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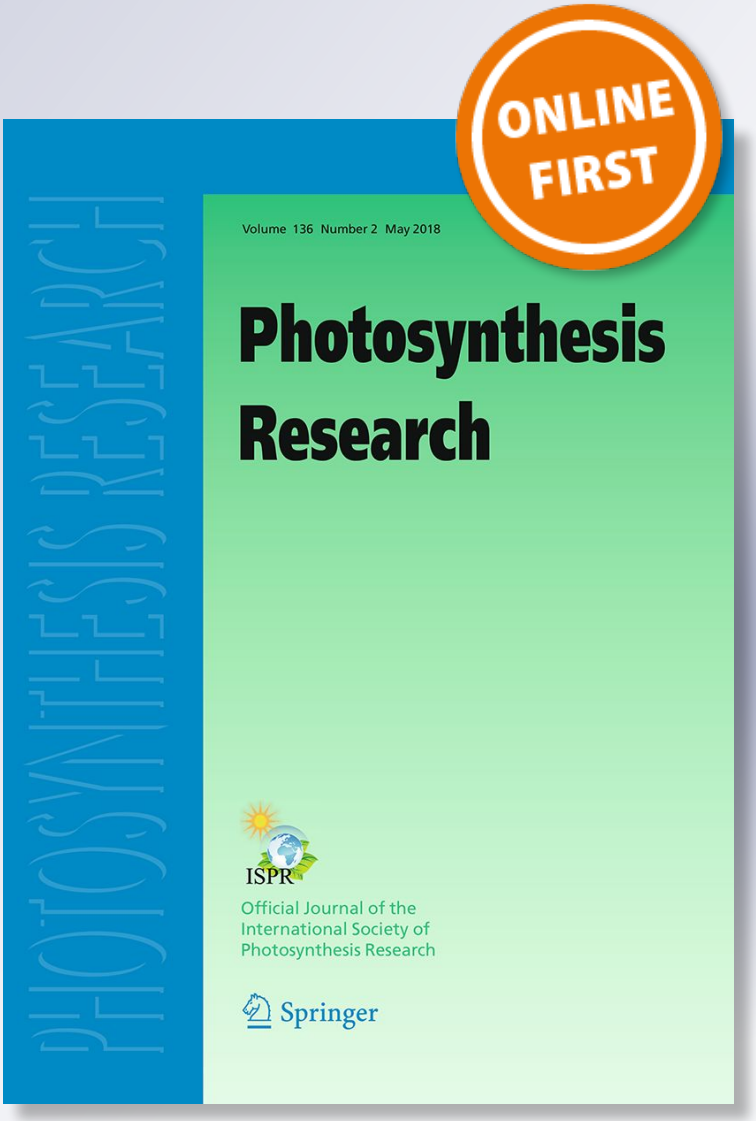
Photosynthesis Research
Official Journal of the International Society of Photosynthesis Research

ISSN 0166-8595

Photosynth Res
DOI 10.1007/s11120-018-0563-3



Springer

Photosynthesis Research
https://doi.org/10.1007/s11120-018-0563-3

ORIGINAL ARTICLE

Photosynthetic heat tolerance of shade and sun leaves of three tropical tree species

Martijn Slot[1] · G. Heinrich Krause[1,2] · Barbara Krause[1] · Georgia G. Hernández[1] · Klaus Winter[1]

Received: 7 May 2018 / Accepted: 21 July 2018

Abstract

Previous studies of heat tolerance of tropical trees have focused on canopy leaves exposed to full sunlight and high temperatures. However, in lowland tropical forests with leaf area indices of 5–6, the vast majority of leaves experience varying degrees of shade and a reduced heat load compared to sun leaves. Here we tested whether heat tolerance is lower in shade than in sun leaves. For three tropical tree species, *Calophyllum inophyllum, Inga spectabilis*, and *Ormosia macrocalyx*, disks of fully developed shade and sun leaves were subjected to 15-min heat treatments, followed by measurement of chlorophyll *a* fluorescence after 48 h of recovery. In two of the three species, the temperature causing a 50% decrease of the fluorescence ratio F_v/F_m (T_{50}) was significantly lower (by ~ 1.0 °C) in shade than in sun leaves, indicating a moderately decreased heat tolerance of shade leaves. In shade leaves of these two species, the rise in initial fluorescence, F_0, also occurred at lower temperatures. In the third species, there was no shade-sun difference in T_{50}. In situ measurements of photosynthetic CO_2 assimilation showed that the optimum temperature for photosynthesis tended to be lower in shade leaves, although differences were not significant. At supra-optimal temperatures, photosynthesis was largely constrained by stomatal conductance, and the high-temperature CO_2 compensation point, T_{Max}, occurred at considerably lower temperatures than T_{50}. Our study demonstrates that the temperature response of shade leaves of tropical trees differs only marginally from that of sun leaves, both in terms of heat tolerance and photosynthetic performance.

Keywords Chlorophyll fluorescence · Global warming · Heat tolerance · Photosynthetic temperature response · Shade acclimation · Tropical forest

Introduction

Tropical forests contain two-thirds of live biomass on the planet and are responsible for at least one-third of global net primary productivity (Pan et al. 2013), yet the carbon sink of intact tropical forests is declining (Brienen et al. 2015). As temperatures increase photosynthetic carbon uptake by tropical vegetation decreases (Doughty and Goulden 2008; Slot and Winter 2017a; Tan et al. 2017) and tropical forests may come close to, or exceed their thermal threshold (Doughty and Goulden 2008; Mau et al. 2018). Decreases in photosynthesis in response to moderate increases in temperature above the optimum temperature are primarily regulated by stomatal conductance (Slot and Winter 2017b) and are thus reversible. However, exceeding the upper temperature limit of heat tolerance leads to irreversible leaf damage, with potentially severe consequences for the carbon balance of trees.

More than 150 years ago, Sachs (1864) examined the heat tolerance of leaves from a range of species from different climate zones growing at his university in Germany. He first warmed the leaves and then monitored them for subsequent leaf damage. He observed that irreversible leaf damage occurred at an air temperature of ~ 51 °C. Since then the mean global temperature has increased by ~ 1.0 °C and will likely have increased by > 2.0 °C by the year 2100 (Pachauri et al. 2014). More importantly, extreme events

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11120-018-0563-3) contains supplementary material, which is available to authorized users.

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Published online: 27 July 2018

such as heat waves are occurring with greater frequency and severity (Perkins et al. 2012) and this trend is predicted to continue in the future (Meehl and Tebaldi 2004; Diffenbaugh et al. 2017). The rise in global temperature and related weather extremes has increased the interest in determining patterns of leaf heat tolerance (e.g., O'Sullivan et al. 2017; Sastry and Barua 2017; Sastry et al. 2018; Zhu et al. 2018). Already today, sun-exposed outer canopy leaves of tropical trees can experience temperatures up to 48 °C during the diurnal cycle, especially when midday-stomatal closure reduces transpirational cooling (Zotz et al. 1995; Krause et al. 2010; Slot et al. 2016).

In most contemporary studies, heat tolerance is assessed by using techniques based on chlorophyll *a* (Chl *a*) fluorescence, first described by Schreiber and Berry (1977). Generally, leaf damage is determined either by the temperature-dependent increase of initial fluorescence (F_0), or by the decrease of the ratio of variable to maximum fluorescence (F_v/F_m), a measure of potential photosystem II (PSII) efficiency. Both parameters have been shown to correlate with the development of visible leaf necrosis as used by Sachs (1864) (e.g., Bilger et al. 1984; Krause et al. 2010, 2013) and both are less time-consuming than the necrosis test, but the F_v/F_m approach provides more reliable data (Krause et al. 2010). Chl *a* fluorescence emission is based not only on the physiochemical properties of PSII, but also on leaf optical properties, such as the amount of light absorbed by leaves and by PSII reaction centers. When a stress treatment affects leaf optical properties independent of physiochemical properties, as might be the case at high temperatures (Takahashi and Badger 2011), the use of fluorescence ratios (F_v/F_m) rather than absolute values (F_0) is recommended, as the ratios are likely to cancel out potential errors (Baker 2008).

A recent study based on F_0 measurements of sun leaves reported an 8 °C increase in T_{Crit} (the critical temperature at which PSII inactivation is imminent) from the Arctic to the tropics (O'Sullivan et al. 2017). The average T_{Crit} of species tested at the warmest site, in the Peruvian Amazon, was 50.8 °C, remarkably similar to the heat tolerance determined for a series of tropical and temperate plant species by Sachs (1864). The increase in T_{Crit} from the Arctic to the tropics is moderate, given the 20 °C increase in mean maximum daytime temperature, but nonetheless demonstrates adaptation of tropical plants to the higher ambient temperatures.

Leaf functional traits, including heat tolerance, are predominantly investigated on sun-exposed leaves, in part because it facilitates comparisons across studies that measurement on leaves from different shade environments do not. However, forests typically maintain a leaf area index (LAI, projected one-sided leaf area per unit ground area) of 5 m^2 m^{-2} (Asner et al. 2003) and in tropical forests, LAI is often even higher (e.g., direct measurements in Costa Rica yielded a landscape level mean LAI of 6.0 m^2 m^{-2}; Clark et al. 2008). This means that for every sun leaf in the forest, there may be four or five leaves that experience some level of shading, and these rarely studied shade leaves make a significant contribution to carbon uptake and dynamics in the forest (He et al. 2018). Shade leaves typically have lower maximum rates of photosynthesis and dark respiration than sun leaves, but require lower light levels to maintain a positive net carbon balance because shade leaves have a lower light compensation point. Leaf temperature is largely a function of the net radiation input (e.g., Campbell and Norman 2012; Fauset et al. 2018). Maximum temperatures experienced by shade leaves in a tropical forest canopy are therefore much lower than those of sun leaves (Rey-Sánchez et al. 2016; Fauset et al. 2018). Maintaining high heat tolerance may be energetically costly (Wahid et al. 2007), and plants are capable of seasonally downregulating heat tolerance (Sastry et al. 2018). Down-regulated heat tolerance in shade leaves would therefore seem advantageous.

Here we tested the hypothesis that shade leaves of lowland tropical tree species have lower heat tolerance than sun leaves. The limit of heat tolerance was assessed by recording the irreversible decrease in F_v/F_m and increase in F_0 of leaf segments incubated at different temperatures. Furthermore, the relationship of net photosynthesis to temperature was assessed for shade and sun leaves in situ to determine whether heat tolerance differences based on Chl *a* measurements are reflected by differences in the temperature-dependence of photosynthetic CO_2 assimilation.

Materials and methods

Plant material and growth conditions

Shade and sun leaves were studied for *Calophyllum inophyllum* L. (Clusiaceae), *Inga spectabilis* (Vahl) Willd. (Fabaceae), and *Ormosia macrocalyx* Ducke (Fabaceae). *C. inophyllum* is a common ornamental tree native to tropical Asia and the Pacific, where it grows in coastal and lowland forests. It is a high-light demanding, but slow-growing species. *I. spectabilis* is a medium-sized central American nitrogen-fixing tree species common from lowlands to mid-elevation and is used as a shade tree in agroforestry. *O. macrocalyx* is a late-successional nitrogen-fixing tree species distributed from southern Mexico to Brazil. For each species one sun-exposed, free-standing mature tree with branches that could be accessed from the ground was selected, *C. inophyllum* growing in the area of the Tupper Center of the Smithsonian Tropical Research Institute in Panama City, Republic of Panama, *I. spectabilis* and *O. macrocalyx* growing in an open area in Gamboa, 30 km NW of Panama City. All three species are evergreen at these study sites. Mean annual temperature at both sites is 26.9 °C, with mean daily

maximum temperatures of 32.6 °C in Gamboa and 32.2 °C in Panama City. Annual precipitation averages 2250 and 1900 mm year^{-1} in Gamboa and Panama City, respectively. Both sites have a distinct dry season from January to April during which rainfall averages < 100 mm month^{-1}.

Characterization of shade and sun leaves

Morphological and physiological traits were used to characterize the shade and sun leaves. Mature leaves selected at 1.5–2.0 m height from the inner and outer tree canopies were harvested between 7:00 and 9:00 h. Leaf blade area was measured with an LI-3100 leaf area meter (LI-COR, Lincoln, NE, USA) and the blades were dried at 70 °C to determine specific leaf area (SLA). Photosynthetic light response curves were determined on an additional set of leaves in situ with an LI6400XT portable photosynthesis system (LI-COR). Net photosynthesis (A) was measured at 400 µLL^{-1} CO_2 and 30 ± 1.0 °C, first at ambient irradiance, and then at a series of ten light levels. The maximum light level was 1000 µmol photons m^{-2} s^{-1} for shade leaves and 1800 µmol m^{-2} s^{-1} for sun leaves. For each set of measurements, a light-response curve was fitted with a non-rectangular hyperbola as

$$A = \frac{\varphi I + A_{Sat} - \sqrt{\left(\varphi I + A_{Sat}\right)^2 - 4\theta\varphi I A_{Sat}}}{2\theta} - R_d\,, \quad (1)$$

where φ is the apparent quantum yield, I the photon flux density incident on the leaf, A_{Sat} the light-saturated net photosynthesis rate, θ the curvature parameter, and R_{Dark} is the dark respiration rate. By optimizing the fit of these curves, R_{dark} and A_{sat} were determined and the light compensation point (LCP) estimated. Confidence intervals of the parameters describing these curves were determined using the 'nls' function from the 'stats' package in R version 3.3.2. (R Development Core Team 2016). In *I. spectabilis* leaf handling tended to cause stomatal closure, and full curves could not be obtained on single leaves. Instead, at each light level a series of leaves was measured after a short equilibration period, before stomatal conductance strongly declined. A single light-response curve was fitted for the pooled measurements of shade leaves and a second curve was fitted for the sun leaves.

Test of heat tolerance

Mature shade and sun leaves, harvested in the morning, were stored under moist conditions in dim light at ~ 25 °C. Heat tolerance tests were carried out between 9:00 and 15:00 h. Six leaf disks (diameter 2.0 cm), one from each of six shade leaves or six sun leaves, were placed on a wire mesh sheet positioned a few mm below the water surface of a preheated water bath (Lauda RM6/RMS, Analytical Instruments, LLC, Golden Valley, MN, USA). The abaxial leaf surface was fully immersed, whereas the adaxial surface remained dry. Leaf disks were incubated at a preset temperature for 15 min in darkness (to avoid photoinhibition of shade leaves). Adaxial leaf surface temperature matched the water temperature. Leaf temperature was monitored with an infrared thermometer (MiniTemp ®, Raytek, Santa Cruz, CA, USA), calibrated against measurements with a copper-constantan thermocouple. Incubations were performed at 6–8 temperatures between 47 and 54 °C. Untreated disks served as controls. After the heat treatment, the disks were stored on moist filter paper in Petri dishes at 25–27 °C under dim light (5–10 µmol photons m^{-2} s^{-1}). Chl *a* fluorescence was recorded 48 h after heat treatment.

Chlorophyll a fluorescence

High-temperature tolerance of leaves was determined following the protocol described by Krause et al. (2010). In brief, at each temperature, the initial Chl *a* fluorescence emission (F_0), maximum total fluorescence (F_m), and the ratio of variable (F_m–F_0) to maximum fluorescence (F_v/F_m) were recorded on six temperature-treated leaf disks per species and sun-exposure category after dark adaptation for 10 min. Measurements were made with a PAM 2000 fluorometer (Walz GmbH, Effeltrich, Germany). To determine T_{50}, the temperature at which F_v/F_m was reduced to 50% of the untreated control disks, the decrease in F_v/F_m as a function of incubation temperature was fitted with a logistic function in which F_v/F_m asymptotes towards the minimum observed values ($F_v/F_{m,\,Min}$) as

$$F_v/F_m = \frac{F_v/F_{m,\,Max} - F_v/F_{m,\,Min}}{1 + e^{b\times(T_{Leaf} - T_{50})}} + F_v/F_{m,\,Min} \quad (2)$$

where $F_v/F_{m,Max}$ is the average F_v/F_m measured on control leaf disks that were not heat-treated; b is a constant that reflects the rate of F_v/F_m decrease, and T_{Leaf} is the incubation temperature (°C) of the leaf disks. Curves asymptoting to $F_v/F_{m,Min}$ fitted the data better than curves asymptoting to zero, as F_v/F_m generally did not reach zero in the measured temperature range. Curves were fitted and standard errors of the curve parameters were determined with the 'nls' function from the 'stats' package in R (R Development Core Team 2016).

T_{Crit} the critical temperature beyond which F_v/F_m declines and leaves are irreversibly heat damaged, was determined as the temperature at which a line describing the linear part of the F_v/F_m decline (for which we used a 1.5 °C range centered on T_{50}) intersected with a horizontal line indicating $F_v/F_{m,Max}$:

$$T_{Crit} = \frac{F_v/F_{m,\,Max} \; - \; Intercept\ linear\ fit}{Slope\ linear\ fit} \tag{3}$$

T_{Crit} is the highest temperature that not yet causes PSII damage, whereas T_{50} is the temperature at which PSII functionality is reduced by 50%.

T_{50} and T_{Crit} were also determined for F_0. For these curves, we only considered the increase in F_0; data points of the F_0 decrease above ~ 53 °C were not included when fitting logistic curves to the F_0 data.

In situ photosynthesis temperature response

The temperature response of light-saturated photosynthesis in shade and sun leaves was measured in situ with either an LI-6400XT (*I. spectabilis, O. macrocalyx*), or an LI-6800 portable photosynthesis system (LI-COR) (*C. inophyllum*) at a CO_2 concentration of 400 μLL^{-1} entering the leaf cuvette. For shade leaves, the light level in the cuvette was set to 1000 µmol photons m^{-2} s^{-1}; for sun leaves we used 1500 µmol m^{-2} s^{-1}. To maximize the leaf temperature range, we used a combination of ambient temperature variation between 9:00 and ~ 14:00 h, and manipulation of the block temperature of the Peltier-controlled leaf cuvette. For each species, all measurements on shade and sun leaves, respectively, were pooled before fitting the temperature-response curves. Two types of curves were fitted to determine the optimum temperature for photosynthesis (T_{Opt}), the rate of photosynthesis at T_{Opt} (A_{Opt}), and the high-temperature CO_2 compensation point (T_{Max}). First, T_{Opt} and A_{Opt} were determined by fitting photosynthesis according to June et al. (2004) as

$$A(T) \; = \; A_{Opt} \; \times \; e^{-\left(\frac{T_{Leaf} \; - \; T_{Opt}}{\Omega}\right)^2} \tag{4}$$

where A(T) is net photosynthesis per unit leaf area at leaf temperature T_{leaf}; Ω is the difference between T_{Opt} and the temperature at which A is reduced to e^{-1} (37% of A_{Opt}) and thus reflects the width of the peak of the temperature response curve. Then T_{Max} was calculated by fitting the data according to Cunningham and Read (2002) as

$$\mathrm{A(T)}= \mathrm{b} \; \times \; \left(T_{\mathrm{Leaf}} - T_{\mathrm{Min}}\right) \; \times \; \left(1 - \; e^{\mathrm{c} \times \left(T_{\mathrm{Leaf}} \; - \; T_{\mathrm{Max}}\right)}\right) \tag{5}$$

where T_{Min} and T_{Max} are the low- and high-temperature CO_2 compensation points, respectively, and *b* and *c* are constants. All curves were fitted using non-linear least squares with the 'nls_multstart' function in the 'nls.multstart' package version 1.0.0. (Padfield and Matheson 2018) in R, and standard errors were determined for all parameters.

Statistical analyses

Differences between parameters measured under identical conditions in shade and sun leaves were assessed by the Student *t* test. Differences were considered significant at $p < 0.05$. Differences between modeled parameters were assessed by determining whether the 95% confidence intervals overlapped or not.

Results

Characteristics of shade and sun leaves

The leaves selected from the inner- and outer-canopies of the three species exhibited characteristic properties of shade and sun leaves, respectively. The light response curves of net photosynthesis showed very low rates of net CO_2 uptake in shade compared to sun leaves and a tendency for a lower light saturation point (Fig. 1a–c). Shade leaves had significantly higher SLA, while light-saturated net photosynthesis (A_{Sat}), dark respiration (R_{Dark}), and light compensation point (LCP) were significantly lower than in sun leaves (Table 1).

Heat tolerance determined by Chl a fluorescence

Analysis of the decline in F_v/F_m as function of temperature revealed a small but significantly lower heat tolerance of shade compared to sun leaves of *C. inophyllum* (Fig. 2a). T_{50} was significantly lower for shade than for sun leaves, both when calculated from the F_v/F_m decrease and from the F_0 rise (Table 2; Fig. 2a). Significant differences in F_v/F_m between shade and sun leaves were visible after heating to 49, 50, and 51 °C. In leaves of *I. spectabilis,* T_{50} was also significantly lower in shade than in sun leaves (Table 2; Fig. 2b). There was a significant difference in F_v/F_m between shade and sun leaves at 51 °C when shade leaves had already reached values close to zero while F_v/F_m of sun leaves was still ~ 0.35, and at 48 and 49 °C, when the difference in F_v/F_m, although biologically negligible, was statistically significant due to small standard deviations (Fig. 2b). In contrast, in *O. macrocalyx* there was no difference in T_{50} between shade and sun leaves and no significant difference in F_v/F_m at any point between 48 and 53 °C (Fig. 2c). The temperature response of sun leaves of *O. macrocalyx* was similar to that of shade leaves for the other two species studied (Fig. 2), indicating lower heat tolerance of sun leaves in *O. macrocalyx*, without increased heat sensitivity of shade leaves.

Results for T_{Crit} closely resembled those for T_{50}. In *C. inophyllum* and *I. spectabilis,* T_{Crit} was considerably lower in shade leaves than in sun leaves while in *O. macrocalyx* there was no difference (Table 2). On average T_{Crit} values

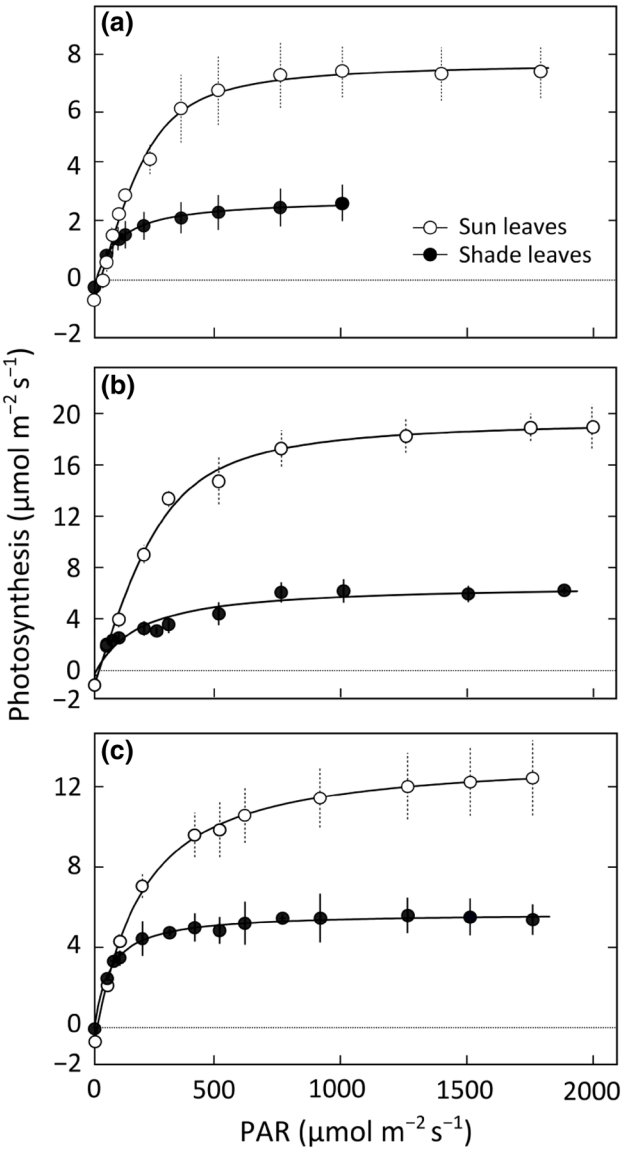

Fig. 1 Net photosynthesis as a function of photosynthetically active radiation (PAR) for shade (closed symbols) and sun (open symbols) leaves of *C. inophyllum* (**a**), *I. spectabilis* (**b**), and *O. macrocalyx* (**c**). Error bars indicate standard deviations of 2–9 replicate measurements on different leaves

were 1.5 °C lower than T_{50} when calculated from F_v/F_m and 1.3 °C lower when calculated from F_0. The T_{50}–T_{Crit} differential was not significantly different between shade and sun leaves, suggesting that the decline in F_v/F_m and the rise in F_0 were not steeper in shade leaves than in sun leaves. This was confirmed by an absence of differences in the *b* parameter of Eq. 2 (data not shown).

The plot of F_0 versus incubation temperature (Fig. 3a–c) supports the results seen in Fig. 2a–c. In shade leaves of *C. inophyllum*, the increase in F_0 began at lower temperature than in sun leaves. (Fig. 3a), i.e., T_{Crit} calculated for F_0 was lower, as was T_{50} (Table 2). Similar effects on F_0 were observed in leaves of *I. spectabilis*, but differences between shade and sun leaves were significant only at 49 °C (Fig. 3b). In contrast to *C. inophyllum* and *I. spectabilis*, and consistent with the F_v/F_m results, the response of F_0 to heating did not differ between shade and sun leaves of *O. macrocalyx* (Fig. 3c).

Following the F_0 increase starting at ~48 °C, F_0 eventually decreased at temperatures at which F_v/F_m was reduced to values that were not significantly different from the final minimum (~52 °C). The decrease in F_0 tended to start at lower temperatures in shade leaves than in sun leaves (i.e., the temperature maximum of F_0 was lower in shade leaves).

Temperature responses of in situ photosynthesis

The optimum temperature for in situ net photosynthesis (T_{Opt}) tended to be lower in shade leaves than in sun leaves (Fig. 4a–c; Table 3), but this difference was not significant (Table 3).

Maximum light-saturated net photosynthesis (A_{Opt}) was significantly different between shade and sun leaves (Fig. 4; Table 3), with peak values for sun leaves of 16.0 ± 0.6, 17.1 ± 0.8, and 15.3 ± 0.4 µmol m^{-2} s^{-1} for *C. inophyllum, I. spectabilis*, and *O. macrocalyx*, respectively (parameter estimate derived from Eq. 4 ± SE of the estimate). A_{Opt} of shade leaves was less than half that of sun leaves with minimal differences among species (Table 3). The high-temperature CO_2 compensation point (T_{max}), estimated with Eq. 5, did not differ significantly between shade and sun leaves, nor did the width (Ω) of the temperature response curves (Fig. 4; Table 3).

Net photosynthesis correlated with stomatal conductance, which decreased as temperatures increased above T_{Opt} of net photosynthesis. This was observed in both shade and sun leaves, except in shade leaves of *I. spectabilis*, which showed a decrease in stomatal conductance across the entire temperature range (Fig. 5a–f). The average T_{Opt} for stomatal conductance (31.4 °C) was remarkably similar to that of net photosynthesis (31.6 °C, see Table 3). The decrease in stomatal conductance with increased temperature was associated with an increase in leaf-to-air vapor pressure deficit (Fig. S1).

Discussion

Species differences and shade tolerance

We tested the hypothesis that shade leaves of tropical trees would have lower heat tolerance than sun leaves. The present study lends partial support for this hypothesis. Heat tolerance, determined as the temperature at which 50% of PSII

Table 1 Specific leaf area, SLA, and characteristics of CO_2 exchange of shade and sun leaves

Species	SLA ($cm^2\ g^{-1}$)	A_{Sat} ($\mu mol\ m^{-2}\ s^{-1}$)	R_{dark} ($\mu mol\ m^{-2}\ s^{-1}$)	LCP ($\mu mol\ m^{-2}$)
C. inophyllum				
Shade	117 ± 9 (6)**	3.15 ± 0.82 (9)**	0.28 ± 0.10 (9)**	9.4 ± 3.3 (9)**
Sun	76 ± 3 (6)	8.73 ± 0.92 (3)	0.96 ± 0.16 (3)	27.4 ± 4.7 (3)
I. spectabilis				
Shade	120 ± 12 (5)**	6.00 ± 0.78 (4)**	0.50 ± 0.12 (5)**	9.8
Sun	84 ± 5 (5)	18.76 ± 1.69 (3)	1.13 ± 0.32 (5)	17.7
O. macrocalyx				
Shade	161 ± 15 (6)**	5.55 ± 0.90 (3)**	0.21 ± 0.07 (3)**	5.7 ± 2.7 (3)*
Sun	97 ± 4 (6)	12.53 ± 1.92 (4)	0.86 ± 0.19 (4)	14.1 ± 2.8 (4)

Means ± SD are presented; in brackets number of leaves tested. Light response curves for *I. spectabilis* were fitted through pooled data; hence, no SD could be determined for LCP

A_{sat} Light-saturated net photosynthesis, R_{dark} Dark respiration, *LCP* Light compensation point

Significant shade-sun differences are indicated: $*p < 0.05$, $**p < 0.01$

functionality is lost (T_{50}), was indeed lower for shade leaves of *C. inophyllum* and *I. spectabilis*, whereas for *O. macrocalyx* there was no difference of heat tolerance between shade and sun leaves (Figs. 2, 3; Table 2). Shade-sun differences in heat tolerance thus appear to be species specific.

The lack of shade-sun difference in heat tolerance of *O. macrocalyx* is not based on a smaller shade-sun contrast compared to the other two species, as is clear from the comparison of light response curves of photosynthesis (Fig. 1), SLA, and other parameters presented in Table 1. It is noteworthy, however, that shade leaves of *O. macrocalyx* did not have elevated heat tolerance compared to the other species tested; rather their sun leaves had lower heat tolerance. For example, mean F_v/F_m at 49 °C was 0.54 for sun leaves of *O. macrocalyx*, whereas sun leaves of *C. inophyllum* and *I. spectabilis* still maintained F_v/F_m values of 0.78 and 0.75, respectively (or > 90% of the values for untreated control leaves). Of the species used in the current study, *O. macrocalyx* is the most shade-tolerant, and full sunlight has been shown to reduce growth of *O. macrocalyx* seedlings compared to conditions of partial shading (Krause et al. 2012). Nonetheless, control leaves of this species that were not heat treated exhibited similarly high F_v/F_m values as the other species (~ 0.80), in the shade as well as in the sun, demonstrating that there was no sustained photoinhibition independent of the temperature treatment that may have influenced the results.

Sastry and colleagues (Sastry and Barua 2017; Sastry et al. 2018) recently reported that heat tolerance of sun leaves of tropical tree species in India varies across species in relation to the species' position on the slow-fast continuum of resource acquisition strategies (sensu Wright et al. 2004), with resource acquisitive species with high SLA having lower heat tolerance than resource conservative species with low SLA. Similarly, Zhang et al. (2012) studying woody savanna species in southern China observed that species with long-lived leaves, generally associated with conservative resource use, had higher heat tolerance than species with shortlived leaves. Despite its status as a late-successional, shade-tolerant species, *O. macrocalyx* had the highest SLA of the three species in the current study (Table 1). While the observation of lower T_{50} in sun leaves of *O. macrocalyx* appears to be consistent with the results of Sastry and Barua (2017) and Sastry et al. (2018), more species should be studied to confirm such a relationship for neotropical tree species. Furthermore, greater replication at the species level would be needed to determine the mechanistic underpinning of species differences in heat tolerance.

Shade–sun differences in heat tolerance and the role of photoinhibition

Although we focus here solely on high-temperature effects, ecologically they cannot always be separated from the effects of irradiance. For example, when sun leaves are illuminated during heat treatment, their heat tolerance, indicated by T_{50}, is significantly higher (by ~ 1 °C) than when leaves are heated in the dark, as shown for the tropical tree species *Ficus insipida* (a pioneer species), and *Calophyllum longifolium* (a late-successional species) (Krause et al. 2015). The positive effect of light likely results from antioxidative heat protection activated by light, e.g., by formation of zeaxanthin from violaxanthin via the violaxanthin cycle (see Havaux and Niyogi 1999; Johnson et al. 2007). In shade leaves, such mechanisms appear to be less active. Königer et al. (1995) found strongly reduced pool sizes of violaxanthin cycle pigments (sum of violaxanthin, antheraxanthin, and zeaxanthin) and considerably less light-induced conversion of violaxanthin to zeaxanthin in shaded understory plants than in sun-exposed canopy leaves. Shade leaves are therefore more prone to photoinhibition of PSII (resulting from the energetic imbalance between light energy absorbed

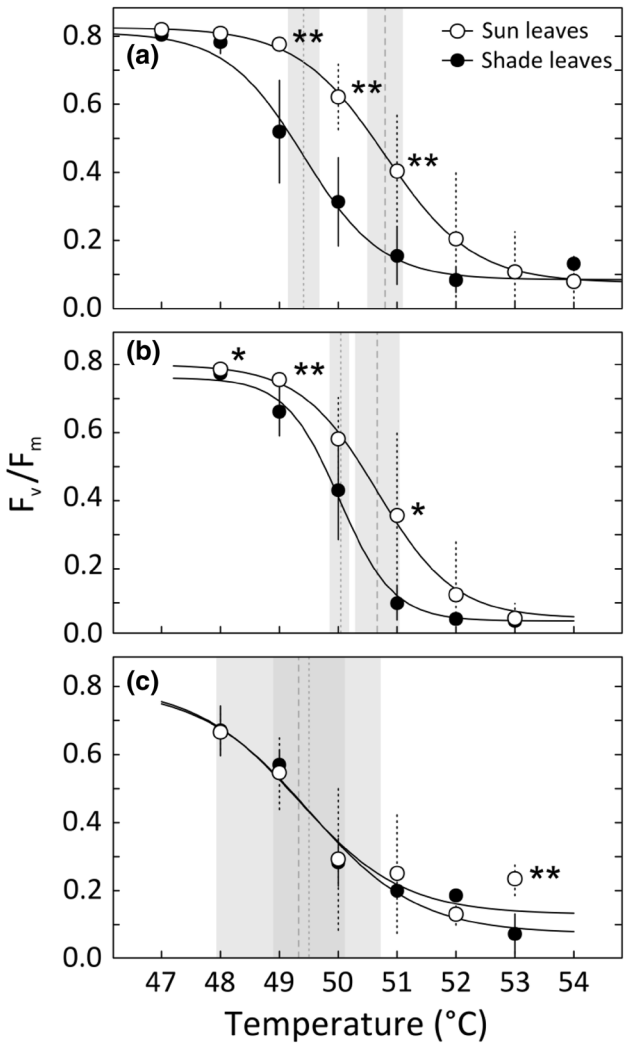

Fig. 2 Ratio of variable to maximum fluorescence (F_v/F_m) in relation to leaf temperature in shade (closed symbols) and sun (open symbols) leaves of *C. inophyllum* (**a**), *I. spectabilis* (**b**), and *O. macrocalyx* (**c**). F_v/F_m ratios of untreated controls of shade and sun leaves, respectively, were **a** 0.812 ± 0.016 and 0.826 ± 0.003, **b** 0.786 ± 0.007 and 0.805 ± 0.009, **c** 0.790 ± 0.008 and 0.805 ± 0.006. Error bars indicate standard deviations of 6 replicate measurements. Vertical-dotted and -dashed lines indicate T_{50} for shade and sun leaves, respectively. Shaded areas represent the 95% confidence intervals of T_{50}. * and ** indicate significant differences between shade and sun leaves with $p < 0.05$, and $p < 0.01$, respectively

Table 2 Heat tolerance traits based on F_v/F_m and F_0 characteristics for shade and sun leaves of three tropical tree species

Species	F_v/F_m		F_0	
	T_{50} (°C)	T_{Crit} (°C)	T_{50} (°C)	T_{Crit} (°C)
C. inophyllum				
Shade	49.4 ± 0.1*	48.0	49.0 ± 0.2*	47.7
Sun	50.8 ± 0.2	49.2	50.6 ± 0.1	48.6
I. spectabilis				
Shade	50.0 ± 0.1*	49.0	49.0 ± 0.3*	47.9
Sun	50.7 ± 0.2	49.3	49.8 ± 0.3	48.7
O. macrocalyx				
Shade	49.5 ± 0.3	47.6	49.0 ± 0.2	47.8
Sun	49.3 ± 0.7	47.5	48.8 ± 0.2	47.5

Shown are T_{50} values modeled with Eq. 2 ± their estimated standard errors, and T_{Crit} values determined with Eq. 3

*Indicates a significant shade-sun difference in T_{50} based on non-overlapping 95% confidence intervals of the parameter estimates

and the amount of energy that can be processed by CO_2 assimilation/photorespiration), which may affect their heat tolerance. Photoinhibition is usually reversible under moderate conditions (Leitsch et al. 1994), but may lead to acute photooxidative damage under sustained high-light at high-temperature exposure, a situation that may occur when shade leaves experience excess light exposure by gap formation or during prolonged sun flecks. For example, in shade leaves of the tropical understory herb *Alocasia macrorrhiza* photoinhibition intensified necrosis at high temperatures (Königer et al. 1998). Furthermore, shade leaves of *A. macrorrhiza* have reduced capacity to recover from high irradiance and associated high temperatures compared to sun leaves (Mulkey and Pearcy 1992). Shade leaves also have lower stomatal conductance and thus lower capacity for transpirational cooling than sun leaves.

The tendency for lower thermal thresholds (Figs. 2, 3; Table 2), the increased risk of compounding effects of photoinhibition (e.g., Königer et al. 1995, 1998; Mulkey and Pearcy 1992), and the limited capacity for transpirational cooling, suggest that shade leaves are particularly vulnerable when exposed to high temperatures. Nonetheless, the highest temperatures in the canopy are experienced by sun leaves (Rey-Sánchez et al. 2016), so the likelihood of heat damage might be lower in shade leaves. However, even in shade leaves occasionally elevated leaf temperatures are typically associated with high-light exposure. Hence, excess irradiation itself could become detrimental in shade leaves.

CO_2 assimilation at high temperatures

While shade and sun leaves exhibited slightly different characteristics with respect to their temperature response of photosynthetic CO_2 assimilation, these differences were not directly linked to differences in T_{50} and T_{Crit} (Fig. 4; Table 2). Net photosynthesis tends to decline with rising temperature considerably below T_{Crit}, primarily owing to a decline in stomatal conductance associated with rising VPD (Fig. 5; Slot and Winter 2017a, b). More detailed measurements on the biochemical and stomatal controls over net photosynthesis would be needed to establish differences in the drivers of the photosynthetic temperature response between shade and sun leaves, and to determine whether

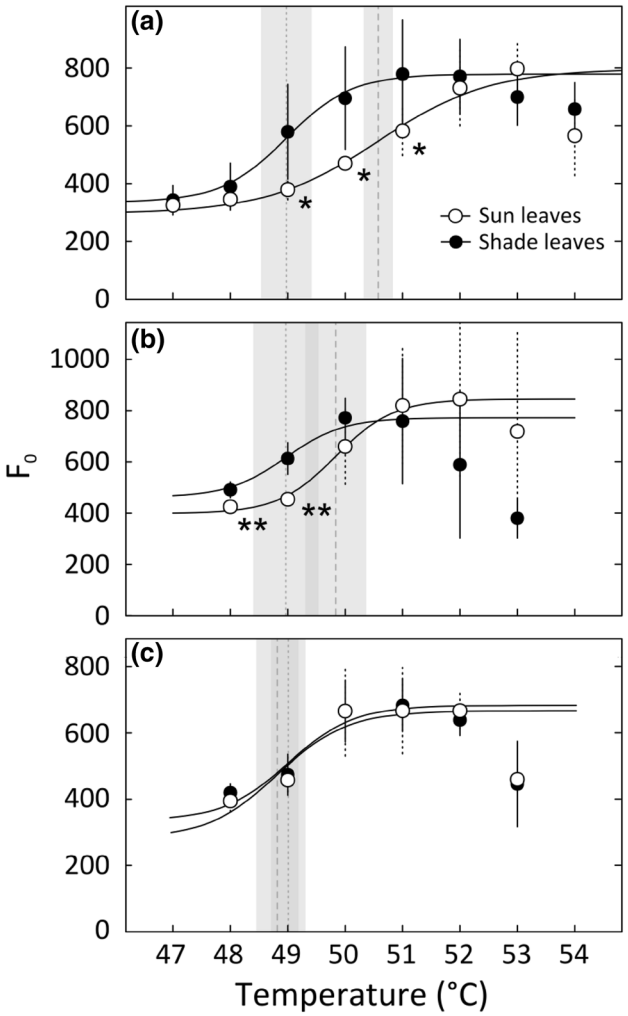

Fig. 3 Initial fluorescence (F_0) in relation to leaf temperature in shade (closed symbols) and sun (open symbols) leaves of *C. inophyllum* (**a**), *I. spectabilis* (**b**), and *O. macrocalyx* (**c**). F_0 of untreated controls (relative units) of shade and sun leaves, respectively, was **a** 335 ± 46 and 297 ± 17, **b** 462 ± 40 and 399 ± 13, **c** 336 ± 22 and 282 ± 21. Error bars indicate standard deviations of 6 replicate measurements. Vertical-dotted and -dashed lines indicate T_{50} for shade and sun leaves, respectively. Shaded areas represent the 95% confidence intervals of T_{50}. * and ** indicate significant differences between shade and sun leaves with $p < 0.05$, and $p < 0.01$, respectively

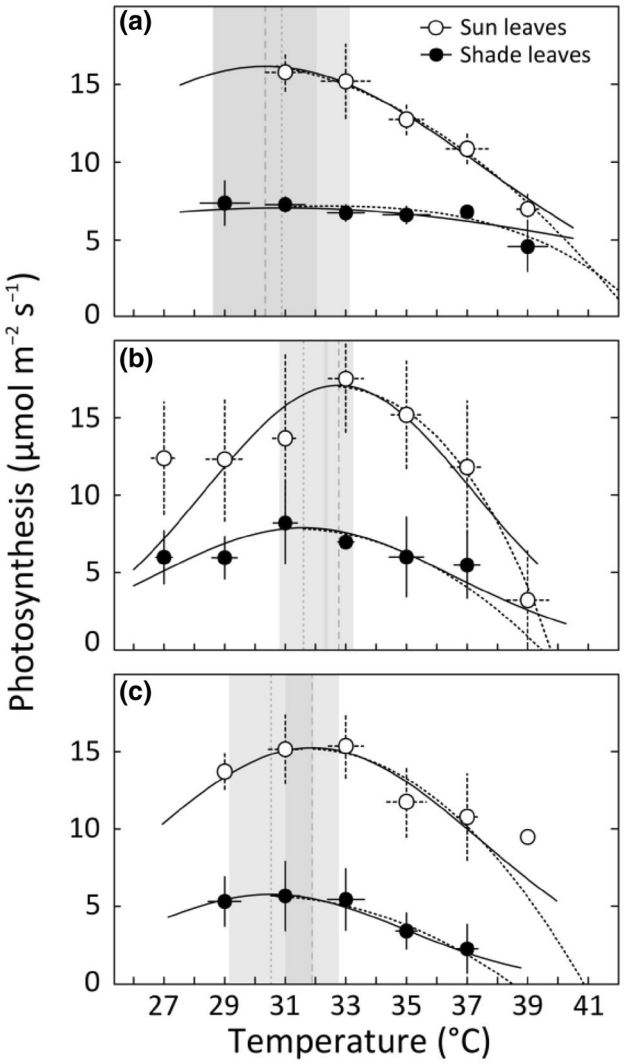

Fig. 4 Light-saturated photosynthesis as a function of leaf temperature in shade (closed symbols) and sun (open symbols) leaves of *C. inophyllum* (**a**), *I. spectabilis* (**b**), and *O. macrocalyx* (**c**). Shown are mean values ± standard deviations by 2 °C-bins. Curves are fitted to the raw data with Eq. 4. Dotted curves above T_{Opt} show the curves fitted with Eq. 5 to calculate T_{Max}. Vertical-dotted and -dashed lines indicate T_{Opt} for shade and sun leaves, respectively. Shaded areas represent the 95% confidence intervals of T_{Opt}

such differences are similarly species specific as the T_{Crit} and T_{50} differences reported here.

Thermal acclimation, heat tolerance, and climate change

Sastry and Barua (2017) reported significant plasticity in photosynthetic heat tolerance among tropical trees in India, with higher T_{50} values in the hot, dry season than in the cooler wet season. The sites these authors studied experience a highly seasonal climate: daily mean temperatures range from ~ 18 °C during winter to > 30 °C in summer. Mediterranean trees that experience considerable seasonal temperature variation similarly exhibit dynamic changes in heat tolerance (Froux et al. 2004). Zhu et al. (2018) recently reported seasonal acclimation of T_{Crit} (determined with the F_0 method) in plants from several thermally contrasting biomes in Australia, including species from a fairly a-seasonal tropical forest in north Queensland. Rapid acclimation of T_{50} was shown for *Eucalyptus parramattensis* trees during a simulated 4-day heatwave in a subtropical climate in New South Wales (Drake et al. 2018). These studies suggest that plants have the capacity to up- or down-regulate heat

Table 3 Parameters describing the characteristics of in situ temperature responses of light-saturated photosynthesis rates in shade and sun leaves

Species	T_{Opt} (°C)	A_{Opt} (μmol m^{-2} s^{-1})	Ω (°C)	T_{Max} (°C)
C. inophyllum				
Shade	30.9 ± 1.2	7.2 ± 0.2*	17.2 ± 3.7	43.0 ± 1.8
Sun	30.3 ± 0.9	16.2 ± 0.5	10.2 ± 1.1	42.5 ± 1.9
I. spectabilis				
Shade	31.6 ± 0.4 *	7.9 ± 0.5*	7.1 ± 1.1	39.4 ± 2.2
Sun	32.7 ± 0.3	17.1 ± 0.8	6.2 ± 0.6	39.8 ± 0.4
O. macrocalyx				
Shade	30.5 ± 0.7	5.8 ± 0.3*	6.3 ± 1.0	38.5 ± 1.2
Sun	31.9 ± 0.4	15.2 ± 0.4	7.9 ± 1.0	40.9 ± 2.3

The optimum temperature for photosynthesis, T_{Opt}; the rate of light-saturated photosynthesis at T_{Opt}, A_{Opt}; the sharpness or width of the peak of the temperature response curve, Ω; and the high-temperature CO_2 compensation point, T_{Max}. T_{Max} was estimated with Eq. 5; all other parameters were determined with Eq. 4. Shown are parameter estimates and their standard errors

*Indicates significant shade-sun difference based on non-overlapping 95% confidence intervals of the parameter estimates

tolerance depending on the environmental conditions and the associated risks of heat exposure. In the current study, we also see a tendency for lower heat tolerance in the cooler shade leaves compared to the warmer sun leaves. However, the magnitude of the acclimation response was remarkably small (Fig. 1; Table 2) considering the fact that leaves in the deep shade rarely experience temperatures > 33 °C (Slot, personal observation), while sun leaves routinely experience temperatures > 40 °C (Krause et al. 2010; Slot et al. 2016). These small changes in heat tolerance are consistent with the study by Sastry and Barua (2017), who observed T_{50} values of sun leaves differing by only ~ 1.0 °C despite large seasonal temperature changes. Although there clearly is some physiological plasticity, there appears to be a limit in the degree of down-regulation of T_{50} in cool seasons or shaded leaves, possibly because even in these instances, occasional direct sun exposure can warm leaves considerably above air temperature. Based on a modeling study, Schymanski et al. (2013) concluded that overheating causing damage to shade leaves during fluctuating irradiance is avoided by appropriate hydraulic capacitance and transpirational cooling. However, given the low stomatal conductance of shade leaves and the strong stomatal control of photosynthesis rates at temperatures well below T_{Crit} (Fig. 5; Slot and Winter 2017a, b), intrinsic heat tolerance as reported here appears to be essential for survival of shade leaves exposed to sunflecks or canopy gap formation.

Recent work has shown that net photosynthesis of tree seedlings from a lowland tropical forest that lacks distinct temperature seasonality can acclimate to warming above current ambient temperatures (Slot and Winter 2017c, 2018). However, in Slot and Winter (2017c), the high-temperature CO_2 compensation point, measured under controlled conditions in the laboratory, did not acclimate and remained the same over a 10 °C growth temperature range. This suggests that T_{Max} is rather inflexible, similar to T_{50} values in the current study. While there is evidence of downregulation of T_{50} during the cooler months in areas with pronounced temperature seasonality, there is very little evidence that T_{50} of tropical plants can be upregulated significantly in response to higher temperatures. Among higher plants in warm climates, only desert succulents seem to have the capacity for strong upregulation of heat tolerance (Nobel and Zutta 2008). The study by Zhu et al. (2018) evaluated acclimation of T_{Crit} to experimental temperature manipulation, but with temperature regimes of 20/15 °C versus 30/25 °C (day/night) the experiment examined the potential for acclimation to cool rather than warm temperatures for the tropical tree species under investigation. Recent studies with *Ficus insipida* Willd. saplings in Panama yielded a moderate increase in T_{50} from ~ 52° in plants grown at ambient temperature and $[CO_2]$ to ~ 53 °C for plants grown at ambient + 6 °C and double ambient $[CO_2]$ (Krause and Winter, unpublished results). This suggests that maximum heat tolerance is a highly conserved trait in plants of the humid tropics and that values in the low 50s reflect a general threshold of thermal tolerance for C_3 plants, unchanged since Sachs reported this in 1864, and unchanging as global temperatures rise. Global warming will increase the likelihood of extreme leaf temperatures, and as $[CO_2]$ rises, the reduction in stomatal conductance and the associated decrease in transpirational cooling will further increase leaf and canopy temperatures (e.g., Kimball and Bernacchi 2006). Limited plasticity of T_{50} as shown by relatively high heat tolerance of shade leaves and a generally moderate capacity for heat tolerance increases suggest that irreversible heat damage could become a significant stress phenomenon in the not-too-distant future, particularly in sun leaves of tropical forest trees.

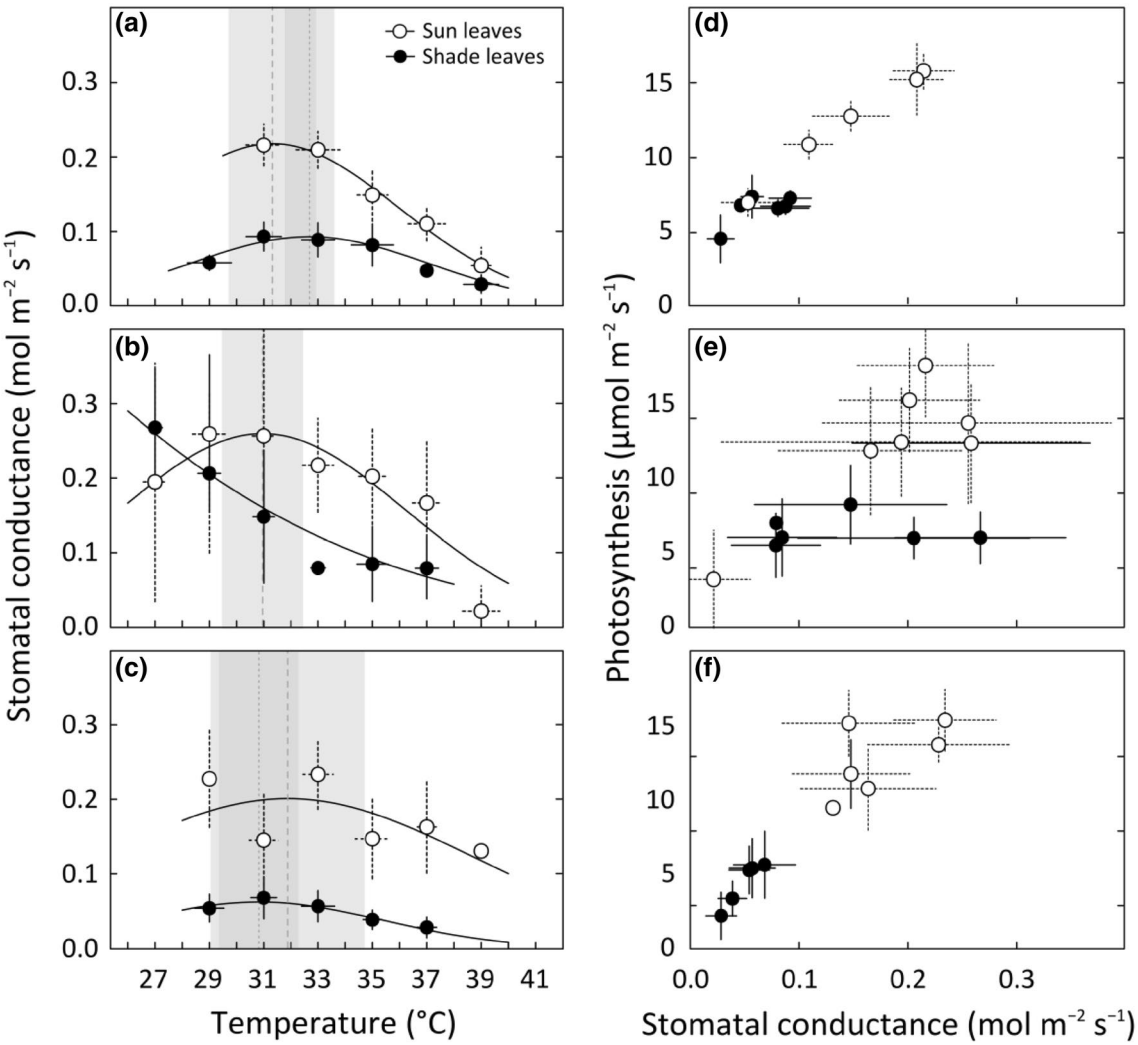

Fig. 5 Stomatal conductance under saturating light as a function of leaf temperature (**a**–**c**) and light-saturated photosynthesis as a function of stomatal conductance (**d**–**f**) in shade (closed symbols) and sun (open symbols) leaves of *C. inophyllum* (**a, d**), *I. spectabilis* (**b, e**), and *O. macrocalyx* (**c, f**). Shown are mean values ± standard deviations by 2 °C-bins. Stomatal temperature response curves are fitted with Eq. 4. Vertical-dotted and -dashed lines and shaded areas in **a**–**c** indicate T_{Opt} of stomatal conductance for shade and sun leaves, respectively, and the 95% confidence intervals of T_{Opt}

Acknowledgements This research was supported by the Smithsonian Tropical Research Institute (STRI). MS was recipient of an Earl S. Tupper–STRI postdoctoral fellowship. GGH received a Hollister-Smith Endowment-funded STRI internship. Aurelio Virgo assisted with the preparation of figures. We gratefully acknowledge helpful comments and suggestions of two anonymous reviewers.

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