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Growth response and acclimation of CO₂ exchange characteristics to elevated temperatures in tropical tree seedlings

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Abstract

Predictions of how tropical forests will respond to future climate change are constrained by the paucity of data on the performance of tropical species under elevated growth temperatures. In particular, little is known about the potential of tropical species to acclimate physiologically to future increases in temperature. Seedlings of 10 neo-tropical tree species from different functional groups were cultivated in controlled-environment chambers under four day/night temperature regimes between 30/22 °C and 39/31 °C. Under well-watered conditions, all species showed optimal growth at temperatures above those currently found in their native range. While non-pioneer species experienced catastrophic failure or a substantially reduced growth rate under the highest temperature regime employed (i.e. daily average of 35 °C), growth in three lowland pioneers showed only a marginal reduction. In a subsequent experiment, three species (Ficus insipida, Ormosia macrocalyx, and Ochroma pyramidale) were cultivated at two temperatures determined as sub- and superoptimal for growth, but which resulted in similar biomass accumulation despite a 6°C difference in growth temperature. Through reciprocal transfer and temperature adjustment, the role of thermal acclimation in photosynthesis and respiration was investigated. Acclimation potential varied among species, with two distinct patterns of respiration acclimation identified. The study highlights the role of both inherent temperature tolerance and thermal acclimation in determining the ability of tropical tree species to cope with enhanced temperatures.

Key words: Functional trait, Panama, photosynthesis, respiration, temperature, tropical forest

Introduction

Lowland tropical forests cover just 10% of all land area (Mayaux et al., 2005) but represent a far larger, and a highly dynamic, component of the terrestrial carbon cycle (Grace, 2004; Pan et al., 2011). Anthropogenic alterations of greenhouse gas concentrations and surface albedo have contributed to recent warming in the tropics (i.e. 0.26 °C per decade since 1975; Malhi and Wright, 2004), with tropical lowland forests soon to see the emergence of temperature regimes unprecedented since the mid Quaternary (Diffenbaugh and Scherer, 2011). Without tolerance of, or acclimation to, such temperatures, species must rapidly migrate to cooler climates along elevational or latitudinal gradients, or face extirpation (Walther et al., 2002; Colwell et al., 2008; Chen et al., 2011).

A species’ thermal niche is the result of both adaptation and acclimation in physiological, morphological, and biochemical traits (Berry and Björkman, 1980; Criddle et al.,
1994; Premoli and Brewer, 2007). Unlike boreal or temperate species, tropical trees exist in a relatively stable climatic envelope with little seasonal variation in temperature (Wright et al., 2009). It is hypothesized that tropical trees have adapted to operate within a narrow range of temperatures with only a limited potential to acclimate (Janzen, 1967; Ghalambor et al., 2006). Indeed, climatic envelope or species distribution models often highlight the narrow range of temperatures to which tropical lowland forests are restricted, emphasizing their vulnerability to increasing temperatures (Colwell et al., 2008; Wright et al., 2009; Laurance et al., 2011; Zelazowski et al., 2011). However, analysis of realized and potential distributions of temperate species over the late Quaternary (Veloz et al., 2012) has shown that care must be taken with extrapolating current distributions to estimates of biologically viable climatic envelopes. Fossil records within eastern Colombia and western Venezuela demonstrate that diverse neo-tropical forests existed during the Paleocene–Eocene Thermal Maximum (PETM; ~55 million years ago) when mean annual temperatures may have been as high as 31–34 °C (cf. current 27 °C) (Jaramillo et al., 2010). Assuming parity between extant and ancestral species, current distributions of at least certain groups must represent a truncated thermal niche (Feeley and Silman, 2010) with no modern analogue of the hot-humid tropics seen in the palaeorecord.

The ability to predict accurately how the structure and function of tropical forests may change is constrained by the paucity of data on tropical plant responses to increasing temperatures (Reed et al., 2012). For example, in a recent meta-analysis on the impact of warming on terrestrial plants, Lin et al. (2010) employed just nine observations of growth and biomass allocation at mean annual temperatures (MATs) >18 °C, as compared with 528 observations at MATs <18 °C. While the lack of data on tropical trees is recognized as a limitation in such an analysis (Lin et al., 2010; Way and Oren, 2010), there has yet to be substantial improvement in the number (or range) of tropical species for which the fundamental response to elevated temperature is known. In the small number of empirical studies that have sought to elucidate fundamental thermal niches of tropical tree species, maximum growth has often been found under the highest temperature regime tested (Herwitz, 1993; Cunningham and Read, 2003; Allen and Vu, 2009; Esmail and Oelbermann, 2011; Cheesman and Winter, 2013). In these cases, the thermal optimum for growth was found to be higher than that predicted by either climatic home range or photosynthetic thermal optimum (Cunningham and Read, 2002).

Thermal acclimation is defined as the ability of an organism to adjust morphological, physiological, and biochemical characteristics in a way which optimizes performance under a given temperature regime (Lambers et al., 2008). In plants, thermal acclimation may include a range of processes, from the alteration of intracellular biochemistry (e.g. expression of heat shock or chaperone proteins, changes in metabolic efficiency, accumulation of osmolytes, and alteration of organelle morphology and membrane fluidity; Billings et al., 1971; Berry and Björkman, 1980; Atkin and Tjoelker, 2003; Hikosaka et al., 2006; Gunderson et al., 2010; Martinière et al., 2011) and organ development (Haman and Traas, 2010), to leaf physiognomy (Royer et al., 2009; Pepe et al., 2011) and biomass allocation (Poorter et al., 2012). The net result is an integrated organismal response to temperature (Gunderson et al., 2010), with component processes often showing distinct response profiles to temperature (Batten and Lahav, 1994).

As demonstrated in recent sensitivity analyses of coupled climate–carbon cycle models (Good et al., 2011; Booth et al., 2012) and in attempts to predict changes in species distributions within the tropics (Feeley et al., 2012), there is a pressing need for improved mechanistic understanding of the responses and potential acclimation in tropical forest species to increasing temperatures. In this study, the fundamental thermal niche in seedlings of 10 neo-tropical tree species was determined and the following questions were addressed. (i) Are tropical tree seedlings operating near their thermal optimum? (ii) Do predicted increases in land surface temperature extend beyond the thermal limit for growth? (iii) To what extent does thermal acclimation of leaf photosynthesis and night-time respiration contribute to the observed response of tropical tree seedlings to increasing growth temperatures?

Materials and methods

Initial experiments into the fundamental thermal niche of seedlings involved the growth of 10 neo-tropical tree species under favourable conditions (i.e. medium light levels, high water and nutrient availability) and a range of temperature regimes within plant growth chambers. Subsequent detailed experiments into thermal acclimation involved growing additional individuals of three species at temperature regimes shown to be sub- and superoptimal for growth. Both developmental and short-term thermal acclimation were studied by testing leaf-level photosynthetic capacity and respiration rates under growth conditions and after 1 week of reciprocal transfer to the alternative thermal regime.

Species selection and initial material

Species were selected representing a range of functional types based upon their known role in Panamanian forests (Condit et al., 2010). Eight species typically occur within the lowlands of Panama and include fast-growing pioneers [i.e. Adenanthera pavonina L. (Fabaceae), Ficus insipida Wild. (Moraceae), Luehea seemannii Triana & Planch. (Tilaceae), Ochroma pyramidale (Cav. ex Lam.) Urb (Malvaceae), Tabebuia guayacan (Seem.) Hemsl. (Bignoniaceae)] and late-successional [i.e. Ormosia macrocalyx Ducke (Fabaceae), Garcinia intermedia (Pittier) Hammel (Clusiaceae)], and the subcanopy species Theobroma cacao L. (Malvaceae). In addition, two species, Lacistema aggregatum (P.J. Bergius) Rusby (Lacistemataceae) and Citharexylum sp. (Verbenaceae), were collected from a tropical montane cloud forest site to investigate their presumed greater susceptibility to elevated temperatures (Colwell et al., 2008). Seeds for the lowland species were collected from trees growing at 0–50 m above mean sea level (amsl) within forests surrounding Panama City, Panama. Seeds of Citharexylum sp. and L. aggregatum were collected from the Cerro Jeffe montane cloud forest at ~1000 m amsl (Pierce et al., 2002). It should be noted that although L. aggregatum was collected from a wild population growing at higher elevation, the species range does extend into the wet lowland tropics (Condit et al., 2010). All seeds were germinated with a standard soil mix, in a ventilated screen-house (70% natural sunlight) under the ambient temperature regimes recorded at the Santa Cruz Experimental
Field Facility, Smithsonian Tropical Research Institute, Gamboa, Panama (Supplementary Fig. S1 available at JXB online).

Further detailed study of thermal acclimation was carried out using two fast-growing pioneer species noted for their high temperature growth optimum (Fig. 1), *F. insipida* and *O. pyramidale*, and a late successional species *O. macrocarpyx* observed to grow at, yet be challenged by, high temperatures.

After emergence of cotyledons and establishment of primary leaves, seedlings were transferred to pots (1.75 or 2.2 litres) containing a non-sterilized mix of native top soil and 20% (by volume) vermiculite. The top soil used was collected from a local orchard and had a pH in water of 5.4, and was base rich with high levels of both anion exchange membrane-extractable phosphorus (17.8 mg kg⁻¹) and salt-extractable inorganic nitrogen (62 mg kg⁻¹; B.L. Turner, personal communication). Mixing with vermiculite (a known source of potassium) resulted in a fertile substrate with high field capacity and rapid drainage.

**Home range conditions**

In lowland Panama, air temperature (Tₐ) shows limited seasonality, with diel temperatures at one forested site typical of the lowlands ranging from an average 22–30 °C during the dry season to 24–29 °C during the wet season (Supplementary Fig. S1). In the tropics it is generally assumed that a rise in elevation of 1000 m equates to a decrease in mean Tₐ of ~5 °C due to adiabatic lapse, although is generally assumed that a rise in elevation of 1000 m equates to a decrease in mean Tₐ of ~5 °C due to adiabatic lapse, although

Due to practical constraints, temperature dependence of seedling growth was analysed within species across two time points assuming exponential growth. To allow for comparison between species at a similar ontogenetic stage (*Poorter et al., 1990*), final harvest occurred between 30 d (i.e. *O. pyramidale*) and 97 d (i.e. *G. intermedia*). Prior to final harvest, leaf temperatures of three fully expanded unshaded leaves of each plant (all leaves >2 cm²) were measured 2 h after the beginning of the light period using an MT6 hand-held infra-red sensor (Raytek, Santa Cruz, CA, USA), with values shown to be consistent with copper/constantan thermocouples attached to the leaf lamina. At harvest, height to apical meristem was determined and plants were separated into leaf lamina, stem+petioles, and roots. Total leaf area was determined using a Li-3100 leaf area meter (Li-Cor, Lincoln, NE, USA) and roots were separated from soil by hand sorting, dry sieving, and final washing. Samples were dried to constant mass at 70 °C, weighed, and stored under ambient lab conditions until further analysis. Biomass and leaf area data were used to calculate average leaf mass per area (LMA g m⁻²), biomass allocation patterns, and mean relative growth rates (RGR, g g⁻¹ d⁻¹) using Equation 1 (Blackman, 1919) and assuming an exponential growth rate is sufficient to describe non-limited seedling growth.

\[
RGR = \frac{\ln(\text{mass}_t) - \ln(\text{mass}_0)}{\text{time}}
\]  

**Fundamental thermal niche**

Over several experimental iterations, seedlings of the 10 study species were randomly assigned to one of four GC15 plant growth chambers (Environmental Growth Chambers, Chagrin Falls, OH, USA) housed at the Tupper Center of the Smithsonian Tropical Research Institute in Panama City. Chambers were maintained with a 12 h light period [Philips TL841 fluorescent bulbs, with 500 μmol photosynthetically active radiation (PAR) m⁻² s⁻¹ at plant level]. Although not reaching the high, but transient, levels of illumination observed in clearings in the tropics (i.e. 2000 μmol PAR m⁻² s⁻¹, Supplementary Fig. S1 at JXB online), the use of 500 μmol PAR m⁻² s⁻¹ results in an integrated daily light input of 21.6 mol PAR m⁻² d⁻¹ which is comparable with levels seen under natural illumination, while avoiding photooxidation in more shade-tolerant late-successional species. Temperature regimes were chosen to represent current lowland conditions in Panama and a range of elevated temperatures up to a maximum daily average potentially seen during the PETM (i.e. 35 °C), with diel temperature range (DTR) set at 8 °C. Day/night temperatures were 30/22, 33/25, 36/28, and 39/31 °C. The chambers were ventilated with well mixed lab air which was itself continuously replenished from outside to maintain [CO₂] at ~400 μl l⁻¹. Plants were set out in a uniform pattern within each chamber and individuals were rotated every 3 d to ensure that light levels, at pot height, were standardized between temperature treatments. Plants were maintained with adequate water supply by the addition of tap water every 2 d, or as required.

**Thermal acclimation**

An additional 12 seedlings of each of three target species, grown in 2.2 litre pots, were assigned to either sub- or superoptimal growth temperature regimes. Temperatures were chosen to bracket the optimal temperatures (Tₜₐₐ) derived in the first set of experiments, with *F. insipida* and *O. pyramidale*, noted for maintaining growth at high temperatures,
assigned to two chambers set at 33/25 °C and 39/31 °C and *O. macrocalyx* assigned to chambers set to 30/22 °C and 36/28 °C. Final measurements were initiated after 48, 49, and 90 d in *O. pyramidalis*, *F. insipida*, and *O. macrocalyx*, respectively. Temperature dependence of leaf-level gas exchange in all plants was measured using a Li-6400 portable gas-flow analyser (Li-Cor Biosciences) (see below for details). Three of the six plants within each chamber were maintained under their original temperature regime, while three plants were transferred to the lower or higher temperature regime, respectively. This resulted in four populations: plants grown under suboptimal temperatures and maintained at suboptimal (LL) or transferred to superoptimal temperatures for 1 week (LH), and plants grown under superoptimal temperatures and maintained at superoptimal (HH) or transferred to suboptimal temperatures (HL) for 1 week. After 1 week, temperature dependence of leaf-level gas flux was again determined on the previously targeted leaves.

**Leaf level gas exchange**

Plants were transferred ~6 h before the beginning of the scheduled light period to a common GC15 growth chamber with Tair set at 22 °C. Plants were acclimated for 1 h before the net respiration rate (*R*<sub>n</sub>) was determined in a single recently mature fully developed leaf on each plant using an Li-6400 analyser. The temperature of the cuvette (T<sub>cuv</sub>) was set to that of the growth chamber T<sub>air</sub> and reference [CO<sub>2</sub>] regulated at 400 µmol l<sup>−1</sup>. T<sub>air</sub> of the growth chamber and T<sub>cuv</sub> were then raised through sequential temperature increases, with *R*<sub>n</sub> measured at 25, 28, and 31 °C. Plants were allowed to equilibrate for 30 min at each set point, with measurements made on the same leaves in approximately the same location. Chamber lights were then turned on and T<sub>air</sub> was set to 30 °C. After 2 h equilibration, a similar procedure was employed to determine the temperature dependence of net photosynthesis under chamber conditions using an external light source (red/blue LED) at 500 µmol PAR m<sup>−2</sup> s<sup>−1</sup> (*A*<sub>iso</sub>) and T<sub>cuv</sub> of 27, 30, 33, 36, and 39 °C. Plants were allowed to equilibrate for at least 30 min at each temperature stage before measurement of leaf-level gas exchange.

**C, N, and stable isotope analysis**

Leaves used for gas exchange measurements were dried and ground to a fine powder before being analysed for elemental concentrations of C and N, as well as δ<sup>13</sup>C and δ<sup>15</sup>N, with an elemental analyser (ECS 4010, Costech Analytical Technologies, Valencia, CA, USA) coupled to an isotope ratio mass spectrometer (Delta XP, Finnigan MAT, Bremen, Germany).

**Data analysis**

**Fundamental thermal niche**

The temperature dependence of growth rates was analysed using a simple analysis of variance (ANOVA), with comparisons between treatments made using Tukey HSD post-hoc analysis. The temperature dependence of mean RGR was modelled using a simple third-order polynomial with one optimum and zero growth at Tmin and Tmax (Equation 2).

\[
RGR_T = \alpha T (T - T_{min})(T_{max} - T)
\]  

(2)

Based upon work by van der Heide *et al.* (2006), this approach was found to be more robust than using a simple inverse parabolic curve. The model was fitted using generalized least squares with standard initial parameters [T<sub>min</sub>=10, T<sub>max</sub>=40, and α (an empirical scaling constant)=2]. Rearrangment of the fitted parameters allows for derivation of T<sub>opt</sub> (Equation 3).

\[
T_{opt} = \frac{1}{3} T_{max} + T_{min} + \sqrt{\frac{T_{max}^{2} - T_{min} \cdot T_{max} + T_{min}^{2}}{2}}
\]  

(3)

T<sub>opt</sub> was then compared between plant functional types (i.e. early-successional, late-successional, and montane) using non-parametric statistics (Kruskal–Wallis test).

To explore generalized changes in morphological characteristics (i.e. LMA and biomass allocation) across the fundamental thermal niche, a mixed-effects model was applied, with treatment and plant functional type as fixed factors and species as a random factor. To improve homoscedasticity, an assumption of heterogeneous variance (varident function) was used. The Akaike information criterion (AIC) was employed to select the most parsimonious model and derive appropriate P-values (Crawley, 2007).

**Thermal acclimation**

Mean RGR and biomass allocation were found to show no significant difference between plants maintained under their original growth conditions and those transferred to the alternative temperature regime for 1 week. Therefore, a simple t-test within each species based upon a plant’s original assignment (i.e. sub- or superoptimal temperature regime) was applied to determine the significance of any differences observed in growth characteristics.

The temperature dependence of *R*<sub>n</sub> on leaf temperature was modelled using a modified Arrhenius equation (Equation 4) described by Lloyd and Taylor (1994).

\[
R_n(T_1) = R_n(T_2) e^{\left(\frac{E_0}{R} \left(\frac{1}{T_1} - \frac{1}{T_2}\right)\right)}
\]  

(4)

where *R*<sub>n</sub>(T<sub>1</sub>) and *R*<sub>n</sub>(T<sub>2</sub>) are respiration rates at different leaf temperatures, T<sub>1</sub> and T<sub>2</sub> (K); E<sub>0</sub> (J mol<sup>−1</sup> K<sup>−1</sup>) is the activation energy, and R is the gas constant (8.314 J mol<sup>−1</sup> K<sup>−1</sup>). The model was fitted to individual leaves using non-linear generalized least squares solving for both E<sub>0</sub> and *R*<sub>n</sub> at the reference temperature, T=25 °C. Variance in E<sub>0</sub> and *R*<sub>n</sub> was explored using ANOVA within each species, with developmental temperature and short-term exposure temperature (at the second time step) as fixed factors. Given the degree of variation seen in individual leaves across temperature tested, the temperature dependence of *A*<sub>iso</sub> was compared in plants grown at sub- and superoptimal temperatures and between LL, LH, HH, and HL plants after a further week’s growth using a generalized linear mixed model. In this approach, ‘leaf temperature’ and ‘treatment’ were considered as fixed factors and plant identity as a random factor. Interaction terms were dropped to provide the most parsimonious model if warranted by only a limited change in AIC (i.e. ΔAIC <2) (Crawley, 2007).

**Results**

**Fundamental thermal niche**

All species studied grew under all temperature regimes, with the exception of the two species collected from a montane site (*L. aggregatum* and *Citharexylum* sp.) which did not survive under the highest temperature treatment (Fig. 1; Supplementary Table S1 at JXB online). Mean RGR ranged from 0.009 to 0.1 g g<sup>−1</sup> d<sup>−1</sup>, and in all species, with the exception of *A. pavonina*, there was a significant (ANOVA, P < 0.05) effect of growth temperature on mean RGR (Fig. 1). *Adenanthera pavonina* appeared relatively insensitive to growth temperature, with mean RGR ranging from 0.091 to 0.097 g g<sup>−1</sup> d<sup>−1</sup> between the 30/22 °C and 36/28 °C treatments. In three of the species (*A. pavonina*, *F. insipida*, and *O. pyramidalis*), growth under the highest temperature treatment was not significantly reduced,
compared with lower growth temperatures (Fig. 1), although a non-significant decrease was observed. In the three late-successional species, there was a significant and substantial reduction in mean RGR under the highest temperature regime when compared with the optimal temperature treatment 33/25 °C (43, 55, and 31% reduction in mean RGR in G. intermedia, O. macrocalyx, and T. cacao, respectively). Additionally, G. intermedia grown under the highest temperature regime showed signs of progressive stress (i.e. yellowing) that may have resulted in death given a longer study period. *Luehea seemannii* showed an unusually high degree of variation in mean RGR (Fig. 1), but still demonstrated a classically ‘domed’ response curve to growth temperature. The temperature dependence of mean RGR for each species was described using a third-order polynomial (Equation 2), with derived parameters used to model $T_{\text{opt}}$ for growth (Equation 3). The resulting $T_{\text{opt}}$ showed significant differences (Kruskal–Wallis test, $\chi^2=6.91, P < 0.05$) between plant functional types, averaging 32.0 28.9, and 28.7 °C in early-successional, late-successional, and montane species, respectively (Table 1).

Leaf temperatures ($T_{\text{leaf}}$) in well-watered plants, measured 2 h after the start of the daylight period, varied greatly between species, as a result of variation in traits associated with heat exchange (i.e. leaf physiognomy, leaf aspect, absorbance, and evaporative cooling). The relationship between chamber $T_{\text{air}}$ and measured $T_{\text{leaf}}$ across chambers was described with a simple linear regression. This was used to calculate the temperature of a leaf grown and maintained at a $T_{\text{air}}$ of 30 °C [$T_{\text{leaf}}(30)$] and the rate of change seen in $T_{\text{leaf}}$ with increasing ambient $T_{\text{air}}$ ($\frac{dT_{\text{leaf}}}{dT_{\text{air}}}$) (Table 1). There was a significant negative correlation between $T_{\text{opt}}$ and $T_{\text{leaf}}(30)$ (Spearman rho = –0.71, $P < 0.05$), suggesting a role of leaf-level cooling in allowing plants to maintain growth at higher air temperatures.

Biomass allocation and leaf characteristics varied with increasing temperature (Fig. 2, Table 2), with LMA and shoot mass ratio showing highly significant changes ($P < 0.001$) with growth temperature. LMA showed only a marginally significant difference between plant functional types ($P < 0.1$) as a result of variation in species selected, but there was a general decrease with increasing temperature (Fig. 2A). In early-successional species, LMA decreased from an average 41.1 g m$^{-2}$ to 38.5 g m$^{-2}$ between the lowest and highest temperature regimes, while in late-successional species LMA decreased from 64.7 g m$^{-2}$ to 57.1 g m$^{-2}$. Montane specimens showed no clear trend; with maximum LMA occurring in plants grown under the 33/25 °C regime. The shoot to total biomass ratios were not significantly different between plant functional types yet increased significantly with increasing temperature, rising from 71% and 68% to 73% and 74% in early- and late-successional species, respectively (Fig. 2B).

### Table 1. Average diel temperature optimum for growth and leaf-level cooling characteristics in 10 tropical tree species under experimental chamber conditions

<table>
<thead>
<tr>
<th>Species</th>
<th>$T_{\text{opt}}$ (°C)</th>
<th>$T_{\text{leaf}}(30)$ (°C)</th>
<th>$\frac{dT_{\text{leaf}}}{dT_{\text{air}}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early successional</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. pavonina</td>
<td>31.5</td>
<td>29.8</td>
<td>0.89</td>
</tr>
<tr>
<td>F. insipida</td>
<td>32.4</td>
<td>29.1</td>
<td>0.78</td>
</tr>
<tr>
<td>L. seemannii</td>
<td>31.8</td>
<td>27.8</td>
<td>0.87</td>
</tr>
<tr>
<td>O. pyramidale</td>
<td>32.8</td>
<td>27.0</td>
<td>0.85</td>
</tr>
<tr>
<td>T. guayacan</td>
<td>31.5</td>
<td>29.8</td>
<td>0.95</td>
</tr>
<tr>
<td>Late successional</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G. intermedia</td>
<td>28.7</td>
<td>30.3</td>
<td>0.99</td>
</tr>
<tr>
<td>O. macrocalyx</td>
<td>29.3</td>
<td>31.1</td>
<td>0.96</td>
</tr>
<tr>
<td>T. cacao</td>
<td>28.7</td>
<td>33.7</td>
<td>1.05</td>
</tr>
<tr>
<td>Montane</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citharexylum sp.$^b$</td>
<td>28.3</td>
<td>30.2</td>
<td>0.71</td>
</tr>
<tr>
<td>L. aggregatum$^b$</td>
<td>29.0</td>
<td>28.2</td>
<td>1.08</td>
</tr>
</tbody>
</table>

* Rate of change in leaf temperature with changing air temperature.  
  b Leaf temperature coefficients calculated using only surviving plants.

![Figure 2](image-url)  
Fig. 2. Leaf mass per area (A) and shoot mass ratio (B) of tropical tree seedlings grown under four temperature regimes. Symbols represent functional type averages ± 1SE. Open circles, five early-successional species; filled circles, three late-successional species; squares, two montane species.
Thermal acclimation

Seedlings for the thermal acclimation study were successfully grown at sub- and superoptimal temperatures, resulting in similarly sized plants despite a 6 °C difference in average growth temperature. *Ficus insipida* and *O. macrocalyx* showed no significant (P > 0.05) difference in final biomass between temperature regimes (Table 3), while *O. pyramidale* showed a small but significant difference (t-test = 3.16, df = 10, P < 0.05), with plants grown at superoptimal temperatures attaining slightly larger final biomass (7.9 ± 0.7 g) as compared with those grown at suboptimal temperatures (6.2 ± 1.1 g). The LMA of the latest fully developed leaf targeted for gas-exchange measurements showed no significant difference at sub- and superoptimal growth temperatures in *F. insipida* and *O. pyramidale* (Table 3), while *O. macrocalyx* had a significant (t-test = 2.63, df = 10, P < 0.05) decrease from 68.6 ± 5.6 g m⁻² to 60.0 ± 5.9 g m⁻². Molar ratios of C:N in *O. macrocalyx* did not differ significantly between sub- and superoptimally grown plants (Table 5), averaging 0.66 and 0.80 μmol CO₂ m⁻² s⁻¹ in *F. insipida* and *O. macrocalyx*, respectively. In *O. pyramidale*, there was a clear difference in Rn (25), with leaves developed at superoptimal temperatures having significantly lower Rn (25) (0.66 ± 0.03 μmol CO₂ m⁻² s⁻¹) than leaves developed at suboptimal temperatures (1.10 ± 0.04 μmol CO₂ m⁻² s⁻¹). The average activation energy, E₀, derived from fitting of the modified Arrhenius equation, was not significantly different between sub- and superoptimally grown leaves in *O. macrocalyx*, but differed significantly (P < 0.05) in the other two species (Table 5). *Ochroma pyramidale* plants grown under suboptimal temperatures had a lower E₀ (34.5 ± 3.7 kJ mol⁻¹ K⁻¹), as compared with those grown under superoptimal temperatures (61.4 ± 5.4 kJ mol⁻¹ K⁻¹). Yet, in *F. insipida* this pattern was reversed; E₀ was significantly higher in plants grown under suboptimal conditions (110.6 ± 13.5 kJ mol⁻¹ K⁻¹) than in plants grown under superoptimal conditions (64.6 ± 7.1 kJ mol⁻¹ s⁻¹). *Ormosia macrocalyx* also showed no significant difference in temperature response characteristics of Rₙ between sub- and superoptimally grown plants when data were converted to a leaf mass basis.

Nighttime respiration in leaves grown under sub- and superoptimal temperatures showed a range of responses to temperature in the three species tested (Fig. 4 A–C, Table 5). In *F. insipida* and *O. macrocalyx*, Rₙ (25) did not differ significantly between sub- and superoptimally grown plants (Table 5), averaging 0.66 and 0.80 μmol CO₂ m⁻² s⁻¹ in *F. insipida* and *O. macrocalyx*, respectively. In *O. pyramidale*, there was a clear difference in Rₙ (25), with leaves developed at superoptimal temperatures having significantly lower Rₙ (25) (0.66 ± 0.03 μmol CO₂ m⁻² s⁻¹) than leaves developed at suboptimal temperatures (1.10 ± 0.04 μmol CO₂ m⁻² s⁻¹). The average activation energy, E₀, derived from fitting of the modified Arrhenius equation, was not significantly different between sub- and superoptimally grown leaves in *O. macrocalyx*, but differed significantly (P < 0.05) in the other two species (Table 5). *Ochroma pyramidale* plants grown under suboptimal temperatures had a lower E₀ (34.5 ± 3.7 kJ mol⁻¹ K⁻¹), as compared with those grown under superoptimal temperatures (61.4 ± 5.4 kJ mol⁻¹ K⁻¹). Yet, in *F. insipida* this pattern was reversed; E₀ was significantly higher in plants grown under suboptimal conditions (110.6 ± 13.5 kJ mol⁻¹ K⁻¹) than in plants grown under superoptimal conditions (64.6 ± 7.1 kJ mol⁻¹ s⁻¹). *Ormosia macrocalyx* also showed no significant difference in temperature response characteristics of Rₙ between sub- and superoptimally grown plants when data were converted to a leaf mass basis.

Table 2. Results of mixed effects model of plant growth characteristics in 10 tropical tree species grown under a range of temperature treatments treated as a continuous variable

<table>
<thead>
<tr>
<th>LMA (g m⁻²)</th>
<th>Shoot mass ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
</tr>
<tr>
<td>Growth temperature</td>
<td></td>
</tr>
<tr>
<td></td>
<td>69.2</td>
</tr>
<tr>
<td>Functional type</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Table 3. Growth and leaf characteristics of three tropical tree species grown at sub- and superoptimal temperature regimes

<table>
<thead>
<tr>
<th>Species</th>
<th>Growth temperature</th>
<th>Final biomass (g)</th>
<th>LMA (g m⁻²)</th>
<th>δ¹³C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>F. insipida</em></td>
<td>Suboptimal</td>
<td>5.42 ± 2.13</td>
<td>65.2 ± 7.8</td>
<td>−28.7 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>Superoptimal</td>
<td>3.11 ± 1.46</td>
<td>65.3 ± 9.1</td>
<td>−29.6 ± 0.5</td>
</tr>
<tr>
<td><em>O. macrocalyx</em></td>
<td>Suboptimal</td>
<td>3.06 ± 1.05</td>
<td>68.7 ± 5.6</td>
<td>−26.3 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>Superoptimal</td>
<td>2.5 ± 0.74</td>
<td>60 ± 0.9</td>
<td>−27.5 ± 0.4</td>
</tr>
<tr>
<td><em>O. pyramidale</em></td>
<td>Suboptimal</td>
<td>6.24 ± 1.06</td>
<td>55.3 ± 5.8</td>
<td>−30.6 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>Superoptimal</td>
<td>7.91 ± 0.74</td>
<td>54.7 ± 8.4</td>
<td>−30.6 ± 0.4</td>
</tr>
</tbody>
</table>

Values equal mean (n=6) ± 1SD, with values in bold indicating significant differences within species (P < 0.05).
The response of \( R_n \) to short-term (1 week) changes in temperature varied between the species tested (Fig. 4 D–F, Table 6). In \( O. \) macrocalyx there continued to be no significant difference in the temperature response characteristics [i.e. \( R_{n(25)} \) and \( E_0 \)] between populations, with \( R_{n(25)} \) averaging 0.72 \( \mu \)mol CO\(_2\) m\(^{-2}\) s\(^{-1}\) across all plants (i.e. comparable with 0.80 \( \mu \)mol CO\(_2\) m\(^{-2}\) s\(^{-1}\) determined a week previously). \( F. \) insipida, previously found to show a significant difference in \( E_0 \) as a result of development temperature, no longer demonstrated significant differences in either \( E_0 \) or \( R_{n(25)} \), although plants developed under and maintained at suboptimal temperatures were generally found to have higher \( R_n \) across all temperatures. \( O. \) pyramidale, in a manner similar to initial measurements, was found to have a significant difference in \( R_{n(25)} \) as a result of developmental temperature (ANOVA, \( F=23.2, P < 0.001 \)), but there was no significant impact shown as a result of short-term differences in temperature regime.
Fundamental thermal niche

Under this study’s favourable conditions (i.e. relatively high light, nutrient, and water availability), all species showed substantial growth at air temperatures above those seen in their native range. This included certain species able to tolerate, and thrive at, an average daily temperature of 35 °C, close to the upper limit estimated for mean surface temperatures during the PETM (Jaramillo et al., 2010). Yet, there was a range in thermal responses shown between species, with both $T_{\text{opt}}$ and the magnitude of change observed in mean RGR varying between plant functional groups. Differences in $T_{\text{opt}}$ appear to be related to leaf-level energy balance, in that species able to reduce $T_{\text{leaf}}$ in a given situation demonstrated higher $T_{\text{opt}}$. In well-ventilated growth chambers, it is likely that heat transfer by forced convection and latent heat exchange is maximized due to a reduction in the leaf-level boundary resistance (Jones and Rotenberg, 2011), with the expectation that species with high transpiration rates (a trait often associated with early-successional species) would be able to better buffer leaf tissues from the effects of increasing $T_{\text{air}}$.

At the global scale, climate models and empirical data would suggest that relative humidity remains stable during climate warming (Held and Soden, 2000; Dessler and Sherwood, 2010).
2009; Dessler and Davis, 2010); yet it is highly likely that relative humidity at the regional level will vary substantially with changing climate and precipitation patterns (Li et al., 2006; Dessler et al., 2008; Lintner et al., 2012). In the experiments presented here, the use of pre-conditioned air within the growth chambers resulted in a generally much higher VPD within chambers than found currently in the moist tropical lowlands, ranging from ~2.3 kPa at 30 °C to ~5 kPa under the 39 °C daytime treatment. Leaf-level δ¹³C values obtained for these early-successional species (i.e. F. insipida, O. pyramidale, and T. guayacan) and one late-successional species (T. cacao) from the initial study (data not shown) and of plants in the acclimation study (Table 3) suggest that stomatal limitation did not increase as a consequence of high VPD (Farquhar et al., 1989; Zhang and Nobel, 1996). Indeed, high VPD actually conferred an advantage to well-watered high-transpiring seedlings by enhancing leaf-level cooling. Further studies are required to elucidate the interactive role VPD and Tₐₗ in determining T_leaf and growth of tropical tree species.

The two species collected from a montane cloud forest, L. aggregatum and Citharexylum sp., were unable to tolerate the highest growth temperature regime used, yet still had T_opt higher than that which would be expected from their immediate home. Growth rates at T_opt were similar to those seen in early-successional species, but showed dramatic declines beyond an average growth temperature of 32 °C. This suggests that neither intrinsic growth rate nor T_opt are in themselves a good predictor of the temperature above which growth is severely curtailed. Both L. aggregatum and Citharexylum sp. can be found within the tropical lowlands. It is therefore interesting to speculate that the apparent temperature sensitivity observed in this study may in fact reflect local adaptation or epigenetic variation (Bräutigam et al., 2013) within the species. Further detailed studies using a broad range of seed sources would be required to investigate the interspecific variation in thermal adaptation and acclimation potential.

Significant changes in leaf characteristics and patterns of biomass allocation across temperature were observed (Fig. 2), although the biological relevance of, for example, the observed reduction in LMA with temperature increase is unclear. LMA is known to increase with elevation both between (van de Weg et al., 2009) and within (Körner et al., 1986; Cordell et al., 1998) species, but it is unknown whether or not this reflects adaptation to temperature per se. In their recent meta-analysis of warming treatments, Way and Oren (2010) showed no significant trend in specific leaf area (the inverse of LMA) across a broad range of plant functional types. The reductions in LMA within LMA of early- and late-successional species observed here (6% and 12% across the total temperature range tested) may simply be a result of non-linear temperature-related changes in leaf morphology at very high temperatures (Kogami et al., 2001; Poorter et al., 2009; Muhl et al., 2011). When considering changes in biomass allocation such as shown in Fig. 2B, ontological effects during seedling development must be considered. Nevertheless, a significant increase in the shoot:root biomass ratio and an increase in internode length within stems (data not shown) are consistent with previous observations of increased tree height at higher growth temperatures (Way and Oren, 2010; Esmail and Oelbermann, 2011). Dependent upon whether this is caused by changes in cell elongation and/or the promotion of apical meristem activity, it is likely to have profound implications upon mechanical stability, and competitive interaction of stems during growth.

At the leaf level, constitutive differences in plant traits associated with heat exchange (i.e. emissivity, leaf physiology, leaf angle, transpiration rates, and canopy structure) have long been recognized as determining T_leaf under particular environmental conditions (Lange, 1959), resulting in, for example, large differences within temperatures of temperate forest tree canopies (Leuzinger and Körner, 2007). The acclimation of functional traits has been shown to maximize leaf-level cooling in the model herbaceous species Arabidopsis thaliana when grown at high temperatures (Crawford et al., 2012), and the present results suggest that similarly, differences between species and the ability of a species to modify such factors may be key to predicting tropical tree seedling responses to increasing temperatures.

**Thermal acclimation**

Previous evidence for thermal acclimation in plants has recognized acclimation in both existing tissue subjected to a sustained change in temperature and tissues developed under novel temperatures (Campbell et al., 2007). Acclimation in both pre-existing (PE) and newly developed (ND) tissue may involve changes in intracellular biochemistry, while the acclimation potential of PE is constrained by previously determined morphology, the acclimation of ND tissue may also include alteration in organ development and physiology (Royer et al., 2009; Hamant and Traas, 2010; Peppe et al., 2011). In the three species tested, it was demonstrated that seedlings exhibited a range of acclimation potentials; from acclimation of ND but not PE (i.e. O. pyramidale), to acclimation of ND and PE (i.e. F. insipida), to an apparent lack of any thermal acclimation (i.e. O. macrocalyx)

While O. macrocalyx showed no significant acclimation of Rₑ in leaf tissue developed under or exposed for a short period to the alternative temperature regime, the two pioneer species F. insipida and O. pyramidale showed substantial acclimation as a result of growth temperature. Analysis at the first time point (Table 5, Fig. 4A–C) showed patterns consistent with the two acclimation types codified by Atkin and Tjoelker (2003). In F. insipida, as in previous work (Krause et al., 2013), a so-called type I acclimation was observed, in which the rate of change in Rₑ across temperature is down-regulated in plants grown under higher temperatures. In contrast, in O. pyramidale, a pattern consistent with type II acclimation was observed, in which the Rₑ at all temperatures is reduced. Evidence for this distinction in acclimation type between species is also provided in the patterns observed at the second time point (Table 6, Fig. 4D–F). Differences in O. pyramidale, as a result of developmental temperature, were maintained despite subsequent changes in temperature regime, while F. insipida showed evidence for short-term acclimation. Type I acclimation is believed to be the result of short-term
changes in metabolic capacity and efficiency, whereas type II acclimation is putatively linked to changes in mitochondrial density and ultrastructure (Atkin and Tjoelker, 2003). As a result, type I acclimation is considered a more rapid process, and is likely to be the net result of acclimation to both developmental and recent temperature regimes (Fig. 4D), while type II acclimation (exhibited in *O. pyramidalis*) is tied to developmental processes (Armstrong et al., 2006) and is therefore inflexible under short-term changes in temperature regime (Fig. 4F).

Consistent with previous studies in other plant functional groups (i.e. forbs, grasses, and evergreen trees/shrubs; Campbell et al., 2007), thermal acclimation of photosynthesis and respiration was asynchronous in that the three tropical species showed relatively stable biomass accumulation between treatments and exhibited only limited alteration in the temperature response of $A_{500}$. In *O. pyramidalis* and *O. macrocalyx*, there were significant differences in the absolute rates of $A_{500}$ between plants grown under sub- and superoptimal temperatures. However, this may have been a consequence of greater sink strength (high $R_s$ in all tissues) under the higher temperature regime as opposed to a true ‘acclimation’ of photosynthetic processes (Körner, 2003; Sala et al., 2012).

In this study, clear, and novel, evidence is provided that certain tropical trees do have an ability to maintain growth as seedlings at temperatures above those of their current home range, with a fundamental thermal niche generally broader than that realized in the field. Clear evidence is also provided that thermal acclimation of leaf-level processes may play an important role in this response. However, although thermal acclimation in tropical species may help buffer the adverse effects of increasing temperatures, it currently is not possible to quantify how the acclimation potential shown here in seedlings may translate to the performance of intact forests. Physiological processes and biotic interactions uniquely associated with adult trees are highly dependent upon the temperature regime, from the thermal sensitivity of plant gametophytes and thereby reproductive success (Hedhly, 2011) to the interdependence of temperature and drought severity (Cowling and Shin, 2006). It is clearly apparent that further work is needed on the physiological impact of increasing temperature in mature tropical trees, but the potential for thermal acclimation must be considered if we are to better predict the fate of forests under future climate scenarios.

**Acknowledgements**

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**References**


**Supplementary data**

Supplementary data are available at *JXB* online.

Figure S1. Average diel cycle in (a) temperature, (b) photosynthetically active radiation, and (c) vapour pressure deficit at STRI’s Santa Cruz Experimental Field Facility typical of lowland Panama during wet (February) and dry (July) seasons.

Table S1. Growth and biomass allocation of 10 tropical tree species grown under four different temperature regimes.

Table S2. Derived growth characteristics of 10 tropical tree species grown under four different temperature regimes.

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