

High-temperature tolerance of a tropical tree, *Ficus insipida*: methodological reassessment and climate change considerations

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Abstract. In view of anthropogenic global warming, heat tolerance of a neotropical pioneer tree, *Ficus insipida* Willd., was determined. Sections of sun leaves from a mature tree and from seedlings cultivated at ambient and elevated temperatures were heated to 42–53°C. Leaves from a late-successional tree species, *Virola sebifera* Aubl., were also studied. Widely used chlorophyll *a* fluorescence methods based on heat-induced rise of initial fluorescence emission, F_o , and decrease in the ratio of variable to maximum fluorescence, F_v/F_m , were reassessed. F_v/F_m determined 24 h after heat treatment was the fluorescence parameter most suitable to assess the lethal temperature causing permanent tissue damage. Thermo-tolerance was underestimated when F_o and F_v/F_m were recorded immediately after the heat treatment. The limit of thermo-tolerance was between 50 and 53°C, only a few °C above peak leaf temperatures measured *in situ*. The absence of seasonal changes in thermo-tolerance and only marginal increases in thermo-tolerance of plants grown under elevated temperatures suggest little capacity for further heat acclimation. Heat-stress experiments with intact potted seedlings also revealed irreversible leaf damage at 51–53°C, but plants survived and developed new leaves during post-culture.

Additional keywords: biomass, growth, photosynthetic pigments, tropical forest, xanthophyll cycle.

Introduction

Investigations into the heat tolerance of plants have gained renewed high interest in recent years. Anthropogenic global surface warming by 1.8–3.6°C in the 21st century has been projected based on multiple models. Increased frequency and amplitude of extreme events such as heat waves and extended drought periods are also predicted (Meehl *et al.* 2007). For tropical America, a rise of annual mean temperature by 2.5–4.7°C from 2000 to 2100 was projected based on climate change scenarios derived from four different climate models (Cramer *et al.* 2004). Further model calculations suggest that the surface temperature in the region of wet tropical forests will rise even more due to deforestation caused by the combined effects of increased atmospheric CO₂ levels and reduced surface evaporation in deforested areas (Zhang *et al.* 2001). Extended dry seasons during El Niño years may aggravate drought and high-temperature stress on plants in certain regions of the tropics (National Oceanic and Atmospheric Administration 2010). There is concern that global warming will adversely affect plants from temperate (e.g. De Boeck *et al.* 2008) to tropical climate zones (Cramer *et al.* 2004). Growth, development and productivity of plants (Kipp 2008) including important crop species such as cereals (Barnabás *et al.* 2008) and rice may be affected (Peng *et al.* 2004; Fitzgerald and Resurreccion 2009; Wassmann *et al.* 2009).

Heat stress is frequently associated with other adverse abiotic constraints such as excessive light and drought, which are known to exacerbate the effects of heat stress (e.g. Havaux 1992; Ladjal *et al.* 2000; Rizhsky *et al.* 2004; Mittler 2006; Zavalloni *et al.* 2009). Under high light, midday depression of net CO₂ assimilation associated with stomatal closure and strong photoinhibition of PSII are commonly observed in tropical tree canopies (Zotz *et al.* 1995; Krause *et al.* 2006). Owing to limited transpirational cooling, leaf temperatures may rise to values substantially above air temperature (Hamerlynck and Knapp 1994; Krause *et al.* 2006). It has been suggested that biomass production of tropical forests might approach a high-temperature threshold as global warming causes stronger and more frequent events of depressed CO₂ uptake in full sunlight (Doughty and Goulden 2008). Elevated atmospheric CO₂ associated with increased temperatures may have contributed to mass extinction of higher plant species at the Triassic-Jurassic boundary, as hypothesised by McElwain *et al.* (1999).

The heat tolerance limit of leaves, i.e. the temperature that causes permanent leaf damage, has in most cases been determined by means of chlorophyll *a* (chl *a*) fluorescence. At room temperature, fluorescence is emitted predominantly by chl *a* antennae of PSII (Krause and Weis 1991). Photosynthetic electron transport driven by PSII is one of the most heat-sensitive processes in green leaves (Krause and Santarius

1975; Smillie 1979; Berry and Björkman 1980; Havaux *et al.* 1991). In comparison, PSI, the chloroplast envelope and enzymes of the chloroplast stroma are less heat labile. A rapid routine assay to determine PSII damage was established by Schreiber and Berry (1977). The authors recorded chl *a* fluorescence emission in very low exciting light ('initial fluorescence', F_o) during continuous heating of leaves by $\sim 1^\circ\text{C}$ per minute. The onset of a dramatic F_o increase was taken as the critical temperature, T_c , of incipient PSII damage. This method was applied in many later studies. A drawback of the method is the summation of heat exposure time, as the heating temperature increases. In several species, a correlation was found between T_c and 50% necrotic leaf damage observed during long-term storage of leaves after 30 min heat treatments (Bilger *et al.* 1984). A close correlation between T_c and a strong decline of net CO_2 assimilation was reported by Downton *et al.* (1984) and Seemann *et al.* (1984, 1986).

However, in several investigations applying the method of continuous heating, published T_c values appear to be relatively low (e.g. Smillie and Nott 1979; Terzaghi *et al.* 1989; Braun *et al.* 2002; Weng and Lai 2005). In some studies the decline of potential PSII activity, F_v/F_m (the ratio of maximum variable to maximum total fluorescence), was used to determine the temperature limit of heat tolerance (Méthy *et al.* 1997; Bigras 2000; Ladjal *et al.* 2000; Cunningham and Read 2006). Only a few investigations considered reversibility of heat-induced fluorescence changes (Schreiber and Berry 1977; Méthy *et al.* 1997; Bigras 2000).

Most data on limits of heat tolerance of leaves were obtained from species of temperate climate zones. Data on desert plants (Downton *et al.* 1984; Seemann *et al.* 1984, 1986) and tropical species (Smillie and Nott 1979; Terzaghi *et al.* 1989; Cunningham and Read 2003, 2006; Weng and Lai 2005) are relatively rare. In many cases, studied plants were greenhouse-grown (e.g. Havaux 1992; Bigras 2000; Cunningham and Read 2003, 2006). More information is needed to determine whether leaves of tropical forest trees, particularly sun-exposed outer canopy leaves, may suffer damage and whether carbon storage or even survival of trees will be affected when exposed to increased maximum temperatures in a warmer climate.

In the present study, heat tolerance of leaves of a neotropical rainforest tree, *Ficus insipida* Willd., a fast-growing pioneer species, has been investigated in detail. We reassessed the chl *a* fluorescence method evaluating both F_o rise and F_v/F_m decline after heating of leaf sections at a series of temperatures from 42 to 53°C. For the heat treatment, mature sun leaves were collected from the outer canopy of a tall tree accessible from of a construction crane in a seasonably dry forest near Panama City. Tests were done in the late rainy season and early and late dry season. To single out heat effects, treatments were performed in the dark or dim light, avoiding excess light and drought stress. Fluorescence was recorded shortly after heat treatment and again after ~ 24 h (or a longer period) in order to assess reversibility of fluorescence changes. Fluorescence data were compared with visible damage of leaf tissue observed during storage of leaf disks for 11 days. The critical temperature of tissue damage was compared with maximum leaf temperatures recorded *in situ*. To assess the capacity of heat acclimation, seedlings of *F. insipida* and the late-successional tree species *Virola sebifera* Aubl. were grown under full solar irradiance at both ambient and

elevated air temperatures. Heat tolerance tests of mature sun leaves were performed after several months of growth under those contrasting conditions. We also subjected entire seedlings of *F. insipida* to heat treatment in order to examine temperature effects on growth and long-term survival at the whole-organism level.

According to our assays, the decline in F_v/F_m recorded 24 h after heat treatment is the fluorescence parameter most closely related to permanent leaf damage. The data indicate that under current ambient conditions, sun leaves of *F. insipida* frequently operate close to their limit of heat tolerance and have only marginal capacity for further heat acclimation.

Materials and methods

Plant material

Mature outer canopy sun leaves of *Ficus insipida* Willd. (Moraceae) were accessible from a construction crane in the seasonably dry secondary forest of Parque Natural Metropolitano, Panama City. Leaves were harvested in the morning and used for heat tolerance tests on the same day. Sun leaves of seedlings of *F. insipida* and *Virola sebifera* Aubl. (Myristicaceae) were obtained from potted plants cultivated at the Santa Cruz Experimental Field Station in Gamboa near Panama City.

Ficus insipida plants were grown from seed. On 20 November 2008, small seedlings were planted into 18 L pots (height 37 cm), one plant per pot in soil (topsoil from a local orchard without fertiliser added) mixed with 20% rice husks. They were placed at a fully sun-exposed site.

Virola sebifera plants were collected as germinating seeds from the forest floor. For 17 months, seedlings were grown at a partly shaded site. On 12 November 2008 they were transplanted into 18 L pots as described above and stepwise acclimated to higher light. On 15 December 2008 the pots were transferred to full solar irradiance.

In the early dry season (14 January 2009) the seedlings of *F. insipida* and *V. sebifera* were divided in two groups. One remained at the fully sun-exposed site; the other was placed under an adjacent metal frame (length 4 m, width 1 m, height 1.8 m) covered with Aclar foil (0.038 mm Aclar 22A, Honeywell, Pottsville, PA, USA) to raise the growth temperature during the day. Aclar transmits $\sim 95\%$ PAR and 90% UV-B and UV-A radiation (Krause *et al.* 2007). Openings in the Aclar cover near the top and bottom of the metal frame allowed for limited ventilation. Air temperatures under the Aclar-covered frames and outside were recorded using copper-constantan thermocouples connected to a data logger CR10X (Campbell Scientific Logan, UT, USA). Heat tolerance of leaves was assayed after 52 days (*V. sebifera*) and 70 days (*F. insipida*). Mature leaves developed during these periods were used for the tests.

Photosynthetic pigment contents were determined in leaf disks (area 0.9 cm^2) by HPLC (Färber *et al.* 1997; Krause *et al.* 2003).

Heat tolerance tests of leaves

Leaf disks (diameter 2.4 cm) were wrapped in stripes of Mira cloth to avoid possible anaerobiosis effects during heating (one layer on the adaxial and three layers on the abaxial leaf

surface). It has been reported that anaerobiosis enhances the heat-induced F_o rise (Schreiber *et al.* 1976; Schreiber and Berry 1977). In the absence of Mira cloth, we observed irregular F_o increases at relatively low heating temperatures. Five wrapped leaf disks from different leaves were placed into closed plastic bags (one disk per bag) and immersed for 15 min in a preheated water bath (Lauda RM6/RMS, Analytical Instruments, LLC, Golden Valley, MN, USA). Leaf disks reached water temperature within 2 min, as shown by measurements with a fine-wire thermocouple. Calibration of the water bath temperature was performed with a calibrated fractional-degree thermometer, ranging from -1 to 101°C , scale 0.1°C , system uncertainty 0.043°C (ThermoFisher Scientific, Dubuque, IA, USA). Subsequent to heat exposure, the disks were dark-adapted for ~ 15 min and chl *a* fluorescence was monitored with a PAM 2000 fluorometer (Walz, Effeltrich, Germany). Initial fluorescence emission, F_o , and the ratio of maximum variable to maximum total fluorescence, F_v/F_m , served as indicators of heat effects on PSII. F_o was recorded in low actinic light when stable emission under weak infrared irradiation (IR) was reached. A saturating pulse of white light was applied to obtain maximum emission, F_m , and the ratio F_v/F_m . Untreated leaf disks served as controls. All leaf disks were stored in Petri dishes on moist tissue paper under dim daylight. Fluorescence recording was repeated after 10 min dark adaptation, ~ 24 h after heat exposure. Visual tissue damage (dark coloration and necrosis) was monitored for 11 days. The percentage of damaged leaf area of each leaf disk was estimated and averaged. Necrosis (tissue death) reflects the collapse of cellular organisation and functioning, but the exact mechanism of necrotic damage induced by critical heat exposure of leaves is not well known.

Recording of leaf temperature

The temperature of canopy sun leaves of *F. insipida* was measured with an infrared thermometer (MiniTemp MT, Raytek, Santa Cruz, CA, USA). Random measurements were done from a distance of 30–40 cm on the adaxial leaf surface. Accuracy of the infrared thermometer was verified with a fine-wire thermocouple.

Heat tolerance test with intact *F. insipida* seedlings

Soon after germination, seedlings of *F. insipida* were planted in pots (height 9 cm, width 9–12.5 cm; one plant per pot) on 31 January 2009. For 30 days, plants were cultivated at a site that was strongly sun-exposed in the early afternoon for ~ 2 h (air temperature 31 – 33°C). Groups of three plants were heated in an incubator (Isotemp Incubator, Fisher Scientific, Pittsburgh, PA, USA) to pre-set temperatures. Plants were well watered and the pots sealed with aluminium insulation foil to minimise heating of roots and stem bases. Leaf temperatures were measured with a thermocouple attached to the abaxial leaf surface. After stable leaf temperature was reached, heat treatment lasted for 15 min. The plants were then returned to previous growth conditions. Chl fluorescence was recorded on two leaves per plant as described above. Total dry mass and leaf area of heat-treated and control plants were determined 26 days after heat exposure.

Results

Heat tolerance test of leaf sections

Figure 1 shows the response of chl *a* fluorescence to 15 min heating of *F. insipida* leaf sections from canopy sun leaves of a mature tree. The critical temperature of F_o increase, $T_c(F_o)$, was significantly increased, when measured after 24 h compared with 15 min after heating (Fig. 1a). Eleven days subsequent to heating, the F_o rise (of visually undamaged tissue) was much less pronounced. In leaf sections heated to 53.3°C (the highest treatment temperature), no undamaged green tissue remained after 11 days of storage, and F_o had declined close to zero. Similar trends as for F_o applied to F_v/F_m ratios (Fig. 1b). F_v/F_m ratios recovered partly during 24 h after heating and more so after

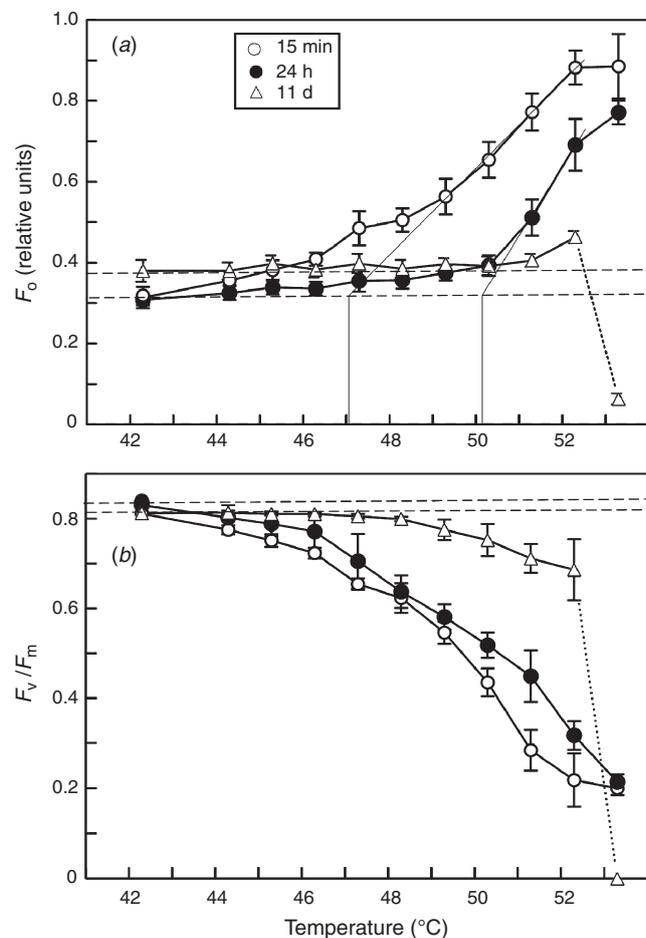


Fig. 1. Effects of 15 min heat exposure of leaf sections of *Ficus insipida* on chl *a* fluorescence emission. The experiment was performed with outer canopy sun leaves of a mature tree in the early dry season (22 January 2009). (a) Initial fluorescence, F_o : extrapolations to obtain critical temperatures, $T_c(F_o)$, are depicted; (b) ratio F_v/F_m . Open circles, recording 15 min after heat treatment; closed circles, recording 24 h after heat treatment; triangles, recording 11 days after heat treatment (measurement on non-necrotic tissue, except for samples heated to 53.3°C). Means \pm s.d. ($n=5$, sections from different leaves) are shown. Control values of untreated leaf sections are denoted as broken horizontal lines. F_o of controls: 15 min, 0.319 ± 0.015 ; 24 h, 0.322 ± 0.021 ; 11 days, 0.373 ± 0.028 . F_v/F_m of controls: 15 min, 0.840 ± 0.004 ; 24 h, 0.835 ± 0.003 ; 11 days, 0.812 ± 0.005 .

11 days; in fully necrotic tissue (heating to 53.3°C), no variable fluorescence was detected (i.e. $F_v/F_m = 0$). In Fig. 2, the progress of visible damage of leaf segments during 11 days of storage of leaf samples subsequent to heating is demonstrated. Heating to 53.3°C caused 100% tissue damage within 8 days, whereas heating to 52.3°C caused ~50% damage within 11 days.

Critical temperatures of F_o increase, $T_c(F_o)$, and temperatures leading to 50% decrease in F_v/F_m ratios, $T_{50}(F_v/F_m)$, are given in Table 1. Visually estimated critical 'lethal temperatures', T_1 causing 50% visible tissue damage 11 days after heating, are also shown. Data from five experiments with *F. insipida* sun leaves are presented, three of them with outer canopy leaves from a mature tree in the rainy season, early and late dry season, respectively; two experiments were done with potted seedlings grown at a fully sun-exposed site either at ambient temperature or at elevated day temperature (see 'Materials and methods').

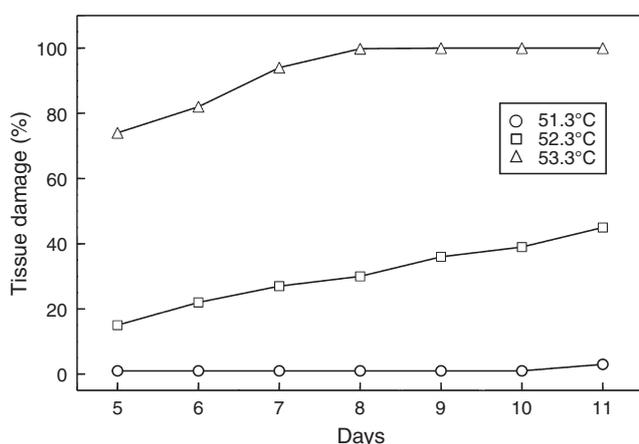


Fig. 2. Estimated visible damage (mean percentage of dark-coloured zones of five leaf sections from different leaves) as a function of storage time (days) after heating; leaf sections of the experiment in Fig. 1; temperatures during 15 min heating: 51.3°C (circles), 52.3°C (squares), and 53.3°C (triangles). No tissue damage was visible in stored non-heated control sections. Whole leaf blades (with petioles and major veins removed) showed necrotic effects only after 32 days storage under a moist atmosphere in dim light.

The mean maximum day temperature at the site of the *F. insipida* tree was only slightly higher in the late dry than in the rainy season, but there was a more than 50% increase in daily mean solar irradiance (Table 2). This implies substantially lengthened periods of full sun exposure at canopy level and, thus, of heat stress during the dry season. However, the data in Table 1 do not indicate significant differences in heat tolerance of canopy leaves between rainy and dry season.

To obtain information on maximum leaf temperature of *F. insipida* canopy leaves *in situ*, random measurements were done on the upper (adaxial) leaf surface under exposure to full solar radiation at a wind-shielded canopy side (Fig. 3). Under these conditions, most leaves showed temperatures of 40–42°C, i.e. well below critical temperatures of tissue damage or fluorescence changes. However, in some leaves, peak temperatures were above 46°C, close to the temperature causing damage (cf. Fig. 1 and Table 1).

Data obtained with mature leaves of seedlings were not significantly different from those with canopy leaves. For seedlings of *F. insipida* and *V. sebifera*, air temperature in the Aclar-covered chambers was substantially higher during the day than for seedlings cultivated outside (Fig. 4). Daily maximum temperature under the Aclar cover was frequently above 40°C (Fig. 4a), whereas the maximum ambient temperature rarely reached 33°C. During many days, mean air temperature under the Aclar was at least 5°C higher than outside (Fig. 4b). Even so, growth of *F. insipida* seedlings under elevated day temperature resulted only in a slight tendency of increased heat tolerance (Table 1). Likewise, seedlings of the late-successional tree, *V. sebifera* did not show a strong acclimatory response when grown at elevated day temperature. In Fig. 5, changes in F_v/F_m of leaf sections recorded 24 h after heating are depicted. There was only a slight tendency to higher heat tolerance of leaves grown at elevated compared with ambient temperature. $T_{50}(F_v/F_m)$ was only slightly (less than 1°C) different from those of *F. insipida* grown under same conditions (cf. legend to Fig. 5 and Table 1).

Elevated temperatures did not cause significant changes in the composition of photosynthetic pigments (Table 3). Contents of

Table 1. Heat tolerance of *Ficus insipida*

Critical temperatures (°C) from fluorescence recording following 15 min heat treatment of leaf sections. $T_c(F_o)$, critical temperature obtained from the slope of F_o increase; $T_{50}(F_v/F_m)$, temperature causing 50% reduction in the F_v/F_m ratio. In addition, visually assessed 'lethal temperatures', T_1 , causing 50% tissue damage are shown. The experiments with sun leaves from seedlings were done with potted plants at an open, sun-exposed site ('ambient temperature') or grown at an adjacent site under a frame covered with Aclar film ('elevated day temperature'). ND, not determined

Material	Date	15 min after heating		24 h after heating		11 days after heating
		$T_c(F_o)$	$T_{50}(F_v/F_m)$	$T_c(F_o)$	$T_{50}(F_v/F_m)$	T_1
Mature tree	9 December 2008 (Rainy season)	48.6	50.8	49.6	52.4	ND
	22 January 2009 (Early dry season)	47.1	50.4	50.1	51.4	52.3
	20 March 2009 (Late dry season)	48.0	51.0	49.9	52.5	52.8
Seedlings	24 March 2009 (Ambient T)	46.5	48.0	48.8	50.1	51.5
	26 March 2009 (Elevated day T)	46.1	48.3	49.1	50.6	52.4

Table 2. Mean daily maximum air temperature (max T) and mean daily total solar irradiance recorded for 7 days before heat tolerance tests of mature canopy sun leaves of *Ficus insipida*
Data were recorded close to the site of leaf collection

Dates of recording	Max T ($^{\circ}\text{C}$)	Total irradiance (MJ m^{-2})
02–08 December 2008	30.4	12.1
15–21 January 2009	31.0	16.6
13–19 March 2009	32.2	20.1

chl $a + b$ and carotenoids (based on chl) were very similar under the two conditions. There were no differences in the de-epoxidation state of xanthophyll-cycle pigments under high PAR. At both growth temperatures, *F. insipida* exhibited a higher chl $a + b$ content, higher β - and lower α -carotene levels and a lower de-epoxidation state than *V. sebifera*.

In all experiments with *F. insipida* leaves, the apparent critical temperatures of fluorescence changes, $T_c(F_o)$ and $T_{50}(F_v/F_m)$, were higher at 24 h than 15 min after heating. $T_{50}(F_v/F_m)$ values were always higher than $T_c(F_o)$. Moreover, $T_{50}(F_v/F_m)$ determined 24 h after treatment was the fluorescence parameter closest to the lethal temperature, T_l , causing 50% visual tissue

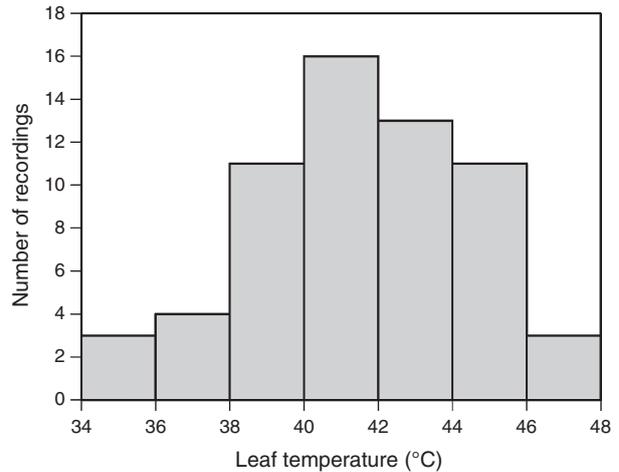


Fig. 3. Temperature of canopy sun leaves of *Ficus insipida* under high solar irradiance at high air temperature. Random measurements were done under low wind conditions in the late dry season (30 March 2009, ~1400 hours) on the upper (adaxial) side of fully sun-exposed mature leaves on a tall tree. PAR was around $2000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$; local maximum air temperature was 33.7°C .

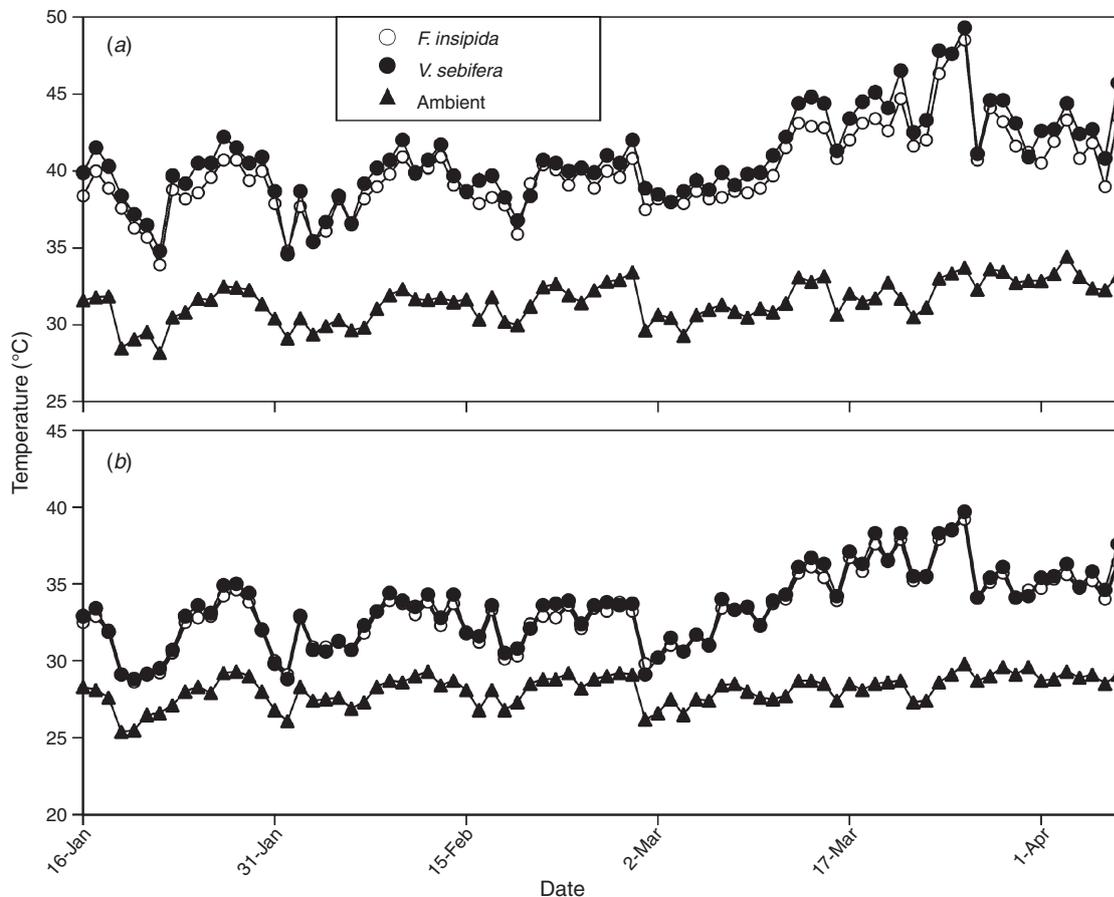


Fig. 4. Growth temperature of *Ficus insipida* and *Virola sebifera* seedlings. (a) Maximum day temperature; (b) mean daytime temperature (0600–1800 hours). Air temperature was recorded under Aclar frames (open circles, *F. insipida*; closed circles, *V. sebifera*) and at the open site (closed triangles, ambient temperatures). Mean ambient night temperature (1800–0600 hours) did not differ significantly from those under the Aclar frames and ranged between ~ 22 and 25°C .

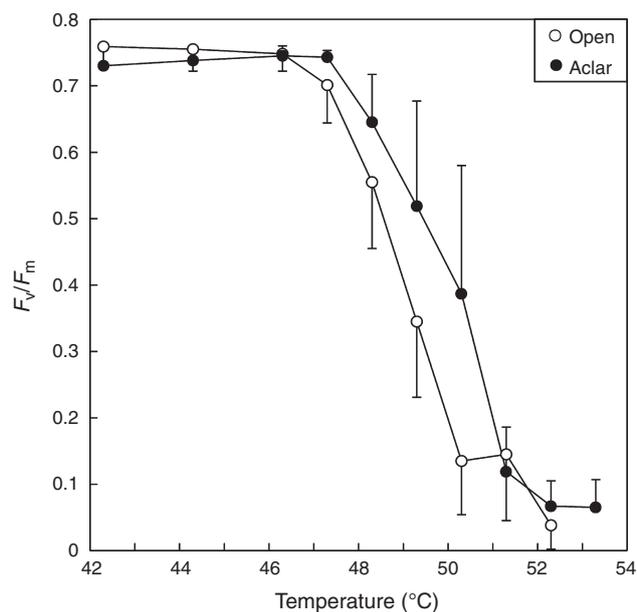


Fig. 5. Effects of 15 min heat exposure of sections of *Virola sebifera* sun leaves on F_v/F_m ratios recorded ~24 h after heating. Open circles, growth at ambient temperature at open site; closed circles, growth at elevated day temperature under Aclar cover (see 'Materials and methods'). Means \pm s.d. are presented ($n=5$, sections from different leaves). F_v/F_m ratios of controls (leaf sections kept at room temperature) were 0.764 ± 0.024 and 0.728 ± 0.031 , respectively. $T_{50}(F_v/F_m)$ values, denoting 50% reduction in F_v/F_m , were 48.4°C (open site), 48.9°C (Aclar cover) 15 min after heating, and 49.1°C (open site) and 50.3°C (Aclar cover) 24 h after heating.

damage in necrosis tests (Table 1). The means (\pm s.d.) of four experiments (Table 1) were $T_1 = 52.2 \pm 0.5^\circ\text{C}$ and $T_{50}(F_v/F_m) = 51.2 \pm 1.0^\circ\text{C}$.

Heating of whole *F. insipida* sun leaves resulted in similar fluorescence responses as in leaf sections. Measurements 15 min after heating gave $T_c(F_o) = 48.5^\circ\text{C}$ and $T_{50}(F_v/F_m) = 49.6^\circ\text{C}$ (cf. Table 1). However, storage of detached whole leaves under dim daylight in a humid chamber resulted in irreversible tissue damage, which started in petioles and spread via major veins to the leaf blades within a few days. In contrast, whole leaf blades (petioles and middle veins removed) did not show visible damage during 32 days storage. Heating of leaf sections for different periods resulted in lower response of fluorescence after 10 min than after 15 min heating. Responses of F_o and F_v/F_m after 30 min heating were stronger, but critical

temperatures were similar as after 15 min heating (data not shown).

Ficus insipida seedlings grown at elevated day temperature did not show reductions of biomass production and leaf growth (Table 4). Rather, the data show an increased growth. Dry mass of stems, roots and total dry mass were significantly greater at elevated than at ambient temperature. For leaves, the dry mass difference was not significant; leaf area per seedling was considerably larger at elevated temperature, but leaf dry mass per area (LMA) was smaller, indicating thinner leaf blades. Stems grew ~50% higher at elevated temperature, but there was no difference in the shoot to root dry mass ratio.

Heat tolerance test with whole seedlings

To assess the response of intact plants to heat stress, potted seedlings of *F. insipida* were heated in an incubator for 15 min. The pots were well watered and insulated to avoid heating of roots and stem bases. Due to transpiratory cooling, measured leaf temperatures were ~6–15°C lower than air temperatures (Fig. 6). The potential efficiency of PSII (F_v/F_m ratio) was strongly reduced at the two highest leaf temperatures: 51.5 and 52.8°C (Fig. 7). From fluorescence data obtained 15 min and 24 h after heating, $T_{50}(F_v/F_m)$ values of 51.8 and 52.0°C , respectively, were deduced. After 24 h, partial recovery of PSII efficiency was observed in plants exposed to lower temperatures (Fig. 7b). In agreement with the $T_{50}(F_v/F_m)$ value, strong leaf damage was visible after only one day and more so after 1 week following heating to 51.5 and 52.8°C (Fig. 8a). All leaves were strongly injured at 52.8°C . Following heat exposure at 51.5°C , most severe damage occurred in the youngest leaves at the top of the shoot, whereas some of the older leaves survived. In part, the damaged leaves were shed. Leaf temperatures of 43.7 , 45.9 and 49.4°C caused slight damage only on the youngest, not yet unfolded leaves. Damage was barely detectable at 43.7°C . No visible damage occurred at 42.3°C (air temperature 49.5°C).

During further culture all plants survived. Even the seedlings most severely damaged formed new leaves, as documented in the photograph taken 22 days after heat exposure (Fig. 8b). However, total biomass and leaf area determined 26 days after heat exposure were substantially reduced in plants heated to the two highest temperatures (Fig. 9). After heating to 52.8°C , biomass and leaf area remained below the initial values of control seedlings. Slight leaf damage did not significantly affect growth of seedlings.

Table 3. Contents of photosynthetic pigments in leaves of *Ficus insipida* and *Virola sebifera* seedlings grown at ambient and elevated temperatures. Samples were taken between 0930 and 1030 hours; PAR $1800\text{--}2000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. Means \pm s.d. ($n=5$) of chl $a+b$ ($\mu\text{mol m}^{-2}$) and sum of xanthophyll-cycle pigments, viola-, anthera- and zeaxanthin (VAZ), lutein (L), and α - and β -carotene (α -Car, β -Car) based on chl (mmol mol^{-1} chl $a+b$) are presented. In addition, the de-epoxidation state of xanthophyll-cycle pigments (DEPS), defined as $(0.5A+Z)/VAZ$, is given

Species	Growth temperature	Chl $a+b$	VAZ	DEPS	L	α -Car	β -Car
<i>F. insipida</i>	Ambient	226 ± 23	100 ± 3	0.63 ± 0.04	81 ± 3	2.7 ± 0.5	106 ± 4
	Elevated	208 ± 35	91 ± 8	0.66 ± 0.10	82 ± 9	2.4 ± 0.2	94 ± 5
<i>V. sebifera</i>	Ambient	142 ± 15	102 ± 22	0.89 ± 0.04	141 ± 8	16.5 ± 1.8	76 ± 7
	Elevated	154 ± 44	104 ± 25	0.91 ± 0.04	126 ± 23	19.7 ± 4.7	61 ± 6

Table 4. Dry mass and growth parameters of *Ficus insipida* seedlings after ~3 months growth (14 January–12 April 2009) at open, sun-exposed sites at ambient or elevated day temperatures

Means \pm s.d. are presented ($n=10$ plants). Significance of dry mass accumulation and growth under the two temperature regimes is denoted by the probability of error, P -value (n.s., not significantly different). At the start of the experiment, control plants ($n=3$) had three leaves and a total leaf area of 3.27 ± 0.46 cm²; total dry mass was 13.6 ± 0.5 mg. LMA, leaf dry mass per area

	Ambient temperature	Elevated temperature	P -value
<i>Dry mass (g)</i>			
Leaves	4.13 ± 1.39	4.90 ± 1.06	n.s.
Stems	1.55 ± 0.58	2.56 ± 0.48	<0.01
Roots	2.66 ± 0.88	3.54 ± 0.66	<0.05
Total	8.34 ± 2.79	11.00 ± 1.89	<0.05
<i>Growth parameters</i>			
Stem height (cm)	28.3 ± 4.7	43.6 ± 5.7	<0.01
Leaf area (cm ²)	625 ± 153	902 ± 184	<0.01
LMA (g m ⁻²)	64.9 ± 10.2	54.2 ± 2.1	<0.01
Shoot to root ratio ^A (g g ⁻¹)	2.15 ± 0.26	2.14 ± 0.42	n.s.

^ARatio of shoot to root dry mass.

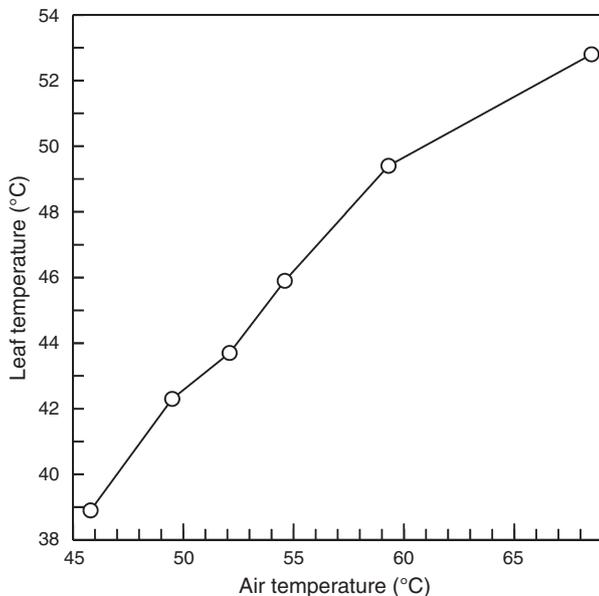


Fig. 6. Leaf temperature as a function of air temperature during 15 min heating of whole *Ficus insipida* seedlings.

Discussion

Fluorescence assay and tissue damage

According to the review by Ducruet *et al.* (2007) and the detailed study and model simulation by Kouřil *et al.* (2004), the heat-induced F_o increase in leaves is a complex process, but clearly results from alterations in PSII. A major factor involved in the mechanism of the F_o rise is an inhibition of electron transport from the primary quinone electron acceptor, Q_A , to the secondary acceptor, Q_B , of PSII, causing accumulation of Q_A^- . Back transfer of electrons from Q_B to Q_A related to damage of the

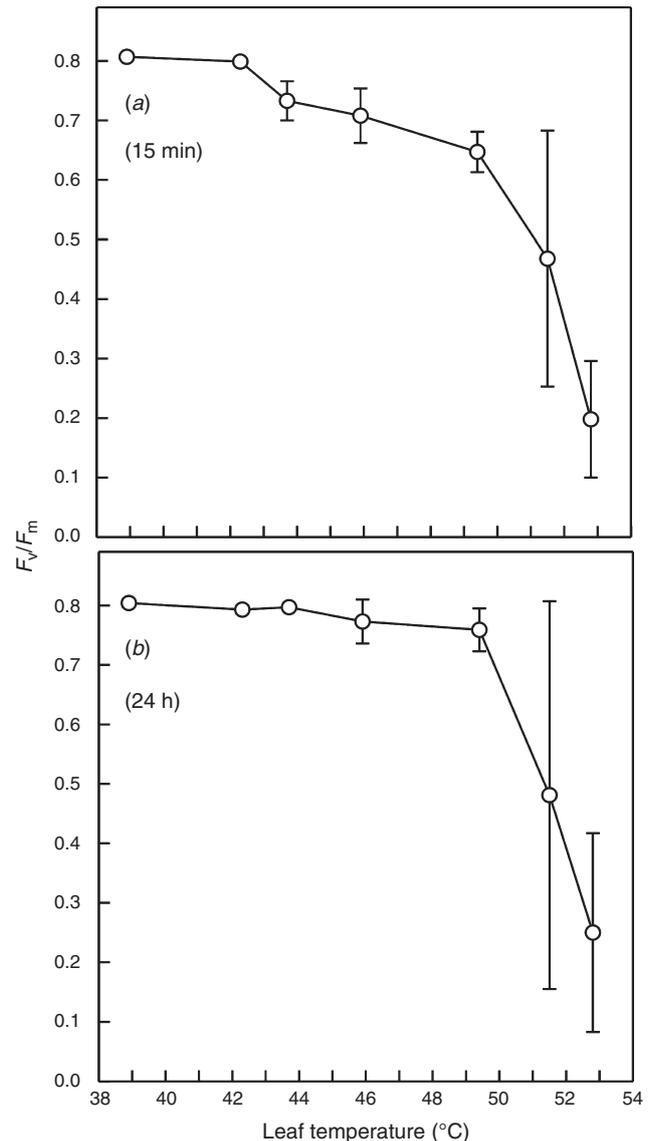


Fig. 7. Chlorophyll fluorescence ratio F_v/F_m recorded 15 min (a) and 24 h (b) after 15 min heat treatment of whole *Ficus insipida* seedlings, depicted as a function of leaf temperature during heating. Means \pm s.d. are shown ($n=6$; different leaves from three plants). Values of $T_{50}(F_v/F_m)$ obtained from the graphs are (a) 51.8°C and (b) 52.0°C.

oxygen evolving complex and an associated change in the redox potential of Q_A may also contribute to Q_A^- accumulation. In our experiments, reduction of Q_A via plastoquinone during heating in the dark (see Kouřil *et al.* 2004) should have been largely excluded by routinely applying PSI exciting far-red light (FR) before recording F_o (see 'Materials and methods'). We observed that FR substantially diminished the rise in F_o when fluorescence was assayed shortly after heat treatment, whereas FR had little or no effects on the F_o signal 24 h after heat exposure (data not shown).

The mechanism of the heat-induced decline of F_v/F_m , the potential efficiency of PSII, has been less well studied. In our tests, F_v/F_m declined as F_o rose (Fig. 1) and was associated with a



Fig. 8. Photographs of *Ficus insipida* seedlings taken (a) 8 and (b) 22 days after heat treatment. Groups of three plants are depicted. Top left, untreated controls. Recorded leaf temperatures during 15 min heating were: 49.4°C (top right); 51.5°C (bottom left), 52.8°C (bottom right).

decrease in both variable fluorescence, F_v , and maximum fluorescence, F_m (data not shown). As discussed by Kouřil *et al.* (2004), the decrease in F_m may be caused by heat-induced detachment of the light-harvesting complex from the core of PSII, reducing the PSII absorption cross-section. Thus, both types of PSII alterations manifested by the F_o increase and the F_m decline, respectively, result in the decrease in F_v/F_m , indicating a decline in potential PSII efficiency.

The mean \pm s.d. of $T_c(F_o)$ from five experiments with *F. insipida* (Table 1), determined 15 min after heating, was $47.3 \pm 1.0^\circ\text{C}$, which is close to previous studies on tropical plant species using the method of continuous heating by $\sim 1^\circ\text{C min}^{-1}$. Smillie and Nott (1979) reported $T_c(F_o)$ values (means \pm s.e.) of $46 \pm 0.63^\circ\text{C}$ for 10 tropical species, most of them grown outdoors in late summer in North Ryde, NSW, Australia. Terzaghi *et al.* (1989) reported mean $T_c(F_o)$ values of $44\text{--}46^\circ\text{C}$ for seven *Piper* and one *Erythrina* species grown in greenhouses at $28/23^\circ\text{C}$ (day/night). In contrast, Weng and Lai (2005) obtained $T_c(F_o)$ values varying widely, between 35 and 48°C , for 18 tropical and subtropical species grown outdoors in Taiwan at mean July temperature of 29.8°C .

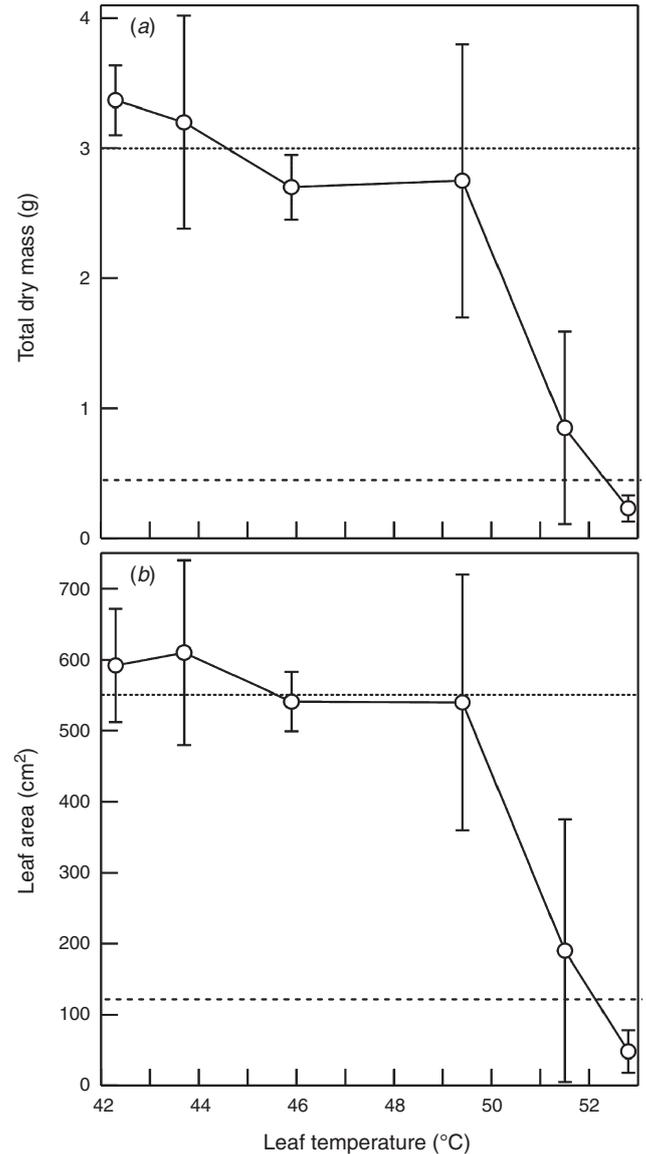


Fig. 9. Total dry mass and leaf area of *Ficus insipida* seedlings determined 26 days after 15 min heat treatment of whole plants. Means \pm s.d. are shown ($n=3$ plants). Untreated control plants had a total dry mass of 2.99 ± 0.12 g and leaf area of 551 ± 16 cm² (dotted lines). Control plants at the time of the heating experiment had a total dry mass of 0.45 ± 0.06 g and leaf area of 119.8 ± 17.3 cm² (dashed lines).

Changes in F_o and F_v/F_m recorded shortly after heat treatment were partly reversible within 24 h and even more so after 11 days (Fig. 1; Table 1). In our experiments (Table 1), $T_c(F_o)$ increased during 24 h to $49.5 \pm 0.5^\circ\text{C}$. Values of $T_{50}(F_v/F_m)$ increased by 1.0 to 2.3°C between 15 min and 24 h after heating. Recovery of F_v/F_m was previously observed in two Mediterranean species, *Quercus ilex* and *Pinus halepensis* (Méthy *et al.* 1997) and the temperate spruce species *Picea glauca* (Bigras 2000). In these and our studies (see Fig. 7), the degree of F_v/F_m recovery subsequent to heat treatment depended on the extent of initial F_v/F_m decrease. A high degree of recovery was observed, when the initial F_v/F_m

reduction was low; less or no recovery occurred when F_v/F_m was initially strongly affected. We found similar recovery patterns in *F. insipida* also for F_o (Fig. 1). These observations show that partial recovery of PSII from heat-induced changes is possible. In contrast to assumptions made in several articles (e.g. Smillie and Nott 1979; Seemann *et al.* 1984, 1986; Terzaghi *et al.* 1989; Weng and Lai 2005), apparent temperature limits of heat tolerance determined by fluorescence shortly after heat treatment may not indicate permanent leaf damage. In the study by Bigras (2000), the critical temperature of F_o rise measured 30 min after heat exposure of *P. glauca* needles was considerably lower than the temperature causing 50% needle damage. In our assay, the temperature causing 50% reduction of F_v/F_m , recorded 1 day subsequent to heat treatment, was very close to the temperature leading to 50% visible leaf damage in the long-term (Figs 1, 2; Table 1). Thus, $T_{50}(F_v/F_m)$ determined 24 h after heat exposure of leaf samples proved to be the most suitable fluorescence parameter to assess the temperature limit of thermotolerance.

Heat acclimation

Various studies have shown that plants grown under controlled conditions acquire significantly increased heat tolerance when exposed for short (minutes) or longer periods (days to months) to increased air temperature (e.g. Santarius and Müller 1979; Havaux 1993; Volkova *et al.* 2009; Wang *et al.* 2009). Changes in the temperature limit of heat tolerance by several degrees centigrade have been observed in the natural habitat of plants during the course of seasons, e.g. in desert plants (Seemann *et al.* 1986) and grapevine cultivars (Zsófi *et al.* 2009) or even during the course of the day (Braun *et al.* 2002). An increase in thermotolerance may also be induced by drought (Ladjal *et al.* 2000) or excess light (Havaux and Tardy 1996). In contrast, our investigation indicated a very low capacity of heat acclimation of the tropical tree species assayed (Fig. 5; Table 1). There was no enhancement of heat tolerance of *F. insipida* canopy leaves in the dry compared with the rainy season, despite a much higher impact of solar irradiance and slightly increased maximum temperatures during the dry season (Table 2), which presumably prolonged periods of stomatal closure (Zotz *et al.* 1995) and, thus, restricted transpiratory cooling. Peak temperatures of 46–48°C (Fig. 3) recorded *in situ* for *F. insipida* leaves in the late dry season, were close to the limit of heat tolerance, at ~50–53°C (Table 1). We do not know whether heat tolerance of *F. insipida* can increase significantly above 53°C when plants are grown (e.g. in controlled environment chambers) at much higher diel mean temperatures than used in this study, combined with brief exposures to near-lethal temperatures.

Seedlings of *F. insipida* and *V. sebifera* showed only marginally improved heat tolerance (Table 1) when grown, in the absence of water shortage, at temperatures substantially higher than ambient (Fig. 4). Likewise, young trees of the fast-growing neotropical species *Swietenia macrophylla* (Meliaceae) cultivated in glass chambers under temperature regimes similar to those shown in Fig. 4, did not exhibit significant differences in heat tolerance. Lethal temperature, T_l , was 50.8°C at near-ambient and 51.4°C at elevated temperature; $T_{50}(F_v/F_m)$ values determined 24 h after heating

were 51.5 and 52.3°C, respectively (K. Winter and G. H. Krause, unpubl. data). The only significant response of *F. insipida* to increased growth temperature was the lowered LMA indicating thinner leaves (Table 4), which may facilitate heat release by convection. The enhanced growth and biomass production under elevated temperature (Table 4) probably is a side effect of the Aclar cover that provided shielding from wind and heavy rainfall.

Heat acclimation of leaves is a highly complex phenomenon, which, among many molecular processes (Kotak *et al.* 2007; Wahid *et al.* 2007; Barua *et al.* 2008; Guy *et al.* 2008), comprises changes in photosynthetic pigment composition such as increases in lutein, α - and β -carotene (Volkova *et al.* 2009) and can alter interactions between xanthophyll-cycle pigments and thylakoid membranes (Havaux and Tardy 1996). The absence of elevated temperature-related changes in contents of photosynthetic pigments and in the de-epoxidation state (DEPS) of xanthophyll-cycle pigments under high PAR in *F. insipida*, *V. sebifera* (Table 3) and *S. macrophylla* (K. Winter and G. H. Krause, unpubl. data) is consistent with the lack of heat acclimation. Evidently, sun leaves of the tropical plants tested are operating close to the limit of their acclimation potential. Based on studies of net photosynthesis of Australian rainforest trees, Cunningham and Read (2003) concluded that tropical species possess a narrower temperature tolerance than temperate species. The authors discussed this difference as being an adaptation to larger seasonal temperature fluctuations in the temperate compared with the tropical climate.

The higher α -carotene content (cf. Krause *et al.* 2001; Matsubara *et al.* 2009) and DEPS of *V. sebifera* compared with *F. insipida* (Table 3) are in agreement with the late-successional, less light-demanding character of *V. sebifera*. In sun leaves of this species, high-light exposure caused chronic photoinhibition, as shown by lowered F_v/F_m values in untreated control leaves (cf. legends to Figs 1 and 5). *V. sebifera* belongs to a heterogeneous group of taxa possessing the lutein epoxide (Lx) cycle (Matsubara *et al.* 2008, 2009). Under both growth regimes, leaves contained ~20 mmol Lx mol⁻¹ chl *a+b* at dawn, which was almost totally de-epoxidised to lutein under high PAR (data not shown).

Heat treatment of intact plants

Leaf temperatures were reduced considerably below air temperatures (Fig. 6) during heating of whole *F. insipida* seedlings (with roots and stem bases protected from heat). This indicates strong transpirational cooling in the dark (cf. Kappen 1981). Extremely high air temperatures (above 60°C) were needed to induce strong leaf damage, which occurred in the same range of leaf temperatures (~52°C) as in the water-bath experiments using leaf sections (Figs 7, 8; cf. Table 1). It is remarkable that this temperature is similar to that causing a biomass and leaf area reduction of ~50% determined 26 days after heat treatment (Fig. 9); plants that experienced only slight visible damage (at 43.7–49.4°C) were capable of fast recovery. Moreover, the plants that suffered the most damage (leaf temperatures 51.5 and 52.8°C) survived in post-culture and produced new leaves (Fig. 8b). This observation suggests that, despite the reassessed and improved fluorescence procedure, the

heat tolerance determined for individual leaves is only an approximation of the heat tolerance of the plant.

Conclusions

F_v/F_m determined ~24 h after heat exposure is a reliable indicator of heat-induced leaf tissue damage. In contrast, fluorescence data obtained within a few minutes subsequent to heat treatment yield unrealistically low temperature values for heat tolerance. This is particularly true when heat tolerance is tested by means of F_o rise. Sun leaves of tropical trees frequently operate close to the threshold of permanent leaf damage when high solar irradiance and stomatal closure cause leaf temperature to rise markedly above air temperature around midday. Given the observed low acclimation capacity, it is conceivable that more frequent and more extreme heat and drought events predicted from global warming may negatively affect tropical rainforest canopy trees.

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