorenzo | *Aspidosperma spruceanum* Benth. ex Müll. Arg.| Moraceae            | E          | Both      |                     |
|                             | *Prottium panamense* (Rose) I.M. Johnst.     | Burseraceae         | E          | Envelope  |                     |
|                             | *Manilkara bidentata* (A.DC.) A. Chev.       | Sapindaceae         | E          | Envelope  |                     |
|                             | *Pourouma bicolor* Mart.                    | Urticaceae          | FD         | Abaxial   | Envelope            |
|                             | *Vochysia ferruginea* Mart.                 | Vochysiacae         | E          | Abaxial   | Whole leaf          |

For species in bold, shade leaves were measured in addition to sun leaves.

*Not native to Panama.

Sampling and measurement protocol

Branches were collected in the morning, when possible from multiple individuals (range = 1–3, mean = 1.5, median = 1). There is no evidence for strong intraspecific variation in cuticle conductance of undamaged leaves of similar age at a given environment, so we prioritised sampling more species at each position along the rainfall gradient over sampling more individuals of fewer species. The branches were transported to the laboratory in opaque plastic bags with moist tissue paper, where they were kept in the dark until further processing. Cuticle conductance can be determined very precisely on isolated cuticles, but direct comparison by Schuster et al. (2017) suggests that minimum conductance derived from drying detached leaves with sealed petioles ($g_{\text{min}}$) is a good proxy of cuticle conductance; see Kerstiens (1996) and Schuster et al. (2017) for more details on different methods to determine the rate at which water can move through leaf cuticles, and the different units to express these rates. We determined $g_{\text{min}}$ using the ‘bench drying method’ (Sack & Scoffoni, 2011). We surface cleaned leaves with distilled water, pat dried them with Kimwipes®, and measured leaf area with an LI-3100C leaf area meter (Li-Cor Inc., Lincoln, NE, USA). These leaves were then hung on a horizontal bar above two upward-blowing fans, and leaves were repeatedly weighed to determine the rate at which water was lost. After the last leaf mass was recorded, leaf area was measured again. The fans, and the spacing of the leaves, minimised the boundary layer around the leaves. $g_{\text{min}}$ was calculated as the rate of water loss per unit leaf area per unit time (‘transpiration’), divided by the vapour pressure deficit, in which the vapour pressure deficit was calculated from air temperature, relative humidity, and atmospheric pressure using the Tetens equation following Monteith & Unsworth (2013).

We calculated $g_{\text{min}}$ in units of mmol m⁻² s⁻¹, for easy comparison with stomatal conductance and transpiration data. Minimum leaf conductance can also be expressed as ‘permeance’, with units of m s⁻¹, which is not affected by atmospheric pressure, as water transport is assumed to take place in the solid phase of the cuticle (see Kerstiens, 1996; Schuster et al., 2017). Permeance is commonly used when minimum conductance is determined on isolated cuticles. We use $g_{\text{min}}$ in mmol m⁻² s⁻¹ throughout, but indicate what the equivalent permeance values would be when reporting the range of values observed across species.

We used two different approaches for determining cuticular water loss. The basic approach of Sack & Scoffoni (2011) involved sealing the petioles, for which we used Parafilm®, and removing the initial curvilinear part of the water-loss curve, which is indicative of stomatal contribution to water loss. Furthermore, leaves were dried in the dark to avoid light-stimulated stomatal opening. This approach, from this point forwards referred to as the ‘whole-leaf method’, was used for 13 species (see Table 1) and for all shade leaves. The other approach
involved folding strips of aluminium tape around the leaves, leaving an area exposed of theastomatous, adaxial side (Diar et al., 2021); all species in our study were hypostomatous. This approach effectively seals off the side of the leaf with stomata, thereby reducing the pathway of water loss to the exposed, stomata-free leaf area. After repeatedly weighing these ‘envelopes’ containing the leaves, the exposed leaf area was measured with a LI-3100C leaf area meter. For 15 species we used this envelope method (Table 1). Of the four species for which both methods were used, three had measurements across a wide enough temperature range (≥ 25°C) to compare the approaches; \( g_{\text{min}} \) appeared moderately, but nonsignificantly lower with the envelope method for one species (Gliricidia sepium), with no differences observed for the other two species, and no obvious differences in temperature responses (Supporting Information Fig. S1). The fact that the approach that seals off the stomatal side of the leaves yielded similar results to the whole-leaf method suggested that leaky stomata were an insignificant contribution to measured water loss.

\( g_{\text{min}} \) was determined at 5–11 temperatures between 25 and 50°C (mean: 6, median: 5). Different temperatures were obtained by air drying the leaves in darkened growth cabinets (20–39°C) (Environmental Growth Chambers, Chagrin Falls, OH, USA) and forced convection drying ovens (40–51°C) (Yamato Scientific Co. Ltd, Tokyo, Japan). Air temperature and relative humidity were monitored by built-in growth chamber sensors. Drying ovens had their own temperature sensors as well, while relative humidity was measured with a portable thermo-hygrometer (Casio ID-16, Tokyo, Japan). Not all temperature treatments could be applied on the same day, so every species was collected on multiple days, and the order in which different temperature treatments were applied was randomised.

At each temperature \( g_{\text{min}} \) was determined on 5–24 leaves per species (mean: 11.1, median: 12) and leaf mass was determined 5–11 times (mean: 8.2, median: 9) to generate dry-down curves for each leaf. The time intervals between measurements varied depending on temperature (shorter intervals at higher temperatures), and on the method used (shorter intervals with the whole-leaf method). Leaves were weighed less frequently when using the envelope method, as this method tended to yield water-loss curves that were linear from the first measurement onward. For the five species indicated in Table 1, we also determined \( g_{\text{min}} \) of leaves from the shaded inner tree canopy, using the whole-leaf method at 5–9 different temperatures, with 4–7 leaves per species at each temperature. In total, our dataset contained more than 15,000 leaf mass records.

**Functional traits**

For each species leaf area was measured to the nearest 0.1 cm\(^2\) with a LI-3100C for 5–10 healthy, fully expanded leaves. Leaf fresh mass was measured on a microbalance. Then the leaves were oven-dried at 60°C for ≥ 72 h to determine dry mass. From these measurements we calculated leaf mass per unit leaf area (LMA; leaf mass in g divided by leaf area in m\(^2\)), and leaf dry matter content (oven-dry mass divided by fresh mass).

Deciduousness of the species was based on personal observations, and expert opinion of S.J. Wright & O. Calderón (Smithsonian Tropical Research Institute). The presence or absence of trichomes was examined with a hand lens and dissection microscope. We scored species as having trichomes or not, and whether the trichomes were on the abaxial or adaxial leaf surface, and did not assess trichome length or density. For 12 species we were able to quantify stomatal densities from nail varnish impressions (\( n = 3 \) leaves per species) using light microscopy.

**In situ leaf conductance data**

For 12 of the study species we have previously measured temperature-response curves of photosynthesis (Slot & Winter, 2017a,b; Slot et al., 2019; Hernández et al., 2020). We only used data from species for which photosynthesis was measured on sun-exposed, field-grown trees, to make the comparison with \( g_{\text{min}} \) in the current study relevant. From these curves we extracted leaf conductance (the sum of stomatal and cuticle conductance) at ambient atmospheric CO\(_2\) concentration of c. 400 ppm. Measurements were conducted with either an LI-6400XT or an LI-6800 portable photosynthesis system (Li-Cor Environmental Sciences, Lincoln, NE, USA). To maximise the temperature range during measurements, ambient temperature changes were followed during the morning hours, with additional cooling and heating provided by the Peltier control system in the leaf cuvette. Leaf temperature was monitored abaxially with a Type E thermocouple. We did not attempt to control for vapour pressure deficit, which consequently increased with temperature during these measurements. For more details we refer to the original publications. All species were measured at the study sites from where they were collected in the current study, with the exception of C. elastica and C. caimito, for which photosynthesis was measured in Panama City, but \( g_{\text{min}} \) was determined for samples collected from Parque Nacional Soberanía.

We fitted 2\(^{nd}\) order polynomial curves to the temperature-response data of total conductance using ordinary least squares errors, and linear regressions (one or two, depending on statistical support for a bi-phasic response – see later) to the temperature response of cuticle conductance.

**Phylogenetic and statistical analyses**

We constructed a phylogenetic tree of the 24 species in our study with the V. PHYLOMAKEr package (Jin & Qian, 2019) in R. To test for phylogenetic signals in \( g_{\text{min}} \) at ambient temperature we calculated Pagel’s \( \lambda \) (Pagel, 1999) using the PHYTOOLS package (Revell, 2012). \( \lambda \) is a scaling factor that transforms the phylogeny to fit the trait data to a Brownian motion-based model of trait evolution, and as such it provides a robust assessment of the degree to which related species resemble one another (Münkemüller et al., 2012). To test for differences in cuticle conductance among sites we used analysis of variance. Effects of leaf habit (evergreen vs deciduous), trichomes, and exposure (sun vs shade) were also tested with analysis of variance. Relationships between cuticle conductance and the leaf traits LMA and leaf dry
matter content were tested with ordinary least squares regression. To determine whether the temperature response of cuticle conductance conformed to a bi-phasic response, we performed piecewise regression analysis using the `segmented` package in R (Muggeo, 2008). All analyses were performed in R v.4.0.2 (R Development Core Team, 2020).

**Results**

In all species, water loss increased with increasing temperature in both sun and shade leaves (Fig. 1). The response to temperature was almost identical to the response to vapour pressure deficit (VPD) (Fig. S2). Water loss in shade leaves tended to be lower than that of sun leaves. As temperatures increased, differences in water loss among species became larger, with mean water loss at 50°C ranging from 0.003 mmol m⁻² s⁻¹ in *Swietenia macrophylla* to 0.022 mmol m⁻² s⁻¹ in *Luehea seemannii*.

**Species differences in basal conductance**

Across 24 species $g_{\text{min}}$ at 25°C ($g_{\text{min,25}}$) of sun leaves averaged 4.0 mmol m⁻² s⁻¹, with a median of 2.8 mmol m⁻² s⁻¹ (10–90th percentile: 1.1–7.6 mmol m⁻² s⁻¹). This is equivalent to a mean permeance of $1.0 \times 10^{-14}$ m s⁻¹ and a median of $6.9 \times 10^{-5}$ m s⁻¹ (10–90th percentile: $2.6 \times 10^{-5}$ to $1.9 \times 10^{-4}$ m s⁻¹). $g_{\text{min,25}}$ varied significantly among species ($F_{23,309} = 46.7$, $P < 0.001$), with values ranging from 0.42 ± 0.03 mmol m⁻² s⁻¹ (mean ± SD) in *S. macrophylla* (mahogany) to 15.6 ± 5.1 mmol m⁻² s⁻¹ in *C. elastica* (Panama rubber tree/Mexican rubber tree) (Fig. 2). In some species, such as *P. bicolor* and *C. elastica*, variation among leaves within species was considerable, but reflected a normally distributed range of values and was not driven by obvious outliers. Across sites, $g_{\text{min,25}}$ ranged from 2.3 ± 1.8 mmol m⁻² s⁻¹ in Panama City to 5.2 ± 4.0 mmol m⁻² s⁻¹ in Parque Nacional Soberanía, but overall site differences were not significant ($F_{2,21} = 1.40$, $P = 0.27$) (Fig. 2).

The phylogenetic tree of the species in the study (Fig. 3) shows that there is some clustering of species with similar $g_{\text{min,25}}$, most notably the high values of *P. bicolor* and *C. elastica*, both in the order Rosales. The three sets of congeneric species also tended to have comparable values (see *Calophyllum*, *Clusia* and, to a lesser extent, *Ficus*, in Fig. 3). Nonetheless, formal tests indicated that the phylogenetic signal was weak (Pagel’s $\lambda \approx 0$), and not significant ($P = 1$ for $\lambda$).

**Cuticle conductance in relation to plant traits**

Evergreen species had lower $g_{\text{min,25}}$ than species that were classified as deciduous (including facultative and obligatory deciduous species) ($F_{1,22} = 15.3$, $P < 0.001$), as illustrated in Fig. 4. This figure further shows that species with trichomes on either or both leaf surfaces (see Table 1) had significantly higher $g_{\text{min,25}}$ than species without trichomes ($F_{1,22} = 12.5$, $P = 0.002$).

Across species, $g_{\text{min}}$ tended to decrease with increasing LMA but although this trend was consistent, it was only significant for $g_{\text{min}}$ at 35°C ($F_{1,21} = 6.6$, $P = 0.0178$, $R^2 = 0.23$) and marginally significant ($P < 0.1$) at 32 and 40°C (Fig. 5). $g_{\text{min}}$ did not scale with leaf dry matter content or stomatal density at any temperature (data not shown).

**Temperature response of cuticle conductance**

Across the 25–50°C temperature range, only seven of the 24 species exhibited the expected bi-phasic response of $g_{\text{min}}$ with a significant increase at high temperature (Fig. 6). The temperature at which $g_{\text{min}}$ started to increase averaged 42.1 ± 3.1°C (SD, $n = 7$), and ranged from 37.6°C (34.2–41.0°C, 95% confidence interval) in *C. cainito* to 44.8°C in *A. spruceanum*, *C. inophyllum*, and *F. benjamina* (Fig. 6). Although statistically significant, the breakpoint in *C. cainito* followed a decrease in $g_{\text{min}}$ so despite the significant increase beyond the breakpoint, $g_{\text{min}}$ at 50°C was not higher than it was at 25°C.

**Sun vs shade leaves**

Shade leaves had significantly lower $g_{\text{min}}$ than sun leaves (ANOVA, $F_{1,605} = 134.5$, $P < 0.001$) (Fig. 6). There was no significant interaction between canopy position (sun vs shade) and measurement temperature. Significant breakpoints were observed for *F. insipida* (45.1°C, 95% CI: 39.6–50.6°C) and *G. sepium* (42.2°C, 38.2–46.2°C), while the increase of $g_{\text{min}}$ in *C. rosea* when temperatures reached 48 and 51°C was not significant due to increased leaf-to-leaf variation (Fig. 6). Sun leaves of these species did not exhibit significant two-phase responses, but there was nonetheless no indication that the temperature responses differed significantly between sun and shade leaves (see Fig. 6).

**Temperature responses of leaf conductance measured in the field**

In all 12 species for which we previously measured temperature responses in the field, leaf conductance declined at high temperatures (Fig. 7). For several species no peaked response curve could be fitted because there were not enough measurements below the optimum temperature. Maximum conductance ranged from 80 ± 21 mmol m⁻² s⁻¹ in the late-successional species *M. bidertextata*, to 508 ± 247 mmol m⁻² s⁻¹ in the pioneer species *F. insipida*, both at 29°C (Fig. 7). The temperature at which conductance reached 0, as based on the fitted curves, averaged 40.1 ± 2.1°C, and ranged from 37.3°C in *V. ferruginea* to 45.5°C in *L. seemannii*.

Comparing the fitted curves for *in situ* conductance and $g_{\text{min}}$ determined on the same species in the laboratory shows that while cuticle conductance would account for 100% of total conductance when temperatures exceed 38–45°C and stomata are predicted to be fully closed, total conductance remains relatively low. Even at the highest measurement temperature $g_{\text{min}}$ was more than an order of magnitude smaller than peak leaf conductance (Fig. 7).
Fig. 1 Cuticular water loss in relation to temperature for 24 lowland tropical tree species from wet (purple), intermediate (teal), and seasonally dry tropical forest (yellow). Error bars represent SD (n = 5–24). Grey datapoints in (k), (l), (o), (r) and (u) represent shade leaves.
Discussion

In contrast with our hypotheses, cuticle conductance, \( g_{\text{min}} \), did not differ systematically across a rainfall gradient in Panama (hypothesis 1); differences in \( g_{\text{min}} \) across species were not associated with phylogenetic relationships among the species (hypothesis 2); and \( g_{\text{min}} \) of most species exhibited moderate temperature sensitivity between 25 and 50°C; a significant bi-phasic temperature response was found in only seven of the 24 species (hypothesis 3). Deciduous and trichomous species had higher \( g_{\text{min}} \) than evergreen and nontrichomous species; \( g_{\text{min}} \) at intermediate temperatures decreased with increasing LMA across species; and shade leaves had lower \( g_{\text{min}} \) than sun leaves. Comparing field-measured leaf conductance with \( g_{\text{min}} \) revealed no relationship across 12 species, and \( g_{\text{min}} \) was too low to explain past observations of increased leaf conductance at very high temperatures. Nevertheless, large differences among species in cuticular water loss at high temperature have the potential to contribute to differential mortality during heat waves or drought, phenomena that are increasingly common in the tropics (Rifai et al., 2019).

Cuticle conductance of tropical tree species

Observed \( g_{\text{min},25} \) values were within the range of values reported in recent reviews (Schuster et al., 2017; Duursma et al., 2019), showing that cuticle conductance is not necessarily lower in tropical species than in species from other biomes, as observed in some past studies (Schreiber & Riederer, 1996; Riederer, 2006).

Fig. 2 Cuticle conductance (\( g_{\text{min}} \)) at 25°C across sites (a) and across species (b). PNSL, Parque Nacional San Lorenzo, is the wettest site; PNS, Parque Nacional Soberanía, has intermediate precipitation; PC, Panama City, is the driest site. Boxes show the interquartile range (IQR) from the 1st to the 3rd quartile; horizontal bars represent the median, and whiskers extend to 1.5x IQR. Outliers are shown as open circles.

Similarly, \( g_{\text{min}} \) of the subset 21 tropical forest species in Schuster et al. (2017) was similar to that of all 93 woody shrubs and trees in the meta-analysis combined (median of 1.3 vs 1.6 mmol m\(^{-2}\) s\(^{-1}\), respectively). With comparable \( g_{\text{min},25} \), tropical forest trees may therefore be more prone to cuticular water loss than species from many other biomes, considering the high, and rising ambient temperatures, and the ongoing increase in VPD (Barkhordarian et al., 2019), the driving force of leaf water loss. \( g_{\text{min}} \) is also a parameter in Ball–Berry type stomatal conductance models, but one that is often ignored. Duursma et al. (2019) discussed potential errors that can arise from ignoring this parameter, and provided guidelines for using \( g_{\text{min}} \) in ecosystem models. Given the important role tropical forests play in the global carbon cycle, \( g_{\text{min}} \) of tropical trees should be accurately represented in such models and doing so requires datasets like ours.

Cuticle conductance across a tropical rainfall gradient

In crop species, cuticle conductance is associated with increased survival during experimental drought (Sinclair & Ludlow, 1986), and model simulations suggest similar importance for cuticle conductance in affecting the mortality of trees during drought (Cochard, 2020). Seasonal drought can therefore be expected to favour low cuticle conductance in evergreen species. However, interspecific variation in \( g_{\text{min}} \) did not exhibit clear patterns along the rainfall gradient as observed by Brodribb et al. (2014). A possible explanation is that, even during a 4-month dry season, mature trees as used in the current study may still have access to...
soil water, which would reduce the selective advantage of low cuticle conductance. Species at our driest site are more often deciduous (Bohlman, 2010), tend to have lower leaf area to sapwood area ratios (Meinzer et al., 2008) and are more isohydric than species at the wettest site (Wu et al., 2020), and these adaptations may represent an important first line of defence against desiccation during the dry season, when water is limiting but not completely inaccessible (Holtum & Winter, 2005; Chitra-Tarak et al., 2021). Even in some of the driest habitats on Earth, hot deserts, species with groundwater access do not have particularly low cuticle conductance (Schuster et al., 2016; Bueno et al., 2019). Schuster et al. (2017) found no evidence that cuticular permeability is adapted to ecological conditions in a meta-analysis of 160 plant species. Our results are consistent with this and suggest that precipitation alone is insufficient to predict cuticle conductance, and that future studies may need to consider soil water access as an additional factor shaping patterns in cuticle conductance.

Functional group differences: effects of leaf habit and leaf traits, not phylogeny

We found no statistical support for a phylogenetic signal in cuticle conductance. Duursma et al. (2019) reported differences in...


$g_{\text{min}}$ among plant orders, but no studies have reported phylogenetic patterns. The lack of phylogenetic signal may be explained by considerable phenotypic plasticity in $g_{\text{min}}$. For example, Duursma et al. (2019) reported a strong effect of growth temperature on $g_{\text{min}}$ of *Eucalyptus* *parramattensis*, and in our study $g_{\text{min}}$ differed between sun and shade leaves.

Evergreen species had significantly lower cuticle conductance than deciduous species, consistent with the greater importance of retaining water in species that do not shed their leaves during the dry season. Schuster et al. (2016) also reported lower median conductance in evergreen than in deciduous woody species of temperate and Mediterranean origin but, due to large variation within both categories, the differences were not significant. Similarly, Schuster et al. (2017) found no significant differences between evergreen and deciduous woody plants in a meta-analysis that pooled species from different origins, growth conditions and growth forms. Our tree-focused regional study suggests greater capacity to retain water in evergreen species, but more deciduous species will need to be investigated to establish whether this pattern is consistent in tropical forests.

Like deciduousness, the presence of trichomes was associated with high cuticle conductance. Seven species in our study had trichomes and, with the exception of *A. spraguei* and *C. cainito*, all of these had relatively high cuticle conductance in the 25–40°C range (Fig. 2). The thicker boundary layer of trichomous species may explain why these species can afford to have relatively high cuticle conductance. The extent to which trichomes reduce boundary layer conductance is small relative to the total leaf-to-atmosphere conductance pathway (Bickford, 2016; Amada et al., 2017), but may nonetheless be significant when stomata are closed during drought. Trichomes on upper leaf surfaces may also reflect incoming radiation, thereby moderating leaf temperatures and VPD (e.g. Pierce, 2007), and affecting potential cuticular water loss. However, all four deciduous species have trichomes, and with the relatively small number of deciduous and trichomous species, the independent effects could not be evaluated. Clearly, the relationship between cuticle conductance and presence of trichomes deserves more attention.

LMA is a key leaf functional trait of the leaf economics spectrum (Wright et al., 2004). The observation that high-LMA species have lower potential nonstomatal water loss than low-LMA species is consistent with the conservative growth strategies of high-LMA species. Trait variation within species is often opposite to patterns observed across species (Osnas et al., 2018) and, indeed, shade leaves had lower LMA and also lower $g_{\text{min}}$. Sack et al. (2003) instead found that shade leaves of the vines *Hedera helix* and *Vitis labruscra* had higher $g_{\text{min}}$ than sun leaves, consistent with the higher VPD and associated desiccation risk in the sun. Shade leaves of more species will need to be measured to determine general patterns.

Muchow & Sinclair (1989) reported that cuticle conductance increased with stomatal density among genotypes of *Sorghum bicolor*, and concluded that peristomatal transpiration – the relative high rate of cuticular water loss from surfaces of guard and subsidiary cells (e.g. Maerker, 1965) – represents an important pathway for water loss. Their observation may also suggest stomatal leakiness, which Machado et al. (2021) reported to be important in Cerrado species in Brazil. We did not detect a relationship between stomatal density and cuticle conductance at any temperature. This is consistent with the observation that the envelope method of leaf drying in which stomata are sealed, did not yield significantly lower $g_{\text{min}}$ estimates than the whole-leaf drying method.

**Cuticle vs total leaf conductance**

Field-measured leaf conductance decreased as temperature increased above c. 30°C, and leaf conductance was projected to reach zero at 37–45°C. Cuticle conductance was nonzero across the temperature range, and therefore the contribution of cuticle to total conductance increases to 100% when stomatal conductance is projected to reach zero. For most species conductance in the field was not measured at temperatures > 40°C, but leaf conductance of seedlings studied in the laboratory has been shown to markedly increase at such high temperatures (Slot et al., 2016, 2017c). After assigning this increase to stomatal conductance in 2016, we referred to it as ‘leakage’ in the later publication, as we could not rule out that the observation was caused by a temperature-induced increase in $g_{\text{min}}$. However, the $g_{\text{min}}$ range in the current study suggested that cuticular water loss is insufficient to account for the laboratory observations. For example, conductance of *F. insipida* measured in the laboratory increased by c. 100 mmol m$^{-2}$ s$^{-1}$ between c. 45 and 50°C (see fig. 1 in Slot et al., 2016), while $g_{\text{min}}$ of *F. insipida* was < 10 mmol m$^{-2}$ s$^{-1}$ at 50°C in the current study. It therefore appears that after monotonically decreasing between 30 and 45°C, stomata re-opened when temperatures further increased. Several studies have reported high, or increasing leaf conductance at high temperature (e.g. Urban et al., 2017; Aparecido et al., 2020; Sadok et al., 2021 and references therein), but the underlying mechanisms remain unknown. Stomatal opening may involve active control to stimulate transpirational cooling as lethal leaf
Fig. 6 Cuticle conductance ($g_{\text{min}}$) in relation to temperature for 24 lowland tropical tree species from wet (purple), intermediate (teal) and seasonally dry tropical forest (yellow), where $g_{\text{min}} = \text{cuticular water loss}$. Error bars represent standard deviations ($n = 5–24$). Grey datapoints in (k), (l), (o), (r) and (u) represent shade leaves. Vertical dashed lines and grey bars indicate statistically significant break points and the 95% confidence intervals of the break points, respectively. Solid black lines illustrate the piece-wise regression for which breakpoints were significant.
temperatures are approached (typically 46–55°C; for example O’Sullivan et al., 2017; Slot et al., 2021) or a passive, heat failure response, in which temperature-induced pressure build-up in substomatal cavities causes stomata to burst open (see Aparecido et al., 2020).

Small temperature response of cuticle conductance in tropical species

While \( g_{\text{min}} \) typically increases at high temperatures, not all species studied to date have shown a strong response. For example, Schuster et al. (2016) reported quite moderate increases of \( g_{\text{min}} \) in \textit{Rhazya stricta}, a desert shrub and Bueno et al. (2019) observed no increase in \( g_{\text{min}} \) of the date palm, \textit{Phoenix dactylifera}. Interestingly, despite significant temperature responses, the tropical tree species in Riederer (2006) did not reach high cuticular conductance at high temperatures and had amongst the highest activation energies in the 35–55°C range among the studied species. Species differences in thermostability of cuticular wax layers appeared to be related to the wax and cutin chemistry (Schuster et al., 2016; Bueno et al., 2019). \textit{P. dactylifera} is very conservative in its water use, and with its long-lived leaves and its very hot and arid environment, the advantage of a very effective cuticular water barrier is obvious. It is less clear why more than two-thirds of the tropical forest species in the current study should exhibit high thermostability of their cuticles. Composition of cuticular waxes varies greatly among species (Buschhaus & Jetter, 2011) and more research is needed to understand the variation in cuticle and cutin chemistry among tropical tree species in relation to their cuticle conductance and its temperature sensitivity.

Conclusions

With rising temperatures and increased frequency and intensity of droughts, the capacity to retain water will become increasingly important for survival of tropical trees. Along with other plant hydraulic traits, cuticle conductance and its temperature sensitivity may strongly affect survival during heat waves or hot drought (Cochard, 2020), and our study highlights large differences among species in both. Cuticle conductance at 25°C varied more than 30-fold, and the temperature response was moderate in most, but not in all species. Nonrandom species loss can
significantly reduce carbon storage in tropical forest (Bunker et al., 2005), so it will be crucial to investigate how cuticle properties contribute to drought responses of tropical forest trees. To generalise the patterns in cuticle conductance we observed among tropical trees, more species need to be examined. New tools may prove useful in these efforts, such as DROUGHTBox (Billon et al., 2020), a low-tech, low-cost device for determining the temperature dependence of nonstomatal conductance during drought. Attention should also be paid to shade leaves; with a leaf area index of <6 m² m⁻² in most forests, shade leaves vastly outnumber sun leaves and, collectively, shade leaves contribute significantly to canopy gas exchange. Our intriguing results of lower $g_{\text{min}}$ in shade leaves warrant further studies on the conductance and chemical composition of cuticles of tropical sun and shade leaves.

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Author contributions

MS and KW designed the study, with input from AB; MS, TN and GGH collected the samples; TN and GGH conducted the measurements; MS performed the analyses and wrote the manuscript, with contributions from TN, GGH, AB, MR and KW.

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Data availability

Tables S1 and S2 contain mean, SD and SEM of leaf cuticle conductance data at each temperature for sun and shade species, respectively. Raw leaf mass data are available on FigShare, https://doi.org/10.25573/data.14642499.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Comparison of temperature responses of cuticle conductance determined with two different methods.

Fig. S2 Cuticular water loss in relation to VPD for 24 lowland tropical tree species.

Table S1 Mean, SD and SEM of g_{min} by temperature for sun leaves of 24 tropical tree species.

Table S2 Mean, SD and SEM of g_{min} by temperature for shade leaves of five tropical tree species.

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