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Thermal tolerance, net CO_2 exchange and growth of a tropical tree species, *Ficus insipida*, cultivated at elevated daytime and nighttime temperatures

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ABSTRACT

Global warming and associated increases in the frequency and amplitude of extreme weather events, such as heat waves, may adversely affect tropical rainforest plants *via* significantly increased tissue temperatures. In this study, the response to two temperature regimes was assessed in seedlings of the neotropical pioneer tree species, *Ficus insipida*. Plants were cultivated in growth chambers at strongly elevated daytime temperature (39 °C), combined with either close to natural (22 °C) or elevated (32 °C) nighttime temperatures. Under both growth regimes, the critical temperature for irreversible leaf damage, determined by changes in chlorophyll *a* fluorescence, was approximately 51 °C. This is comparable to values found in *F. insipida* growing under natural ambient conditions and indicates a limited potential for heat tolerance acclimation of this tropical forest tree species. Yet, under high nighttime temperature, growth was strongly enhanced, accompanied by increased rates of net photosynthetic CO_2 uptake and diminished temperature dependence of leaf-level dark respiration, consistent with thermal acclimation of these key physiological parameters.

Introduction

Increases in greenhouse gas concentrations and anthropogenic alteration of land use and surface albedo have resulted in a rapid rise in global surface temperature over the last three decades (Hansen et al., 2010) and are predicted to result in further rises in the near future (IPCC, 2007). Such changes in temperature may have profound implications upon individual species performance and survival, as well as ecosystem functioning (Colwell et al., 2008; Corlett, 2011, 2012; Offord, 2011). In tropical rainforest regions, a mean temperature increase of 0.26 ± 0.05 °C per decade since the mid-1970s has been reported (Mahli and Wright, 2004), with climate projections suggesting that the American tropics may see accelerated temperature rises in the future (Cramer et al., 2004; Diffenbaugh and Scherer, 2011). There is evidence for a greater increase in nighttime temperature as compared to daytime temperature leading to a decrease in the diel temperature range (Kukla and Karl, 1993; Easterling et al., 1997; Alexander et al., 2006; Malamud et al., 2011; Smithsonian Tropical Research Institute Physical Monitoring Program, unpublished data for Barro Colorado Island). Moreover, extreme weather events, such as heat waves combined with drought (Jentsch and Beierkuhnlein, 2008) and changing seasonal patterns of precipitation (Lintner et al., 2012) are thought to become more common, potentially causing leaf temperature to rise beyond the point at which irreversible damage occurs. Information on thermal tolerance and the ability of plant species in the humid tropics to acclimate to altered high temperature regimes is scarce and largely restricted to a small number of important agronomic species (Smillie and Nott, 1979; Weng and Lai, 2005). In these studies and in those focused on non-crop tree species (Cunningham and Read, 2006), thermal tolerance limits range from 35 to 52 °C.

A widely employed method to determine thermal tolerance of plants is the study of changes in Chl *a* fluorescence upon heating of leaf sections or whole leaves. Established by Schreiber and Berry (1977), a standard procedure to determine incipient leaf damage is to monitor heat-induced increase in 'initial fluorescence' (F_0). This fluorescence response was shown to correlate with visible necrotic damage (Bilger et al., 1984) and detrimental effects on photosynthetic CO_2 assimilation (Downton et al., 1984; Seemann et al., 1984). The decline in the ratio of maximum variable to maximum

Abbreviations: A_{sat}, light-saturated net CO_2 assimilation; Chl, chlorophyll; E_0, activation energy; F_0, initial chlorophyll *a* fluorescence; F_v, variable fluorescence; F_v/F_m, ratio of maximum variable to maximum total fluorescence; LAR, leaf area ratio (leaf area per total dry mass); LMA, leaf mass per area; LMF, leaf mass fraction; NAR, net assimilation rate; PAR, photosynthetically active radiation; PSII, photosystem II; R_d, leaf-level dark respiration; RGR, mean relative growth rate; RMS, root mass fraction; SMF, stem mass fraction.

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total fluorescence (F_v/F_m), indicating loss of potential efficiency of photosystem II (PSII), has also been used as a measure of heat-induced leaf damage (Méthy et al., 1997; Bigras, 2000; Ladjal et al., 2000; Cunningham and Read, 2006). F_v/F_m measurements tend to result in higher critical temperatures of leaf damage than measurements of F_0.

Krause et al. (2010) reevaluated the Chl *a* fluorescence method in an investigation of thermal tolerance in two neotropical tree species of Panama: *Ficus insipida* Willd. (Moraceae), a pioneer tree, and *Virola sebifera* Aubl. (Myristicaceae), a late successional tree. Plants growing under local ambient conditions were studied. Results demonstrated that a 50% decline in F_v/F_m, determined ~24 h after a 15 min heat treatment of leaf disks, was the most reliable predictor of irreversible leaf damage which occurred in both species between 50 and 52 °C. Alongside fluorescence data for four Australian species provided by Cunningham and Read (2006), these represent the highest thermal tolerance values reported thus far in tropical trees. The question arises: is this the upper limit of thermal tolerance in tropical tree species?

We examined the capacity for increases in thermal tolerance by growing *F. insipida* seedlings inside controlled-environment chambers under a markedly elevated daytime temperature of 39 °C, approximately 7 °C above current ambient daytime maxima and 10 °C above mean daytime temperatures in lowland Panama (see Krause et al., 2010), and close to the highest temperature the chambers available to us could sustain. The temperature of 39 °C was continuously maintained throughout a 12 h light period, while during the 12 h dark period, seedlings were exposed to either near natural (22 °C) or elevated (32 °C) air temperatures. Thermal tolerance, photosynthetic and respiratory CO_2 exchange and growth of the seedlings cultivated under the two temperature regimes were analyzed. Results demonstrate no enhanced thermal tolerance of seedlings cultivated under elevated daytime and nighttime temperatures. However, the study did highlight significant effects of nighttime temperature on CO_2 assimilation, dark respiration and growth, the most remarkable result being a strong stimulation of biomass production in response to increased nocturnal temperature.

Materials and methods

Plant material and growth conditions

Seeds of *Ficus insipida* Willd. (Moraceae) were collected from mature trees and germinated in January 2012 in Jiffy 7 peat pellets (Jiffy Products of America Inc., Lorain, OH, USA). Individual seedlings with 4–5 leaves were transferred into 15 cm high 2.2 L plastic pots filled with soil ('Potting mix', Miracle Gro Lawn, Marysville, OH, USA). Three seedlings were harvested and measured for initial leaf area and total dry mass, while 36 plants were distributed evenly between two GC-15 controlled environment chambers (EGC, Chagrin Falls, OH, USA). Plants received an initial dose of slow release fertilizer ('Osmocote Plus', Scotts Miracle-Gro, Marysville, OH, USA) and were watered to field capacity daily. Chambers were maintained with a 12 h light period, with photosynthetically active radiation (PAR) between 520 and 590 μmol photons m^{-2} s^{-1} at leaf level (Philips TL841 fluorescent bulbs), which corresponded to about 50% of integrated daily ambient PAR on sunny days. Air temperature was 39 °C during light periods in both chambers. Temperature during the 12 h dark period was maintained at either 22 or 32 °C. Chamber-set air temperatures were confirmed with a shielded 107-L Air Temperature Sensor (Campbell, Logan, UT, USA). Leaf temperature, measured on the adaxial leaf surface with an infrared thermometer (MiniTemp MT, Raytec, Santa Cruz, CA, USA) ranged from 37 to 40 °C during the light period. In both chambers, air was maintained with a dew point of ~18 °C and ambient CO_2 concentration (~400 μL L^{-1}). After 32–42 days, Chl *a* fluorescence, net CO_2 exchange and biomass accumulation and allocation were determined.

Chlorophyll (Chl) a fluorescence

The ratio of maximum variable to maximum total Chl *a* fluorescence (F_v/F_m), measured on mature leaves after 10 min dark adaptation, was used for assessment of potential efficiency of photosystem II (PSII) under both day and nighttime growth conditions. Fluorescence was recorded with a MINI-PAM fluorometer (Walz, Effeltrich, Germany). For details of the measuring procedure see Krause et al. (2006).

Heat tolerance tests

Chl *a* fluorescence (initial fluorescence, F_0, and ratio F_v/F_m) was used to determine high-temperature tolerance of leaves as described by Krause et al. (2010). Leaf disks (diameter 1.1 cm) of fully expanded leaves from five different plants were heated for 15 min in a water bath (Lauda RM6/RMS, Analytical Instruments, LLC, Golden Valley, MN, USA), at 45, 48, 50, 51, 52 or 53 °C. To avoid anaerobiosis, leaf disks were wrapped in small pieces of Miracloth (Calbiochem, La Jolla, CA, USA) and kept dry in plastic bags. Untreated leaf disks without heating served as controls. After heat treatment, leaf sections were kept in the dark for ~10 min, before fluorescence was monitored with a PAM 2000 fluorometer (Walz, Effeltrich, Germany). Adverse effects of heating were indicated by increased F_0 and decreased F_v/F_m. Fluorescence changes were partly reversed upon storing the same leaf disks for ~24 h in petri dishes on moist tissue paper under dim light. In accordance with Krause et al. (2010), only fluorescence data obtained ~24 h after heating are presented.

Photosynthetic CO_2 assimilation and dark respiration

Rates of light-saturated net photosynthetic CO_2 uptake (A_{sat}) and dark respiration (R_d) were measured on mature leaves using a LI-6400 system equipped with a 2 cm × 3 cm leaf cuvette (LI-COR Biosciences, Lincoln, NE, USA). Temperature response of R_d was determined by transferring 4 dark-adapted plants (at the end of the dark period) from each chamber into a third GC-15 growth chamber with air temperature set at 22 °C. Plants were kept for another hour in darkness before R_d was determined in one fully expanded leaf of each plant. The LI-6400 cuvette temperature was set to that of the growth chamber. Reference CO_2 partial pressure was regulated at 400 μL L^{-1}. Growth chamber and cuvette temperatures were then raised to 27 °C. Plants were allowed to equilibrate for 30 min before R_d was measured on the same leaves. This was repeated at 32 °C. For subsequent A_{sat} determination, the same plants were kept for 2 h under standard conditions of the light period (39 °C; PAR, 520–590 μmol photons m^{-2} s^{-1}). To obtain maximal rates under ambient [CO_2], measurements were performed at saturating light (PAR, 1000 μmol photons m^{-2} s^{-1}) and 400 μL L^{-1} CO_2, with the cuvette temperature set at 39 °C. Growth chamber and cuvette temperatures were then reduced stepwise to determine A_{sat} at a range of lower temperatures (35.5, 32, 27, and 22 °C) with plants equilibrated for 30 min at each step.

Growth

Upon harvest of seedlings, leaf area was measured with a leaf area meter LI-3100 (LI-COR, Lincoln, NE, USA). The dry mass of laminae, stems (including leaf petioles) and roots was determined

Table 1
Chlorophyll fluorescence parameters of *F. insipida* grown at 39/32 °C or 39/22 °C (light/dark periods). After six weeks of growth, recordings were done on mature leaves in the light (8.5 h) or dark (11 h). F_0 and F_v are given in relative units. Means ± SD (n = 8, leaves of different plants).

Growth temp.	Condition	F_0	F_v	F_v/F_m
39/32 °C	Light-adapted	328 ± 18	1078 ± 89	0.766 ± 0.019
	Dark-adapted	285 ± 11	1273 ± 40	0.817 ± 0.005
39/22 °C	Light-adapted	318 ± 28	1154 ± 54	0.783 ± 0.015
	Dark-adapted	301 ± 22	1267 ± 71	0.807 ± 0.011

separately after drying for >72 h at 70 °C. Mean relative growth rate (RGR) was calculated as

$$\mathrm{RGR}\ \ (\mathrm{mg\ g^{-1}\ d^{-1}}) = \frac{\ln W_2 - \ln W_1}{t}, \quad (1)$$

where W_1 and W_2 are total dry mass (mg) at start of the experiment and time of harvest, respectively, and t is the duration of the experiment (39 d). Net assimilation rate (NAR) was calculated using W_1, W_2 (g), t, and the initial and final leaf area, A_1 and A_2 (m^2);

$$\mathrm{NAR}\ \ (\mathrm{g\ m^{-2}\ d^{-1}}) = \frac{(W_2 - W_1)\cdot(\ln A_2 - \ln A_1)}{(A_2 - A_1)\cdot t} \quad (2)$$

Initial total dry mass (W_1) and leaf area (A_1) were 0.116 ± 0.017 g and 31.1 ± 1.6 cm^2, respectively (n = 3).

Statistics

Variances in physiological response and growth parameters between plants grown under contrasting nighttime temperatures were assessed by Student's t-tests. Differences between treatments were considered significant at $p < 0.05$. Fluorescence characteristics presented in Table 1 were evaluated by two-way ANOVA, with growth temperature and light/dark acclimated condition as independent factors. The influence of temperature upon leaf level gas fluxes was analyzed by fitting individual tested leaves with model response curves. The mean fitting parameters were then compared between treatments using Student's t-test. The response of R_d to leaf temperature (Fig. 2b) was modeled using a modified Arrhenius equation (Eq. (3)) described by Lloyd and Taylor (1994); see also Dillaway and Kruger (2011):

$$R_{d(T_1)} = R_{d(T_2)} e^{((E_0/R)\times((1/T_2)-(1/T_1)))} \quad (3)$$

where $R_{d(T_1)}$ and $R_{d(T_2)}$ are dark respiration rates at different leaf temperatures, T_1 and T_2 (K); E_0 ($\mathrm{J\,mol^{-1}\,K^{-1}}$) is the activation energy, and R the ideal gas constant (8.314 $\mathrm{J\,mol^{-1}\,K^{-1}}$). The activation energy was calculated through the fitting of Eq. (3) by non-linear generalized least squares solving for both E_0 and R_d at the reference temperature, 25 °C. The thermal response of A_{sat} across the range of temperatures tested was limited (Fig. 2a) and so analyzed using a simple linear relationship (Eq. (4)) with net assimilation at a standard leaf temperature 30 °C ($A_{sat(30)}$) and rate of decline (β), compared between treatments:

$$A_{sat(T)} = A_{sat(30)} - \beta T \quad (4)$$

Curve fitting was carried out using statistical program R, version 2.13 (R Development Core Team, 2011).

Results

Thermal tolerance

Changes in chlorophyll *a* fluorescence emission determined ~24 h after heat treatment are depicted in Fig. 1. Fluorescence values of non-heated control leaves grown at 39/32 °C and 39/22 °C, respectively, were: (a) F_v/F_m, 0.816 ± 0.014 and 0.823 ± 0.006; (b) F_0, 250 ± 33 and 256 ± 17 relative units. Heating to 45 °C and 48 °C did not significantly reduce F_v/F_m (Fig. 1a) in leaves grown at 39/32 °C ($p > 0.05$), and only slightly decreased F_v/F_m in leaves grown at 39/22 °C ($p < 0.01$). Beyond 48 °C, leaf disks of both growth treatments exhibited progressive decline in F_v/F_m (Fig. 2a). The F_v/F_m decline resulted from combined increase in F_0 and decline in maximum total fluorescence, F_m (data not shown). Correlation of F_v/F_m decline with visible tissue damage observed during storage of leaf sections subsequent to heat treatment has been previously established for *F. insipida* leaves (Krause et al., 2010). A decrease in F_v/F_m by 50%, considered as the limit of thermal tolerance, was reached at approximately 51 and 51.5 °C in plants grown at 39/22 °C and 39/32 °C, respectively. The data do not indicate a significant difference between thermal tolerance of plants grown under either temperature regime.

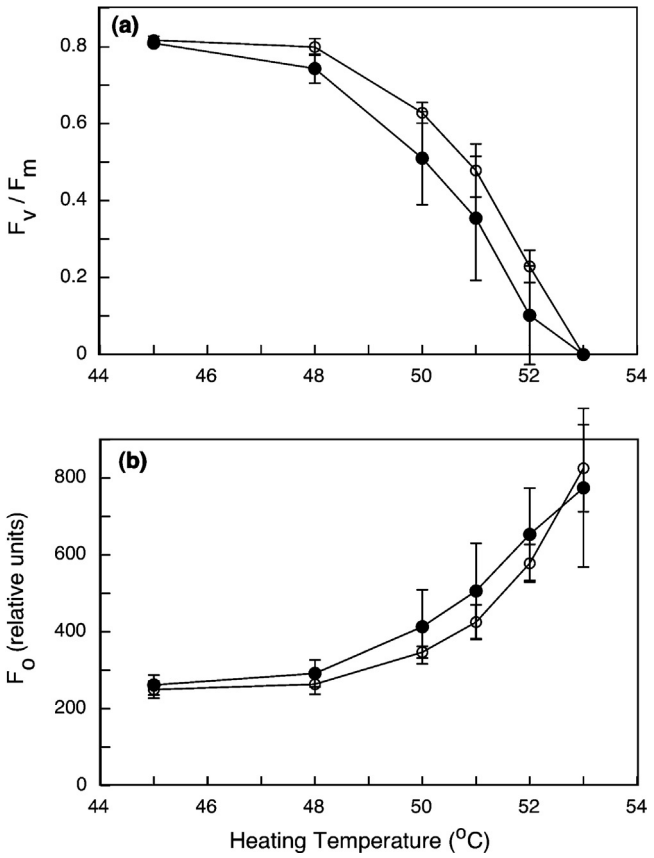

Fig. 1. Effects of 15 min heating of leaf disks of *F. insipida* on Chl *a* fluorescence emission, measured ~24 h after heat treatment. Assays were performed on plants grown for 37–39 d under the specified conditions. (a) Ratio F_v/F_m; (b) initial fluorescence, F_0. Means ± SD are shown (n = 5, leaves from different plants). Open circles, plants grown at 39/32 °C (light/dark periods); closed circles, plants grown at 39/22 °C.

Heat-induced rise in initial fluorescence, F_0 (Fig. 1b), corresponded to the decrease in F_v/F_m. Compared to non-heated controls, heating to 45 and 48 °C did not induce a significant F_0 increase ($p > 0.05$). A strong increase in F_0 was observed upon heat treatment at 51 °C and above in leaf disks from plants of both growth temperature regimes ($p < 0.01$).

Chl a fluorescence, A_{sat} and R_d of intact leaves

Chlorophyll *a* fluorescence parameters, recorded in intact leaves during both dark and light periods, are presented in Table 1. Using two-way ANOVA, the data show a significant effect of illumination as compared to the dark-adapted state; F_0 was increased ($p < 0.001$), and F_v and F_v/F_m were decreased ($p < 0.001$) during the light period.

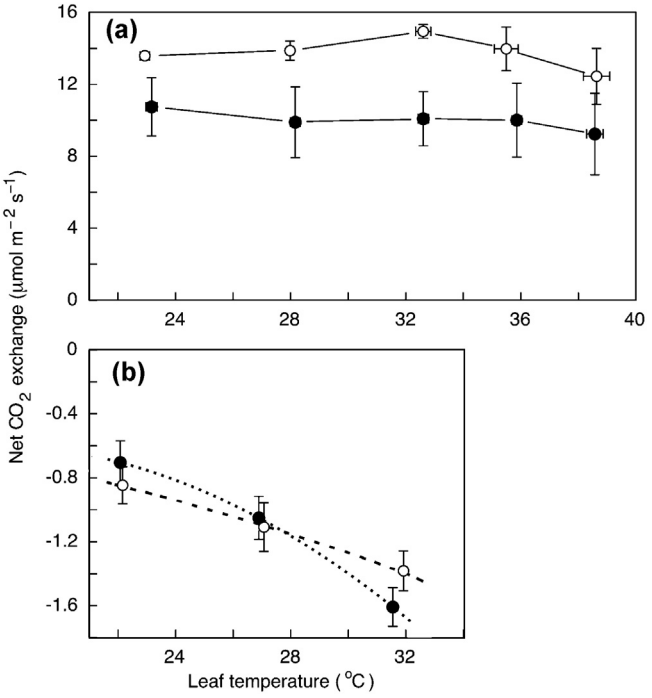

Fig. 2. Net CO_2 exchange in fully expanded leaves of *F. insipida* as function of leaf temperature, determined after 32 d of growth at 39/32 °C (open circles) and 39/22 °C (closed circles). Means ± SD ($n = 4$, leaves of different plants). (a) Net photosynthetic CO_2 uptake under saturating light (A_{sat}); (b) respiration in the dark (R_d). Fitted lines were calculated based on Eq. (3) (see *Materials and Methods*).

Fig. 3. Image of *F. insipida* seedlings after 33 d of growth at day/night temperatures of 39/32 °C and 39/22 °C. Stem heights were 32 and 12 cm, respectively.

There was no significant effect of the growth temperature regime on fluorescence. Interaction between light and temperature condition was significant for F_v/F_m only ($p < 0.01$). The fluorescence changes observed in the light period were fully reversible in the dark.

Light-saturated rates of net CO_2 assimilation (A_{sat}) were considerably higher in leaves of seedlings grown at 39/32 °C as compared to those grown at 39/22 °C at all temperatures tested (Fig. 2a), with modeled $A_{sat(30)}$ significantly ($t_{(6)} = 4.15$, $p < 0.01$) higher in plants grown at higher nighttime temperature, 13.8 ± 0.5 as compared to 10.1 ± 1.7 μmol CO_2 m^{-2} s^{-1}. In plants grown under both temperature regimes, A_{sat} changed little across the wide range of measuring temperatures, with no significant difference in the rate of decline in A_{sat} seen in leaves of plants grown either at 39/22 °C ($\beta = 0.04 \pm 0.10$) or 39/32 °C ($\beta = 0.07 \pm 0.10$).

Rates of R_d increased as leaf temperature increased from ~22 to ~32 °C (Fig. 2b), with a difference in the rate of change observed between plants grown at 39/22 and 39/32 °C. Although plants showed no significant difference in R_d at the reference temperature of 25 °C, plants grown at 39/22 °C did have a significantly higher E_0 ($t_{(8)} = 3.46$, $p < 0.01$) than plants grown under elevated nighttime temperature (63 ± 14 and 37 ± 9 kJ mol^{-1} K^{-1}, respectively). Accordingly, R_d measured at 32 °C in plants grown at 39/22 °C was significantly higher ($t_8 = 2.36$, $p < 0.05$) than in plants grown at 39/32 °C (rates of 1.64 ± 0.20 and 1.34 ± 0.13 μmol CO_2 m^{-2} s^{-1}, respectively).

Growth

Seedlings cultivated at 39/32 °C exhibited much faster growth than seedlings grown at 39/22 °C (Fig. 3). Total biomass accumulation was about three times higher in plants grown at the elevated nighttime temperature, as compared biomass accumulation at 39/22 °C (Table 2), resulting in higher leaf, stem and root mass, leaf area, seedling height, RGR and NAR, but leaf mass per area (LMA) was lower ($p < 0.05$) in plants grown under high nighttime temperature. Mass fractions of leaves, stem and root, as well as leaf area ratio (LAR) and shoot to root ratio were not affected by nighttime temperature (Table 2).

Discussion

Thermal tolerance

Growth of *F. insipida* in controlled-environment chambers at high daytime temperature (39 °C), markedly above temperatures in

Table 2
Growth parameters of *F. insipida* seedlings grown for 39 d either at 39/32 °C or 39/22 °C (light/dark periods). Means ± SD ($n = 5$). Mass fractions of leaves (LMF), stem (SMF) and roots (RMF); leaf mass per area (LMA); leaf area ratio (LAR); mean relative growth rate (RGR); net assimilation rate (NAR).

	Growth temperatures	
	39/32 °C	39/22 °C
Dry mass (g)		
Total**	3.80 ± 0.36	1.17 ± 0.25
Leaves**	1.98 ± 0.28	0.60 ± 0.16
Stem**	0.95 ± 0.16	0.27 ± 0.04
Roots**	0.86 ± 0.15	0.30 ± 0.11
Mass fraction (g g^{-1})		
LMF	0.52 ± 0.03	0.51 ± 0.07
SMF	0.25 ± 0.04	0.23 ± 0.03
RMF	0.23 ± 0.04	0.26 ± 0.06
Other growth parameters		
Leaf area (cm^2)**	473 ± 83	120 ± 35
LMA (g m^{-2})*	42.2 ± 2.1	50.4 ± 7.2
LAR (cm^2 g^{-1})	124 ± 13	103 ± 23
Stem height (cm)**	33.3 ± 4.5	8.80 ± 1.7
Shoot/root (g g^{-1})	3.49 ± 0.71	3.06 ± 1.00
RGR (mg g^{-1} d^{-1})**	89.2 ± 2.5	58.5 ± 6.2
NAR (g m^{-2} d^{-1})**	5.80 ± 0.37	4.08 ± 0.82

Significant differences between parameters at the two growth conditions are denoted (**$p < 0.01$; *$p < 0.05$).

the native habitats of this pioneer tree in Panama, did not lead to an increase in thermal tolerance beyond the upper level (~51 °C) previously observed in outer-canopy leaves from mature forest trees and seedlings cultivated under natural outdoor conditions (Krause et al., 2010). Furthermore, high nighttime temperature (32 *versus* 22 °C) did not significantly enhance thermal tolerance. Instantaneous measurements of F_v/F_m under growth conditions, showed only slight reductions of F_v/F_m during the light period which were reversible in the dark (Table 1), indicating that none of the growth treatments caused sustained photoinhibition of PSII.

The apparent lack of acclimation in thermal tolerance suggests that 51 °C represents a fundamental limit in *F. insipida*. Similar limits of thermal tolerance, based on F_v/F_m analyses, have been observed in leaves of two further neotropical tree species, *Virola sebifera* (see *Introduction*) and *Swietenia macrophylla* (unpublished), as well as in four Australian tropical tree species (Cunningham and Read, 2006). Extension of this analysis to a wider range of tropical forest species is desirable yet it is noteworthy that these observations contrast with the significant acclimation potential of thermal tolerance in plants of biomes with high seasonal and diel temperature variations, notably desert species (Downton et al., 1984; Seemann et al., 1984, 1986) and alpine plants (Braun et al., 2002).

Net photosynthetic CO_2 uptake (A_{sat}) and dark respiratory net CO_2 loss (R_d)

Owing to reduced seasonality in the humid tropics compared to temperate or boreal systems, thermal acclimation potential of photosynthesis and respiration in tropical plants has been postulated to be limited (Janzen, 1967; Cunningham and Read, 2003; Ghalambor et al., 2006). Our study clearly demonstrates significant acclimation in these performance traits to elevated nighttime temperature, as evident from substantial differences in A_{sat} and R_d between plants grown under the two temperature regimes (Fig. 2). In both groups of plants, A_{sat} was relatively stable over a wide range of leaf temperatures (~22–39 °C), but exhibited higher rates in plants grown under elevated nighttime temperature (Fig. 2a). The absence of a clear temperature optimum of A_{sat} in both treatments is notable. Additional experiments with well-watered seedlings of *F. insipida* confirmed high photosynthetic competence over a wide range of leaf temperatures, and demonstrated 66% of A_{sat} at leaf temperatures as high as 44 °C (K. Winter and A. Cheesman, unpublished data). This finding, together with the observation of irreversible leaf damage at 51 °C (Fig. 1) suggests a dramatic decline in rate of photosynthesis above 44 °C. In contrast to *F. insipida*, Cunningham and Read (2003) noted relatively narrow temperature optima and sharp declines of maximum net photosynthesis above 30 °C in three out of four Australian tropical tree species adapted to considerably lower mean annual temperatures than those of lowland Panama. It is too early for generalizations regarding photosynthetic temperature responses of tropical trees. Clearly, species like *F. insipida* seem well adapted photosynthetically to air temperatures above those of their current habitat range.

We demonstrate acclimation of leaf-level R_d, showing a reduced increase in R_d with increasing temperature in plants grown at 39/32 °C compared to growth at 39/22 °C (Fig. 2b). In plants acclimated to the elevated nighttime temperature (32 °C), R_d increased only 1.6-fold when leaf temperature was raised from 22 to 32 °C, whereas in plants grown at 22 °C, R_d increased 2.3-fold over the same temperature range. This pattern of temperature response is consistent with Type I acclimation of R_d as defined by Atkin and Tjoelker (2003), wherein plants acclimated to high temperatures do so by a reduction in the temperature dependence of R_d but may exhibit similar absolute rates as non-acclimated plants at lower temperatures.

The apparent decoupling between acclimation of CO_2 exchange and thermal tolerance indicates that *F. insipida*, within the limits given by thermal stability of leaf tissue and photosynthetic apparatus, is capable of adjusting its metabolism to warmer temperature.

Growth

Although growth responses to temperature were not the primary motivation of the study, its most striking result is the profound increase in biomass accumulation of plants under elevated nighttime temperature (Fig. 3; Table 2). This result contrasts with the widely assumed negative impact of increased nighttime temperature upon tropical tree growth (Clark et al., 2003, 2010) and the general paradigm that warmer temperatures reduce tree growth in the tropics (Way and Oren, 2010; but see Esmail and Oelbermann, 2011). Consistent with the present study, nighttime warming has been shown to stimulate growth in tobacco (Camus and Went, 1952), cotton (Königer and Winter, 1993), and in another tropical pioneer tree species, *Ochroma pyramidale* (A. Cheesman and K. Winter, unpublished data). Furthermore, increased nighttime temperatures have been implicated in increased growth of red oak seedlings in urban environments in upstate New York (Searle et al., 2012).

At this point, we cannot present a definitive mechanism to explain the growth enhancement under elevated nighttime temperature in *F. insipida*. Although rates of R_d at growth-chamber nighttime temperatures (32 *versus* 22 °C) were higher in plants grown at 39/32 °C, this is more than compensated for by concomitant increases in A_{sat} allowing for greater net carbon gain at the leaf level under the higher nighttime temperature regime (Fig. 2). However, it is by no means clear whether this increased carbon gain is the reason or the consequence of increased growth rates (Körner, 2003; Sala et al., 2012), and how tree growth and carbon storage are linked (Wiley and Helliker, 2012). Higher R_d at higher nighttime temperatures could lead to faster breakdown of photosynthate, thereby providing more energy for growth and enhancing the assimilation of new carbon. This view emphasizes the productive features of R_d rather than its CO_2 releasing attributes that negatively impact a plant's carbon budget. Future studies of source–sink relationships, meristematic activity, and carbon use efficiency (Hansen et al., 2009) may provide insight into the mechanism leading to enhanced growth under elevated nighttime temperature.

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References

Alexander LV, Zhang X, Peterson TC, Caesar J, Gleason B, Tank AMGK, et al. Global observed changes in daily climate extremes of temperature and precipitation. J Geophys Res: Atmos 2006;111:D05109.
Atkin OK, Tjoelker MG. Thermal acclimation and the dynamic response of plant respiration to temperature. Trends Plant Sci 2003;8:343–51.
Bigras FJ. Selection of white spruce families in the context of climate change: heat tolerance. Tree Physiol 2000;20:1227–34.
Bilger H-W, Schreiber U, Lange OL. Determination of leaf heat resistance: comparative investigation of chlorophyll fluorescence changes and tissue necrosis methods. Oecologia 1984;63:256–62.
Braun V, Buchner O, Neuner G. Thermotolerance of photosystem 2 of three alpine plant species under field conditions. Photosynthetica 2002;40:587–95.
Camus GC, Went FW. Thermoperiodicity of three varieties of *Nicotiana tabacum*. Am J Bot 1952;38:521–8.
Clark DA, Piper SC, Keeling CD, Clark DB. Tropical rain forest tree growth and atmospheric carbon dynamics linked to interannual temperature variation during 1984–2000. Proc Natl Acad Sci USA 2003;100:5852–7.

Clark DB, Clark DA, Oberbauer SF. Annual wood production in a tropical rain forest in NE Costa Rica linked to climatic variation but not to increasing CO_2. Glob Change Biol 2010;16:747–59.
Colwell RK, Brehm G, Cardelus CL, Gilman AC, Longino JT. Global warming, elevational range shifts, and lowland biotic attrition in the wet tropics. Science 2008;322:258–61.
Corlett RT. Impacts of warming on tropical lowland rainforests. Trends Ecol Evol 2011;26:606–13.
Corlett RT. Climate change in the tropics: the end of the world as we know it? Biol Conserv 2012;151:22–5.
Cramer W, Bondeau A, Schaphoff S, Lucht W, Smith B, Sitch S. Tropical forests and global carbon cycle: impacts of atmospheric carbon dioxide, climate change and rate of deforestation. Philos Trans R Soc Lond B Biol Sci 2004;359:331–43.
Cunningham SC, Read J. Do temperate rainforest trees have a greater ability to acclimate to changing temperatures than tropical rainforest trees? New Phytol 2003;157:55–64.
Cunningham SC, Read J. Foliar temperature tolerance of temperate and tropical evergreen rain forest trees of Australia. Tree Physiol 2006;26:1435–43.
Diffenbaugh NS, Scherer M. Observational and model evidence of global emergence of permanent, unprecedented heat in the 20th and 21st centuries. Clim Change 2011;107:615–24.
Dillaway DN, Kruger EL. Leaf respiratory acclimation to climate: comparisons among boreal and temperate tree species along a latitudinal transect. Tree Physiol 2011;31:1114–27.
Downton WJS, Berry JA, Seemann JR. Tolerance of photosynthesis to high temperature in desert plants. Plant Physiol 1984;74:786–90.
Easterling DR, Horton B, Jones PD, Peterson TC, Karl TR, Parker DE, et al. Maximum and minimum temperature trends for the globe. Science 1997;277:364–7.
Esmail S, Oelbermann M. The impact of climate change on the growth of tropical agroforestry tree seedlings. Agrofor Syst 2011;83:235–44.
Ghalambor CK, Huey RB, Martin PR, Tewksbury JJ, Wang G. Are mountain passes higher in the tropics? Janzen's hypothesis revisited. Integr Comp Biol 2006;46:5–17.
Hansen LD, Thomas NR, Arnholdt-Schmitt B. Temperature responses of substrate carbon conversion efficiencies and growth rates of plant tissues. Physiol Plant 2009;137:446–58.
Hansen J, Ruedy R, Sato M, Lo K. Global surface temperature change. Rev Geophys 2010;48:RG4004.
IPCC. Climate change: the physical science basis. Contribution of Working Group I to the fourth assessment report of the intergovernmental panel on climate change. Cambridge, UK, and New York: Cambridge University Press; 2007.
Janzen DH. Why mountain passes are higher in the tropics. Am Nat 1967;101:233–49.
Jentsch A, Beierkuhnlein C. Research frontiers in climate change: effects of extreme meteorological events on ecosystems. Compt Rend Geosci 2008;340:621–8.
Königer M, Winter K. Growth and photosynthesis of *Gossypium hirsutum* L at high photon flux densities: effects of soil temperatures and nocturnal air temperatures. Agronomie 1993;13:423–31.
Körner C. Carbon limitation in trees. J Ecol 2003;91:4–17.
Krause GH, Gallé A, Virgo A, García M, Bucic P, Jahns P, et al. High-light stress does not impair biomass accumulation of sun-acclimated tropical tree seedlings (*Calophyllum longifolium* Willd. and *Tectona grandis* L.f.). Plant Biol 2006;8:31–41.
Krause GH, Winter K, Krause B, Jahns P, García M, Aranda J, et al. High-temperature tolerance of a tropical tree, *Ficus insipida*: methodological reassessment and climate change considerations. Funct Plant Biol 2010;37:890–900.
Kukla G, Karl TR. Nighttime warming and the greenhouse-effect. Environ Sci Technol 1993;27:1468–74.
Ladjal M, Epron D, Ducrey M. Effects of drought preconditioning on thermotolerance of photosystem II and susceptibility of photosynthesis to heat stress in cedar seedlings. Tree Physiol 2000;20:1235–41.
Lintner BR, Biasutti M, Diffenbaugh NS, Lee JE, Niznik MJ, Findell KL. Amplification of wet and dry month occurrence over tropical land regions in response to global warming. J Geophys Res: Atmos 2012;117:D11106.
Lloyd J, Taylor JA. On the temperature-dependance of soil respiration. Funct Ecol 1994;8:315–23.
Mahli Y, Wright J. Spatial patterns and recent trends in the climate of tropical rainforest regions. Philos Trans R Soc Lond B Biol Sci 2004;359:311–29.
Malamud BD, Turcotte DL, Grimmond CSB. Temperature trends at the Mauna Loa observatory, Hawaii. Clim Past 2011;7:975–83.
Méthy M, Gillon D, Houssard C. Temperature-induced changes of photosystem II activity in *Quercus ilex* and *Pinus halepensis*. Can J For Res 1997;27:31–8.
Offord CA. Pushed to the limit: consequences of climate change for the Araucariaceae: a relictual rain forest family. Ann Bot 2011;108:347–57.
R Development Core Team. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2011.
Sala A, Woodruff DR, Meinzer FC. Carbon dynamics in trees: feast or famine? Tree Physiol 2012;32:764–75.
Schreiber U, Berry JA. Heat-induced changes of chlorophyll fluorescence in intact leaves correlated with damage of the photosynthetic apparatus. Planta 1977;136:233–8.
Searle SY, Turnbull MH, Boelman NT, Schuster WSF, Yakir D, Griffin KL. Urban environment of New York City promotes growth in northern red oak seedlings. Tree Physiol 2012;32:389–400.
Seemann JR, Berry JA, Downton WJS. Photosynthetic response and adaptation to high temperature in desert plants. A comparison of gas exchange and fluorescence methods for studies of thermal tolerance. Plant Physiol 1984;75:364–8.
Seemann JR, Downton WJS, Berry JA. Temperature and leaf osmotic potential as factors in the acclimation of photosynthesis to high temperature in desert plants. Plant Physiol 1986;80:926–30.
Smillie RM, Nott R. Heat injury in leaves of alpine, temperate and tropical plants. Aust J Plant Physiol 1979;6:135–41.
Way DA, Oren R. Differential responses to changes in growth temperature between trees from different functional groups and biomes: a review and synthesis of data. Tree Physiol 2010;30:669–88.
Weng J-H, Lai M-F. Estimating heat tolerance among plant species by two chlorophyll fluorescence parameters. Photosynthetica 2005;43:439–44.
Wiley E, Helliker B. A re-evaluation of carbon storage in trees lends greater support for carbon limitation to growth. New Phytol 2012;195:285–9.