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Light-stimulated heat tolerance in leaves of two neotropical tree species, *Ficus insipida* and *Calophyllum longifolium*

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Abstract. Previous heat tolerance tests of higher plants have been mostly performed with darkened leaves. However, under natural conditions, high leaf temperatures usually occur during periods of high solar radiation. In this study, we demonstrate small but significant increases in the heat tolerance of illuminated leaves. Leaf disks of mature sun leaves from two neotropical tree species, *Ficus insipida* Willd. and *Calophyllum longifolium* Willd., were subjected to 15 min of heat treatment in the light (500 µmol photons m^{-2} s^{-1}) and in the dark. Tissue temperatures were controlled by floating the disks on the surface of a water bath. PSII activity was determined 24 h and 48 h after heating using chlorophyll *a* fluorescence. Permanent tissue damage was assessed visually during long-term storage of leaf sections under dim light. In comparison to heat treatments in the dark, the critical temperature (T_{50}) causing a 50% decline of the fluorescence ratio F_v/F_m was increased by ~1°C (from ~52.5°C to ~53.5°C) in the light. Moreover, illumination reduced the decline of F_v/F_m as temperatures approached T_{50}. Visible tissue damage was reduced following heat treatment in the light. Experiments with attached leaves of seedlings exposed to increasing temperatures in a gas exchange cuvette also showed a positive effect of light on heat tolerance.

Additional keywords: carbon dioxide assimilation, dark respiration, global warming, necrosis, transpiration.

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Introduction

Surface temperatures in the tropics are on the rise (Malhi and Wright 2004; Cramer *et al*. 2004; Diffenbaugh and Scherer 2011). More frequent and prolonged drought periods combined with elevated air temperatures are to be expected (Jentsch and Beierkuhnlein 2008; Lintner *et al*. 2012; Munasinghe *et al*. 2012). As a consequence, average and peak leaf temperatures of tropical plants will increase.

There is a long tradition of testing the heat tolerance of plants. By observing visible damage upon heating plants of a range of species, Sachs (1864) found tolerance limits of ~50−51°C. The results of many later studies were similar. The introduction of modulated chl *a* fluorescence as a method to determine the critical temperature limit (T_c) that leads to irreversible leaf damage provided a means of collecting heat tolerance data easily and quickly. In the original version of the method (Schreiber and Berry 1977), leaves were heated continuously at 1°C min^{-1}; the temperature causing the onset of a steep increase in initial fluorescence emission (F_0) was taken as T_c. A disadvantage of this method is the accumulation of heat doses at temperatures below T_c during the heating procedure, which may result in an underestimation of heat tolerance. Moreover, fluorescence changes induced by heating may be partly reversible when preheated leaves are returned to favourable conditions for extended periods. In later studies, the decline in the ratio of maximum variable to maximum total fluorescence, F_v/F_m, was used and its reversibility was considered to determine heat tolerance limits (e.g. Méthy *et al*. 1997; Bigras 2000). It has been shown for leaves of *Ficus insipida* Willd. that the temperature leading to a 50% decline in F_v/F_m (T_{50}) after 24 h of 'recovery' provides a reliable indicator of the temperature leading to irreversible visible tissue damage (necrosis) observed after long-term (11 days) storage of leaf sections (Krause *et al*. 2010). Reported limits of heat tolerance of tropical agricultural (e.g. Smillie and Nott 1979; Weng and Lai 2005) and rainforest species (Königer *et al*. 1998; Kitao *et al*. 2000; Cunningham and Read 2006; Krause *et al*. 2010, 2013) vary between ~35°C and 54°C, depending on the species tested, the growth regime and test method. Presumably, when F_0 alone is examined, very low T_c values reflect reversible heat-induced fluorescence changes rather than irreversible leaf damage.

It is known for tropical trees that under conditions of full solar irradiance, stomatal closure and strong reduction of photosynthetic CO_2 assimilation may occur (Zotz *et al*. 1995). As a consequence, leaf temperatures may rise considerably above air temperature (Hamerlynck and Knapp 1994; Krause *et al*. 2006). For example, under full solar radiation with little air movement, sun-exposed outer canopy leaves of *F. insipida*

reached temperatures *in situ* up to 46−48°C, only a few degrees below T_{50} (Krause *et al*. 2010). In contrast to temperate species (e.g. Havaux and Tardy 1996), leaves of trees in the humid tropics appear to possess only a low capacity of acclimation to increased heat stress (Cunningham and Read 2003; Krause *et al*. 2010, 2013). Nonetheless, at elevated temperatures below T_{50}, high-temperature acclimation of physiological processes such as photosynthetic CO_2 uptake and dark respiration has been reported for tropical plants (Cheesman and Winter 2013*a*, 2013*b*; Krause *et al*. 2013; Slot *et al*. 2014) The lack of an increase in T_{50} in response to rising temperature may be related to the low seasonal temperature variation in the tropics. As global warming continues, detrimental effects of enhanced peak air temperatures on tropical plants cannot be excluded. It is therefore of interest to determine how leaves respond to increasingly intense heat stress under conditions that restrict photosynthetic energy turnover.

Most previous heat tolerance tests on leaf sections, leaves or whole seedlings were performed by heat treatment in the dark. Under natural conditions, extreme heat stress usually occurs at high levels of solar irradiance that by far exceed the use of absorbed light energy by photosynthetic CO_2 assimilation. Several investigations on the interaction of excess light and heat in leaves have shown that photoinhibition of PSII was enhanced under heat stress (e.g. Gamon and Pearcy 1990; Havaux 1992; Ohira *et al*. 2005; Dongsansuk *et al*. 2013). However, in these studies, applied temperature regimes were below the heat tolerance limit. Evidence that heat-promoted photoinhibition leads to irreversible tissue damage in sun leaves is lacking.

Excess light is known to induce various means of protection, such as formation of zeaxanthin (Z) via the violaxanthin (V) cycle (Demmig *et al*. 1987; Demmig-Adams 1998). Moreover, high light has been shown to enhance heat shock protein (HSP) synthesis in heat-stressed leaves (Barua and Heckathorn 2006). At present, it is not clear how excess light interacts with heat at temperatures that potentially cause irreversible damage to PSII and leaf tissue.

We exposed sections of canopy sun leaves and intact attached leaves of seedlings to high temperatures both in the light and in the dark. Leaves of two neotropical rainforest tree species, *F. insipida* (a pioneer) and *Calophyllum longifolium* Willd. (a late-successional species) were studied. The response of PSII to heat stress was assessed by means of chl *a* fluorescence recording (Krause *et al*. 2010). Heat-induced tissue damage was estimated from the extent of necrosis occurring during long-term storage of heat-treated leaf sections. Our experiments demonstrate that illumination improves the heat tolerance of sun leaves.

Materials and methods

Plant material

Outer canopy sun leaves were collected in the morning from mature trees of *Ficus insipida* Willd. (Moraceae) growing in the Parque Natural Metropolitano, Panama City, Republic of Panama. Tree crowns were accessed using a construction crane. Mature sun leaves of *Calophyllum longifolium* Willd. (Clusiaceae) were harvested from saplings grown in soil in large pots (19 L) at the Santa Cruz Field Station in Gamboa, 30 km from Panama City. The sun-exposed saplings had reached a height of 2–3 m at the time of experiments, January–February 2013. After harvest, the leaves were used for heat tolerance tests within ~5 h.

Seedlings of *F. insipida* and *C. longifolium* were used for experiments with intact attached leaves from February to April 2013. They were cultivated outdoors in tree pots (height 36 cm, width 10 cm). Seedlings of *F. insipida* were ~2 months old and seedlings of the more slowly growing *C. longifolium* were 6–8 months old.

Chl a fluorescence

Measurements of initial chl *a* fluorescence emission (F_0), maximum total fluorescence (F_m) and the ratio of variable to maximum fluorescence (F_v/F_m) served to assess high-temperature tolerance of leaves. Recordings with a PAM 2000 fluorometer (Walz, Effeltrich, Germany) were made after 10 min of dark adaptation of leaf sections or attached leaves (for details, see Krause *et al*. (2010)).

Heat tolerance of leaf sections

Six disks (diameter: 2.0 cm) cut from six detached leaves were placed on a wire mesh sheet located ~2 mm below the water surface of a preheated water bath (Lauda RM6/RMS, Analytical Instruments LLC, Golden Valley, MN, USA). The water bath temperature had been calibrated with a fractional degree thermometer (ThermoFisher Scientific, Dubuque, IA, USA). The abaxial leaf surface was fully immersed, whereas the adaxial surface remained dry. The leaf disks were incubated at a given temperature for 15 min, either in darkness (water bath closed with a lid) or under irradiation with ~500 µmol photons $m^{-2}\,s^{-1}$ supplied by a set of red and blue diodes (120w Extreme Flower LED, Advanced LED Grow Light, Hiwasse, AR, USA). The adaxial leaf surface temperature coincided with the water temperature. Leaf temperatures were controlled with an infrared thermometer (MiniTemp, Raytek, Santa Cruz, CA, USA) calibrated against measurements with a copper-constantan thermocouple. Subsequent to heat treatment, chl *a* fluorescence was recorded (see above). Untreated disks served as controls. The disks were stored on moist filter paper in Petri dishes at 25−27°C under dim light (5–10 µmol photons $m^{-2}\,s^{-1}$). Fluorescence was remeasured 24 h and 48 h after heat treatment. During further storage for up to 14 days (*F. insipida*) and 18 days (*C. longifolium*), visible tissue damage was monitored. The extent of damage was assessed by determining the percentage of necrotic or discoloured leaf disk area.

Heat tolerance of intact attached leaves

Recently fully developed leaves of seedlings of *F. insipida* and *C. longifolium* were inserted into a Peltier temperature controlled GWK-3M gas-exchange cuvette (Walz) connected to a gas exchange system consisting of Walz components and a LI-6252 CO_2 analyser (LI-COR, Lincoln, NE, USA) (Holtum and Winter 2003). Seedlings and the leaf cuvette were placed inside a GC8 controlled-environment chamber (EGC, Chagrin Falls, OH, USA). Air containing 400 µL L^{-1} CO_2 was supplied

at a flow rate of 4.3 L min^{-1} to the cuvette. Depending on the cuvette temperature, the dew point of air entering the cuvette was up to 27°C. PAR of ~900 µmol m^{-2} s^{-1} was provided by a SS-GU300-w LED Grow Light (Sunshine Systems). Leaf temperature was measured with an OS36-RA-T-140F-GMP infrared thermocouple (Omega, Stamford, CT, USA), for which the readings were cross-checked with copper-constantan thermocouples. After CO_2 exchange had stabilised at a moderate temperature, usually 30°C, cuvette temperature was raised in steps of 5°C every 15 min. To reach leaf temperatures >50°C, cuvette air temperature had to be increased up to 70°C, owing to strong transpirational leaf cooling, both in the light and the dark, particularly in *F. insipida*. For cuvette temperatures >50°C, the regular Peltier temperature control system of the cuvette was supplemented with additional heat from a resistance wire. As cuvette temperature was raised, the temperature inside the controlled environment chamber was increased to a maximum of 40°C. Different leaves were used for temperature treatments in the light and dark. After the final treatment at the highest temperature, leaves were detached and kept on moist filter paper in Petri dishes at ~25°C under low light. Fluorescence was recorded 24 h and 48 h after heat treatment. Only the data obtained after optimal recovery (48 h) are presented.

Statistics

Differences between chl *a* fluorescence data from treatments in dark and light under otherwise identical conditions were assessed by the Student's *t*-test (two-level ANOVA); differences were considered significant at $P < 0.05$.

Results

Leaf disks

Disks taken from detached *F. insipida* sun leaves exhibited significantly improved heat tolerance when heat-treated for 15 min in the light in comparison to darkness. This is seen from F_v/F_m recordings 24 h subsequent to heat treatment (Fig. 1*a*), and even more clearly after 48 h (Fig. 1*b*), when further 'recovery' of F_v/F_m had occurred. The critical temperature causing a 50% decline in F_v/F_m (T_{50}), extrapolated from Fig. 1*b*, was shifted in the light by ~1°C (from ~52.5°C to ~53.5°C). Heating to temperatures slightly below T_{50} (51°C and 52°C) caused a considerably greater F_v/F_m decline when heating occurred in the dark. Fluorescence data obtained within ~15 min after heating under illumination were found unsuitable for assessing heat tolerance, due to interference by reversible photoinhibition of PSII contributing to the decline in F_v/F_m (data not shown).

The protective effects of light were also obvious from the F_0 rise (Fig. 2) and F_m decline (Fig. 3) occurring upon heating to 51−53°C in the light compared with darkness. As for F_v/F_m, the differences in F_0 and F_m between darkness and light became more distinct 48 h after heat treatment than after 24 h. At temperatures above T_{50}, F_m was reduced by more than 50% of the control value (Fig. 3), whereas F_0 approached F_m at the highest temperature applied (Fig. 2*b*). Accordingly, F_v/F_m approached zero, as seen by comparison with Fig. 1*b*.

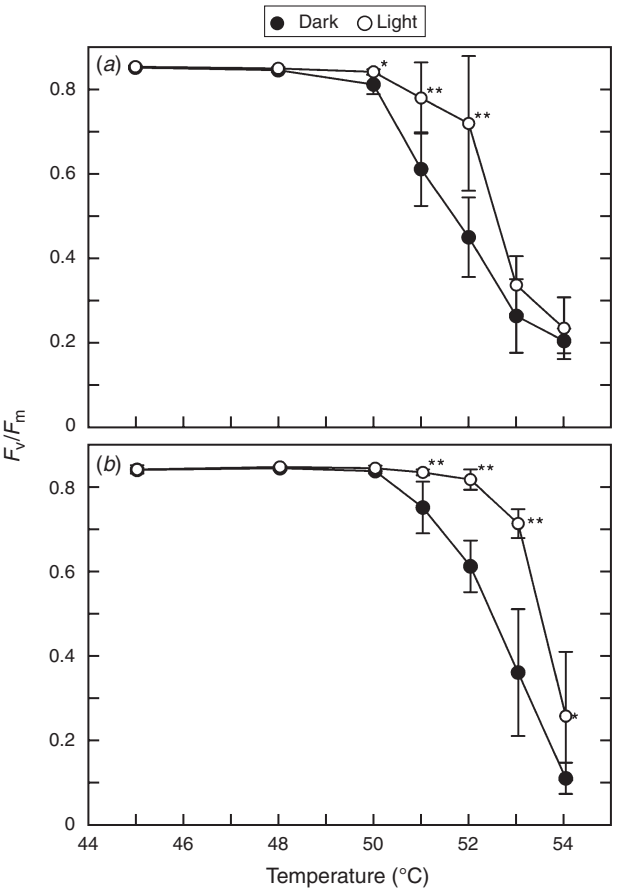

Fig. 1. Response of the ratio of variable to maximum total chl *a* fluorescence (F_v/F_m) to 15 min of heat exposure of leaf disks of *Ficus insipida*. Closed circles, heating in the dark; open circles, heating in the light (500 µmol photons m^{-2} s^{-1}). Recordings were taken (*a*) 24 h and (*b*) 48 h after heat treatment. Means ± s.d. ($n = 6$, sections from different leaves).The F_v/F_m of untreated controls did not significantly differ from values of leaf sections heated to 45°C. Significant differences in data between treatments in dark and light are indicated: **, $P < 0.01$; *, $P < 0.05$.

F_v/F_m values obtained with leaf disks of *C. longifolium* (Fig. 4) showed higher standard deviations than those from *F. insipida*, probably because individual leaves of *C. longifolium* were less homogeneous. Nonetheless, consistent with the responses of *F. insipida*, F_v/F_m declined to a substantially lower degree upon heating to 52°C under illumination than in the dark ($P < 0.01$), as seen from values obtained 24 h (Fig. 4*a*) and 48 h (Fig. 4*b*) after heating. This difference in F_v/F_m resulted from a diminished F_0 rise ($P < 0.01$) and a diminished F_m decline ($P < 0.05$) in the light compared with darkness (data not shown). There was a sharp decline in F_v/F_m between 51°C and 52°C upon heating in the dark, and between 52°C and 53°C upon heating in the light. T_{50} was reached at ~52°C in the dark and at ~53°C in the light. Under both conditions, heating to 51°C did not significantly affect F_v/F_m (Fig. 4*b*, 48 h recovery).

The progress of tissue damage in heat-treated leaf disks of *F. insipida* during 12 days of storage under dim light (Fig. 5) corresponds well with the F_v/F_m data obtained 48 h after heating (cf. Fig. 1*b*). At 45°C, 48°C and 50°C, visible damage was neither

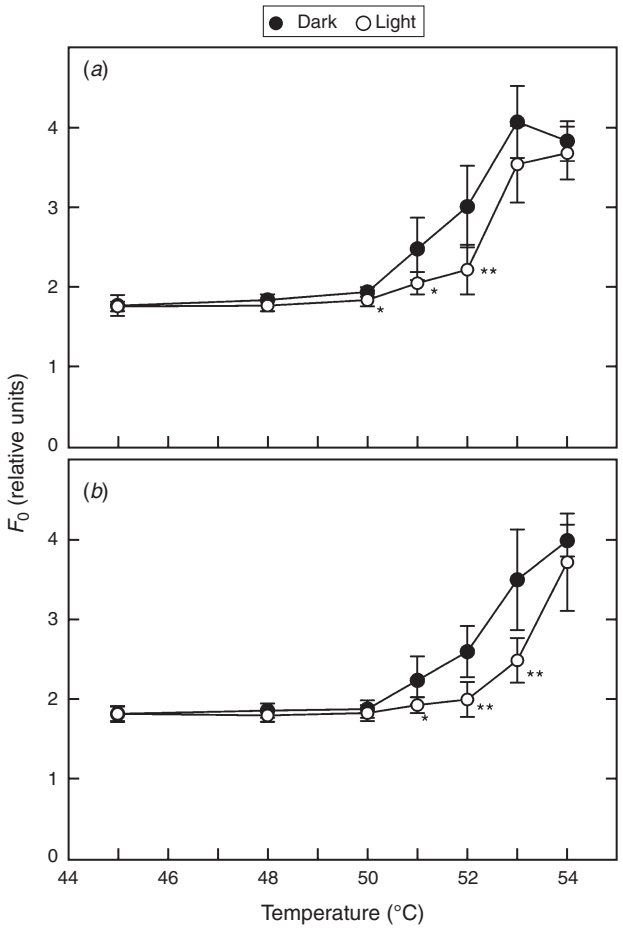

Fig. 2. Response of initial chl *a* fluorescence (F_0) to 15 min of heat exposure of leaf disks of *Ficus insipida* in the dark (closed circles) or light (open circles). Recordings were taken (*a*) 24 h and (*b*) 48 h after heat treatment. Means ± s.d. ($n = 6$, sections from different leaves). The F_0 of untreated controls did not significantly differ from the values of leaf sections heated to 45°C. Significant differences in data between treatments in dark and light are indicated: **, $P < 0.01$; *, $P < 0.05$.

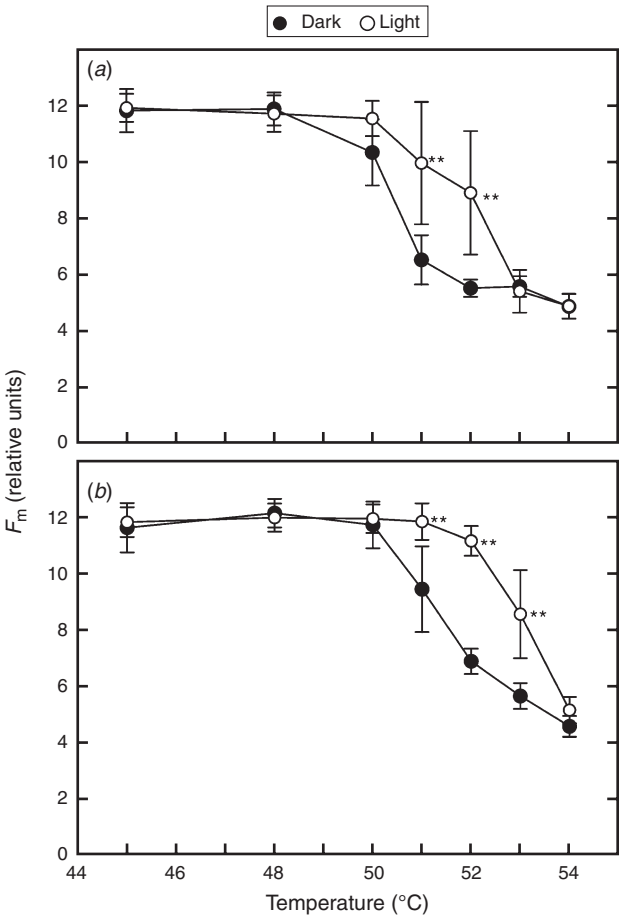

Fig. 3. Response of maximum total chl *a* fluorescence (F_m) to 15 min of heat exposure of leaf disks of *Ficus insipida* in the dark (closed circles) or light (open circles). Recordings were taken (*a*) 24 h and (*b*) 48 h after heat treatments. Means ± s.d. ($n = 6$, sections from different leaves). The F_m of untreated controls did not significantly differ from values of leaf sections heated to 45°C. Significant differences in data between treatments in dark and light are indicated: **, $P < 0.01$.

observed in the light nor in the dark. Untreated leaf disks (controls) and disks heated to 51°C under illumination did not exhibit visible tissue damage; only negligible damage was seen upon heating to 51°C in the dark (Fig. 5*a*). Heating to 52°C in the dark led to substantial damage within 12 days, whereas in the light, the leaf disks remained almost completely intact (Fig. 5*b*). Strong damage, affecting up to ~60% of leaf area, was observed upon heating to 53°C in the dark, but only up to ~20% of leaf area became damaged upon heating in the light (Fig. 5*c*). Images of heat-treated leaf disks of *F. insipida* after ~2 weeks of storage are shown in Fig. 6. Heating up to 51°C in the light and 45°C in the dark did not cause visible damage. Incubation at 52°C in the dark resulted in substantial necrosis, whereas heating to 52°C in the light caused only very minor damage. Upon heating to 53°C, the protective effect of excess light became even more conspicuous. Fig. 7 depicts the degree of tissue damage in leaf disks of *C. longifolium* during 18 days of storage. In contrast to *F. insipida*, untreated controls exhibited slowly progressive tissue damage. Disks pretreated at 30°C in the dark or light behaved like controls. At 52°C, heat treatment in the dark caused substantially faster deterioration of leaf tissue than heat treatment under illumination.

Intact attached leaves

Chl *a* fluorescence data (F_v/F_m), obtained 48 h after heat treatment of leaves of *F.insipida* and *C. longifolium*, are presented in Fig. 8. Leaves of *F. insipida* (Fig. 8*a*) showed a sharp decline in F_v/F_m upon heating in the dark at final leaf temperatures above 53°C, whereas F_v/F_m was not significantly affected at these temperatures upon heating in the light.

Leaves of *C. longifolium* (Fig. 8*b*) heated to 49−50°C showed F_v/F_m values close to those of untreated controls. At higher temperatures, all tests resulted in a more pronounced decrease in F_v/F_m in the dark than in the light. Upon heating to 52°C, this difference was most conspicuous. Heating to ~53°C in both light and dark caused a >50% decline in F_v/F_m, indicating severe leaf damage.

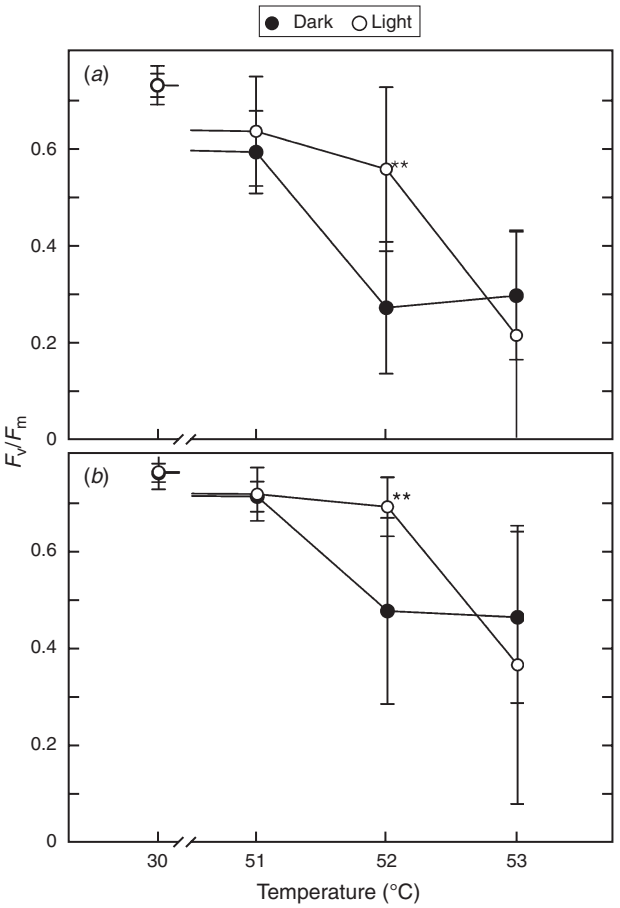

Fig. 4. Response of F_v/F_m ratios to 15 min of heat exposure of leaf disks of *Calophyllum longifolium* in the dark (closed circles) or light (open circles). Recordings were taken (*a*) 24 h and (*b*) 48 h after heat treatments. Means ± s.d. ($n = 6$ for treatment at 30°C; $n = 12$ for treatment at 51°C, 52°C and 53°C, respectively; samples from six different leaves). The F_v/F_m of untreated controls ($n = 6$) were (*a*) 0.764 ± 0.012 and (*b*) 0.782 ± 0.012. Significant differences of data between treatments in dark and light are indicated: **, $P < 0.01$.

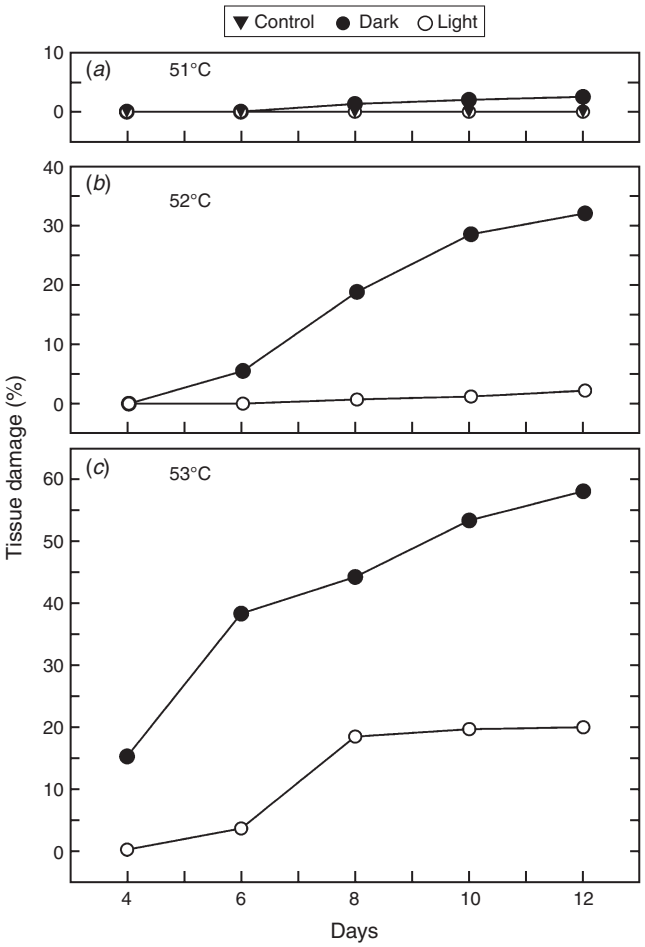

Fig. 5. Visible tissue damage of heat-treated leaf disks of *Ficus insipida* (mean percentage of damaged leaf area of six disks from different leaves) as a function of storage time (days). Heat treatment in the dark (closed circles) or light (open circles). Untreated controls (closed triangles) and treatment temperatures of (*a*) 51°C, (*b*) 52°C and (*c*) 53°C.

Net CO_2 exchange of leaves, determined during the stepwise temperature increases up to the final leaf temperature >50°C, revealed higher overall photosynthesis and dark respiration rates in *F. insipida* (Fig. 9*a*) than in *C. longifolium* (Fig. 9*b*), as well as slightly higher temperature optimum of photosynthesis for *F. insipida* and a higher upper temperature compensation point of net CO_2 exchange in the light in *F. insipida* (~50°C) compared with *C. longifolium* (~46°C). The photosynthetic competence of *F. insipida* was particularly superior to that of *C. longifolium* between 35°C and 40°C. In both species, temperatures causing irreversible heat damage were beyond the upper temperature compensation point of net CO_2 uptake. At these damaging temperatures, rates of net CO_2 loss in the light and dark were of similar magnitude.

Discussion

Contrary to previous suggestions that excess light intensifies high-temperature stress in plants, the present study documents positive effects of illumination on the heat tolerance of sun leaves of two tropical species, a pioneer tree and a late successional tree, characterised by large differences in photosynthetic and respiratory activities (Fig. 9).

Relatively short heating periods (15 min) were chosen, since extreme peak temperatures are usually of short duration in the natural environment. PAR of ~500 µmol photons m^{-2} s^{-1} applied in heat treatments of leaf disks floating on water has to be considered as 'excess light', as most of it is not used in photosynthesis. The leaves of both species possess stomata only on their abaxial side, so floating of leaf sections with their abaxial surface on water is expected to severely reduce photosynthesis. Moreover, as shown for attached intact leaves (Fig. 9), net CO_2 assimilation was strongly or fully inhibited at 45°C and higher temperatures.

Chl *a* fluorescence data from leaf disks of *F. insipida* recorded after 'recovery' periods of 24 h and 48 h showed a clear improvement of heat tolerance when heat treatment was performed under conditions of excess light compared with darkness. The critical temperature leading to a 50% decline in

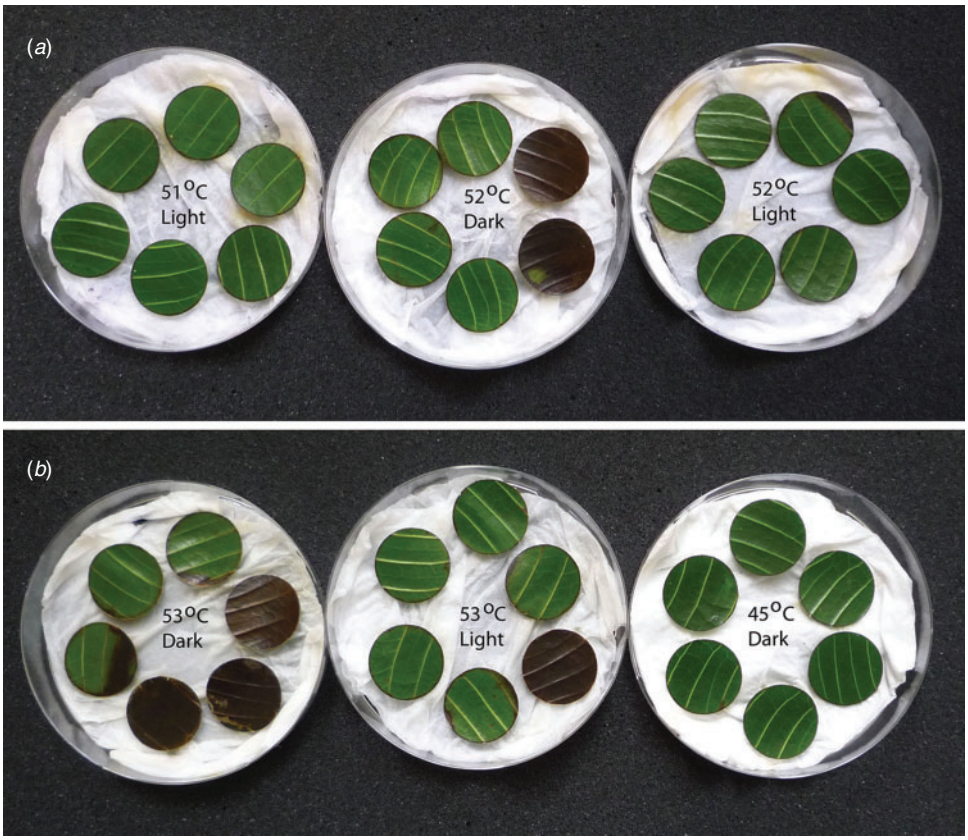

Fig. 6. Images of *Ficus insipida* leaf disks stored subsequent to 15 min of heat treatment for (*a*) 13 days and (*b*) 14 days in dim light on moist tissue paper. Disks from six different leaves are shown. Different degrees of necrotic tissue damage are seen in disks heated to 52°C and 53°C in the dark compared with light. Leaf disks heated to 51°C in the light and to 45°C in the dark served as controls (no visible tissue damage).

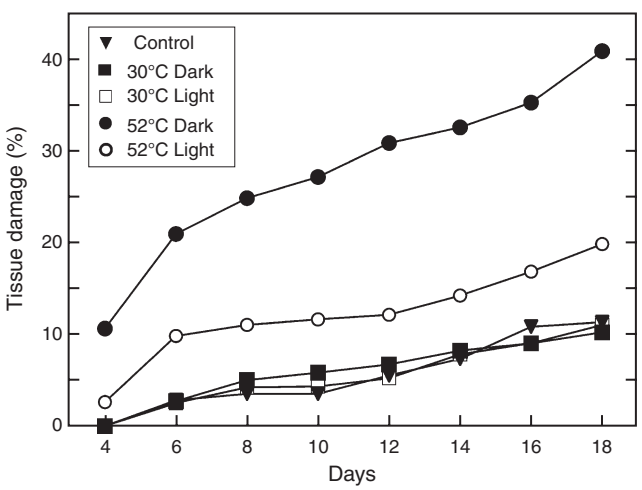

Fig. 7. Visible tissue damage of heat-treated leaf disks of *Calophyllum longifolium* as a function of storage time (days). Values represent the mean percentage of damaged leaf area. Disks are from six leaves (six disks each for controls and treatment at 30°C; 12 disks for treatment at 52°C). Closed triangles, untreated controls; closed symbols, treatment in the dark; open symbols, treatment in the light; squares, treatment at 30°C; circles, treatment at 52°C.

potential PSII efficiency, T_{50}, indicated by the F_v/F_m ratio, was increased by up to 1°C (Fig. 1). Such a temperature increment, although small, may nonetheless critically enhance leaf survival under natural conditions. Moreover, relief of heat stress by light was seen at temperatures below T_{50}, indicated by a markedly reduced decline in F_v/F_m when heating occurred in the light (Fig. 1).

In addition to the F_v/F_m ratio, changes in single fluorescence parameters of *F. insipida*, the heat-induced increase in F_0 and decrease in F_m (resulting in a decline in F_v/F_m) also indicate an improvement in heat tolerance under excess light (Figs 2 and 3). Similarly, the protective effect of excess light was observed in leaf sections of *C. longifolium* upon heating to 52°C under illumination, indicated by a reduced decline in F_v/F_m (Fig. 4), as well as by a diminished F_0 rise and F_m decrease, both after recovery of 24 h and 48 h (data not shown).

As discussed by Kouřil *et al.* (2004) and Ducruet *et al.* (2007), the F_0 rise probably results from accumulation of the reduced form of the primary quinone electron acceptor in the PSII reaction centre; alterations of PSII causing such accumulation are apparently complex. The F_m decline might be related to aggregation of the light-harvesting complexes of PSII, as observed by Tang *et al.* (2007) in heat-stressed spinach (*Spinacia oleracea* L.). Alternatively, a reduction in F_m may

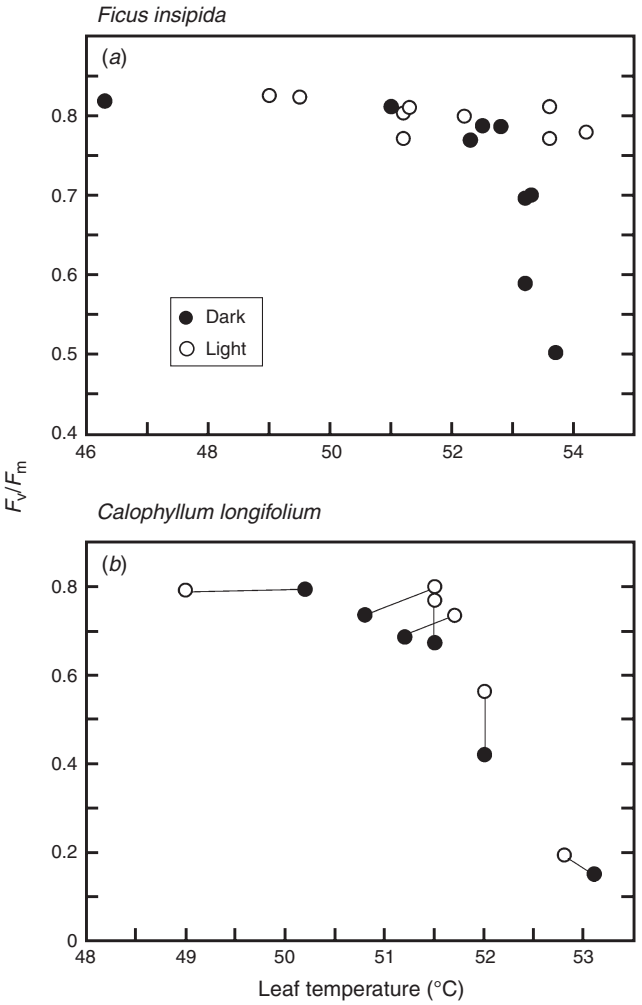

Fig. 8. F_v/F_m in response to final temperature of intact attached leaves of (*a*) *Ficus insipida* and (*b*) *Calophyllum longifolium* exposed to increasing temperatures in a gas exchange cuvette. F_v/F_m was measured 48 h after heat treatment. Heating in the dark, closed circles; heating in the light (900 µmol photons $m^{-2} s^{-1}$), open circles. (*a*) Data from 18 leaves of nine seedlings; (*b*) data from the opposite leaves (connected by solid lines) of six seedlings.

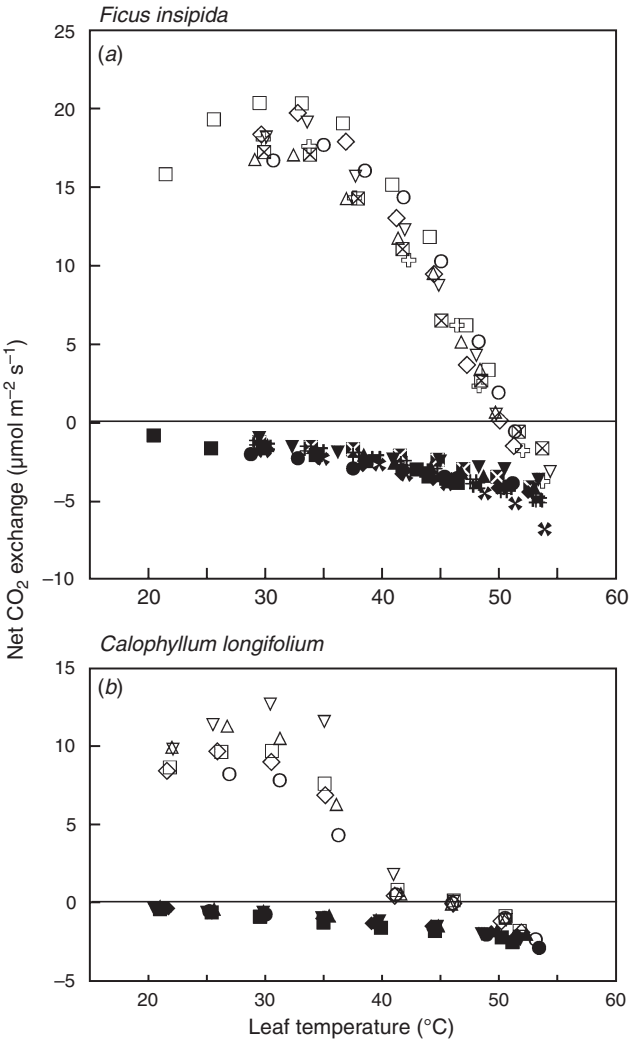

Fig. 9. Net CO_2 exchange of intact attached leaves of (*a*) *Ficus insipida* and (*b*) *Calophyllum longifolium* as function of leaf temperature. Different symbols refer to different leaves. Measurements in the dark, closed symbols; measurements in the light (900 µmol photons $m^{-2} s^{-1}$), open symbols. (*a*) Data from eight plants: nine leaves kept in the dark; seven leaves in the light. (*b*) Data from five leaf pairs of five plants: five leaves kept in the dark; five leaves in the light.

result from initial inactivation of the oxygen evolving complex (Lu and Zhang 2000). Furthermore, accumulation of PSII units containing inactivated D1 protein could cause quenching of F_m. Repair of PSII has been found to be restricted by reactive oxygen species (ROS) under thermal stress as well as light stress due to inhibition of protein (primarily D1) resynthesis (see Murata *et al.* 2007; Allakhverdiev *et al.* 2008). However, the latter effect, resembling photoinhibition of PSII, should be reversible during 'recovery' (see below).

Necrosis and discolouring effects occurring during long-term storage of heat-treated leaf disks (Figs 5–7) are consistent with T_{50} measurements based on the chl *a* fluorescence ratio F_v/F_m (cf. Krause *et al.* 2010). Images of heat-treated leaf disks of *F. insipida* taken after long-term storage (Fig. 6) indicate some variation in heat tolerance between individual samples from six different leaves, as also reflected by increased standard deviations of F_v/F_m at temperatures causing a F_v/F_m decline (cf. Fig. 1).

The much faster progress of necrosis upon heat treatment in darkness compared with light at temperatures close to T_{50} (52−53°C) strongly indicates a protective light effect. These results are in agreement with a recent methodological study by Buchner *et al.* (2013). By means of a visual estimation of leaf damage in two alpine dwarf shrub species, the authors observed significant increases in the lethal temperature when heat treatment was done on attached leaves *in situ* under natural solar irradiance as compared with darkness. In contrast to our study, the protective light effect was seen as a tendency only when the chl *a* fluorescence (F_v/F_m) method was used.

Additional evidence for an improvement in heat tolerance under excess light is provided by chl *a* fluorescence data from attached leaves of *F. insipida* and *C. longifolium* heated in a normal atmosphere (Fig. 8). F_v/F_m, recorded 48 h after heat

treatments, revealed similar results to experiments with leaf sections: at leaf temperatures between 51°C and 54°C, F_v/F_m declined less upon heating in the light than in the dark. The results obtained with intact attached leaves of both species suggest that the protective effect of light observed in sections of detached leaves is not a mere artefact. It should be noted that in the light, T_{50} in leaves *of F. insipida* appeared to be shifted upwards compared with results obtained with leaf sections (Fig. 1*b*). This discrepancy probably resulted from the different heating methods. Final leaf temperatures were reached gradually in the gas exchange cuvette due to the stepwise increases in air temperature of 5°C per 15 min and due to strong transpirational cooling, particularly in *F. insipida* leaves. By contrast, leaf disks were abruptly exposed to one single treatment temperature in the water bath. Furthermore, leaf disks assumed the water bath temperature for essentially the entire 15-min exposure period, whereas leaves in the gas exchange cuvette, because new temperatures were adopted with delay, remained at their final leaf temperatures for somewhat less than 15 min.

The enhancement of photoinhibition of PSII by high temperatures under high light documented in previous reports (see Introduction) does not contradict the improvement of heat tolerance by excess light found in the present study. Photodamage of the PSII complex has been shown to be initiated in the oxygen evolving complex, leading secondarily to inactivation of the PSII reaction centre, particularly of the D1 protein. Upon a return to favourable conditions (i.e. under low light at moderate temperatures), photodamaged PSII can be repaired; in particular, inactivated D1 protein in the reaction centre is degraded, followed by reconstitution of PSII with newly synthesised D1 (see Allakhverdiev *et al*. 2008; Takahashi and Badger 2011; Murata *et al*. 2012). The common reversibility of photoinhibition in low light speaks against a purely destructive mechanism. Rather, photoinhibition has been proposed to be a regulatory process dissipating absorbed light energy in form of heat, thereby preventing uncontrolled oxidative PSII destruction (Öquist *et al*. 1992; Krause and Jahns 2004).

A special type of protection by light against heat damage has been described for leaves of several temperate-climate species (Havaux and Strasser 1990; Havaux *et al*. 1991; Kislyuk *et al*. 2008; Marutani *et al*. 2012). These studies show an inhibition of photosynthetic CO_2 assimilation and O_2 evolution upon moderate heat exposure of leaves in the dark that was ameliorated by low to moderate light. The mechanisms involved in these effects appear to be complex. Inactivation of Rubisco, possibly related to restricted activity of Rubisco activase, and damage to PSII, including degradation of D1 protein, have been found to occur under moderate heat stress in the dark (Marutani *et al*. 2012). It was proposed that in the dark, the introduction of reducing equivalents from the chloroplast stroma into the plastoquinone pool leads to formation of ROS; light may act protectively by inducing cyclic electron transfer around PSI, preventing an over-reduction of plastoquinone. Notably, leaf exposure to moderate heat in the dark caused decreases in F_m and F_v, whereas F_0 remained constant (Havaux and Strasser 1990; Marutani *et al*. 2012). The effect described by these authors is clearly distinct from the observations of the present study, showing a strong increase in F_0 (Fig. 2), indicating detrimental, irreversible heat damage of PSII.

Various mechanisms may contribute to the light stimulation of heat tolerance in PSII and leaf tissue. Stapel *et al*. (1993) reported that HSPs synthesised by heat pretreatment of barley (*Hordeum vulgare* L.) leaves apparently exerted protection against photoinhibition of PSII under combined heat and high-light stress. However, the authors did not examine whether under these conditions high light protected the leaves from irreversible heat damage. According to Debel *et al*. (1994), high light caused post-translational accumulation of plastid HSP 23 in heat-stressed cell cultures of *Chenopodium rubrum* L. In leaves of *Solidago altissima* L., HSP synthesis, particularly formation of HSP 70 and small HSPs, was found to be enhanced by the combined action of heat and high light (Barua and Heckathorn 2006). In *Arabidopsis thaliana* (L.) Heynh, Rossel *et al*. (2002) observed enforced gene expression by high light for several HSPs and other chaperones, including a chloroplast-targeted putative small HSP. In addition, several genes involved in antioxidative defence mechanisms were induced. Within the short heat treatment period of our experiments, significant biosynthesis of additional HSPs and enzymes of defence against ROS in high light seems unlikely but, once induced, they may be beneficial during recovery in low light at moderate temperatures.

A plausible mechanism of protection by high light under heat stress is the proposed antioxidative action of Z. Sun leaves of tropical plants are known to contain high pools of the di-epoxide V in the dark-adapted state (Krause *et al*. 2006, 2010). In excess light, a large proportion of V is de-epoxidised to Z within a few minutes. In addition to the well-known Z-mediated dissipation of excessively absorbed light energy in PSII (Demmig-Adams 1998; Horton *et al*. 1996), Z may diminish lipid peroxidation (Havaux and Niyogi 1999; Johnson *et al*. 2007), and thereby reduce irreversible PSII inactivation and tissue damage.

In shade-acclimated leaves of the tropical herb *Alocasia macrorrhiza* (L.) G.Don, the critical temperature inducing necrosis was found to be considerably lower under high than low light (Königer *et al*. 1998). A lack of effective protection by Z may explain the high-light enhanced necrosis in these heat-stressed shade leaves; their content of V cycle pigments based on chl *a*+*b* was low, and high light resulted in only a moderate degree of V de-epoxidation.

In conclusion, the responses of chl *a* fluorescence parameters and visible tissue damage in sun leaves of two tropical tree species show that excessive light energy exerts a protective function under critical heat stress. At present, the exact mechanism of this protective light effect is unknown. It may primarily involve the action of Z, but also HSPs and defence systems against damage by ROS. Further experiments are required to clarify the mechanism of protection. In particular, Z accumulation and the synthesis of HSPs in response to strong heat stress should be tested. In addition, studies of antioxidative defence systems (e.g. of superoxide dismutase and ascorbate levels) and analysis of lipid peroxidation may help to elucidate the protective light effect. Moreover, cyclic electron transport around PSI should be investigated to test whether it significantly relieves damaging electron accumulation in the plastoquinone pool in illuminated, heat-stressed leaves.

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