

High-temperature inhibition of photosynthesis is greater under sunflecks than uniform irradiance in a tropical rain forest tree seedling

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ABSTRACT

The survival of dipterocarp seedlings in the understorey of south-east Asian rain forests is limited by their ability to maintain a positive carbon balance. Photosynthesis during sunflecks is an important component of carbon gain. Field measurements demonstrated that *Shorea leprosula* seedlings in a rain forest understorey received a high proportion of daily photon flux density at temperatures supra-optimal for photosynthesis (72% at ≥ 30 °C, 14% at ≥ 35 °C). To investigate the effect of high temperatures on photosynthesis during sunflecks, gas exchange and chlorophyll fluorescence measurements were made on seedlings grown in controlled environment conditions either, under uniform, saturating irradiance (approximately $539 \mu\text{mol m}^{-2} \text{s}^{-1}$) or, shade/fleck sequences (approximately $30 \mu\text{mol m}^{-2} \text{s}^{-1}$ /approximately $525 \mu\text{mol m}^{-2} \text{s}^{-1}$) at two temperatures, 28 or 38 °C. The rate of light-saturated photosynthesis, under uniform irradiance, was inhibited by 40% at 38 °C compared with 28 °C. However, during the shade/fleck sequence, photosynthesis was inhibited by 59% at 38 °C compared with 28 °C. The greater inhibition of photosynthesis during the shade/fleck sequence, when compared with uniform irradiance, was driven by the lower efficiency of dynamic photosynthesis combined with lower steady-state rates of photosynthesis. These results suggest that, contrary to current dogma, sunfleck activity may not always result in significant carbon gain. This has important consequences for seedling regeneration processes in tropical forests as well as for leaves in other canopy positions where sunflecks make an important contribution to total photon flux density.

Key-words: *Shorea leprosula*; Dipterocarpaceae; dynamic photosynthesis; photorespiration; understorey.

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INTRODUCTION

Temperature has significant effects upon photosynthesis, with plants having an optimum temperature, above and below which photosynthesis becomes increasingly inhibited (Berry & Björkman 1980). However, the responses of dynamic photosynthesis to temperature have been largely ignored, despite the prevalence of environments in which light is highly heterogeneous and sunflecks are a significant component of total daily photon flux density (PFD) (Baldocchi & Collineau 1994; Pearcy *et al.* 1994).

Sunflecks contribute 60–90% of total daily PFD to plants in the understorey of tropical rain forests, driving up to 65% of total daily carbon gain (Pearcy 1983; Pearcy & Calkin 1983; Chazdon 1988; Pfitsch & Pearcy 1989). However, high PFDs during sunflecks can also result in photo-inhibition, which reduces carbon gain (Le Gouallec, Cornic & Blanc 1990; Watling *et al.* 1997). The leaf temperature of seedlings growing in forest gaps has been reported to exceed 40 °C, resulting in photo-inhibition, photodamage and mortality (Mulkey & Pearcy 1992; Koniger *et al.* 1995; Clearwater 1997; Koniger, Harris & Pearcy 1998). In contrast, leaf temperatures of seedlings growing in the forest understorey have been reported to be much lower (e.g. <24 °C; Pearcy & Calkin 1983). However, sunflecks received by seedlings in the understorey contain not only very high photosynthetically active radiation ($>1500 \mu\text{mol m}^{-2} \text{s}^{-1}$, 400–700 nm), but also longer infra-red wavelengths (>700 nm) which contribute significantly to the radiative energy load (Lee 1987). This has been demonstrated for pot-grown seedlings placed under an Australian subtropical forest canopy, which had high and dynamic leaf temperatures (35–41 °C) in association with sunflecks (J.R. Watling, personal comm.).

The inhibition of steady-state photosynthesis by high temperatures can be attributed to reduced a Rubisco activation state and increased photorespiration. The mechanisms behind these changes are also likely to affect dynamic photosynthesis. The efficiency of dynamic photosynthesis is expressed in terms of lightfleck utilization efficiency (LUE); the ratio of measured carbon gain during a

sequence of sunflecks against modelled carbon gain assuming instantaneous photosynthetic induction gain and loss (Chazdon & Pearcy 1986). Reduced Rubisco activation state at high temperatures is caused by lower activity of Rubisco activase (Law & Crafts-Brandner 1999; Crafts-Brandner & Salvucci 2000). Rubisco activation is essential for photosynthetic induction gain (Portis 1992, 1995) and consequently, photosynthetic carbon gain during sunflecks (Pearcy *et al.* 1994). Therefore, in addition to inhibiting steady-state photosynthesis, high foliar temperature should significantly reduce LUE, via slower rates of induction gain along with more rapid induction loss at higher temperatures. The responses of *Fagus sylvatica* seedlings at 35 °C, compared with 25 °C, were consistent with this hypothesis when grown under half-shade (Küppers & Schneider 1993).

The rate of photorespiration becomes greater with rising temperature as changes occur in the differential solubility of CO₂ and O₂ (Ku & Edwards 1977; Monson *et al.* 1982) and the kinetic properties of Rubisco (Monson *et al.* 1982; Jordan & Ogren 1984; Sage & Sharkey 1987). In shade periods after sunflecks, pools of photosynthetic and photorespiratory pathway intermediates, which accumulate during the high irradiance sunfleck, are metabolized. Changes in post-irradiance gas exchange can significantly impact photosynthetic carbon gain and contribute to changes in growth rate (Sims & Pearcy 1993; Pearcy *et al.* 1994). Increases in the flux through the photorespiratory pathway at higher temperatures may reduce LUE and therefore reduce carbon gain during sunflecks.

The Dipterocarpaceae are the dominant climax trees of lowland rain forest in south-east Asia, with approximately 500 species found over wide geographical ranges and in diverse species assemblages (Symington 1943; Whitmore 1984; Ashton 1988), and, as such, they are the primary determinants of forest structure and function. After gregarious fruiting events, every 3–8 years, mixed species seedling banks develop in the deeply shaded forest understorey. Seedling growth and survival are strongly influenced by light limitation of photosynthetic carbon gain (Chazdon 1988; Fetcher, Oberbauer & Chazdon 1994; Press *et al.* 1996). If temperature affects photosynthesis during sunflecks significantly, changes in carbon gain may impact on seedling growth and survival.

This study aimed to evaluate the impact of temperature on photosynthesis during sunflecks for seedlings of the model dipterocarp species *Shorea leprosula* Miq. First, the relationship between sunfleck PFD and leaf temperature in a forest understorey is reported. Second, the responses of CO₂ assimilation and photosynthetic electron fluxes to temperature were measured by gas exchange and chlorophyll fluorescence under controlled environment conditions. The following hypotheses were tested: (1) leaves of dipterocarp seedlings experience high temperatures in association with sunflecks; (2) steady-state photosynthesis is inhibited by temperatures experienced by seedlings in the forest; and finally, addressing the main aim of the experiment (3) high temperatures reduce photosynthetic carbon gain during sunflecks to a greater extent than dur-

ing steady-state photosynthesis, as a result of lower A_{\max} and LUE.

MATERIALS AND METHODS

Incident PFD, air temperature and leaf temperature of seedlings in the forest understorey

To determine the relationship between incident PFD, air temperature and leaf temperature, simultaneous measurements of the variables were made on the youngest, fully expanded leaf of three *S. leprosula* seedlings. The seedlings were growing at an understorey site with fragmented canopy overhead, within primary, lowland dipterocarp rain forest close to the Danum Valley Field Centre (DVFC), Sabah, E. Malaysia, Borneo (4°58' N, 117°48' E). A PFD quantum sensor (SKP 215; Skye Instruments, Llandrindod Wells, Powys, UK) was levelled to horizontal and adjusted to petiole height, immediately adjacent to the leaf. Simultaneously, measurements of leaf temperature were made using a thermocouple touching the lower leaf surface and air temperature was monitored by a shaded thermistor (SKTS200U & SKH2011; Skye Instruments). All variables were recorded every 10 s by a datalogger, between 0830 and 1800 h, on 3 d per seedling (Datahog2; Skye Instruments).

Plant material

Shorea leprosula seeds were collected from the primary forest surrounding DVFC. They were germinated and maintained for 2 years in a forest nursery (total daily PFD approximately 9.0 mol m⁻² d⁻¹) in polythene pots containing forest soil, prior to transfer to a controlled environment glasshouse in the UK. The seedlings were then transplanted into 2.1 L pots containing 2 : 2 : 1 (v/v) vermiculite, perlite and seed compost, with slow release N : P : K (14 : 13 : 13) fertilizer containing micronutrients (3 g L⁻¹; Osmocote plus, Waardenburg, Holland). The seedlings were maintained for 8 months with maximum and minimum temperatures of 37 and 19 °C, respectively, and a constant relative humidity of approximately 80%. Five seedlings were transferred to a controlled environment cabinet (model SGC097, Fitotron; Sanyo-Gallenkamp, Loughborough, Leicestershire, UK) 1 month prior to the start of the experiment. The PFD was approximately 170 μmol m⁻² s⁻¹, over a photoperiod of 12 h. Mean day and night temperatures were 30.0 and 20.3 °C, respectively. Mean day and night relative humidity was 79.9 and 96.0%, respectively.

Measurement of gas exchange and chlorophyll fluorescence at controlled temperatures under laboratory conditions

All experimental measurements of photosynthesis were made between 0800 and 1300 h on the youngest, fully expanded leaf of five seedlings. There were no confounding effects of circadian rhythm or midday stomatal closure

on photosynthesis over the period of data collection (data not shown). Measurements of CO₂ fixation were made using an open infrared gas analyser (IRGA) system (Model 6400; Li-Cor, Lincoln, NE, USA). Measurements of chlorophyll fluorescence were made simultaneously using a modulated chlorophyll fluorometer (model S5-FL; Opti-Sciences, Tyngsboro, MA, USA). The leaf chamber sealed a leaf area of 6 cm² and flow rates through the chamber were maintained at 700 mL min⁻¹. The time between the injection of air (2% CO₂) into the closed chamber, via a syringe, and the output of the IRGA reaching a peak in CO₂ concentration was 7.5 s, representing the system response time. Gas exchange parameters were calculated using the equations of von Caemmerer & Farquhar (1981). Actinic light was provided, via a fibre optic bundle, by halogen lamps (KL 1500; Schott, Mainz, Germany). Leaf temperature was measured by a thermocouple in contact with the lower leaf surface.

The input gas was bubbled through water and high relative humidity was maintained in the leaf chamber at all times (75–85%; leaf vapour pressure deficit ≤ 1.3 kPa). The apparatus was set up inside a controlled temperature room, allowing air temperatures to be adjusted between 20 and 38 °C. Leaf temperatures could then be controlled, using the leaf chamber Peltier units, between 23 and 43 °C. This design prevented condensation of high humidity air within the IRGA and avoided large differences of temperature inside and outside the leaf chamber.

The chlorophyll fluorescence parameters: (q_p) the proportion of open photosystem II (PSII) reaction centres; (Φ_{PSII}) quantum yield of PSII; (F_v/F_m) maximum quantum efficiency of PSII; (F_v'/F_m') intrinsic efficiency of PSII; (NPQ) nonphotochemical quenching, were defined and calculated as described by Maxwell & Johnson (2000). The apparent electron transport rate (ETR) was calculated according to Schreiber, Bilger & Neubauer (1994). Plants were dark adapted for 1 h prior to measurements. The minimum fluorescence level (F_0) was measured initially, before the maximum fluorescence level (F_m) was measured with a saturating pulse of light (3500 $\mu\text{mol m}^{-2} \text{s}^{-1}$), of 0.8 s duration. After the actinic light used for experimental treatments was switched off, the minimum fluorescence level was determined using far-red illumination (F_0').

Temperature response curves

The temperature response of light-saturated photosynthesis (A_{max}) was measured at a saturating PFD of 539 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at 350 $\mu\text{mol mol}^{-1}$ CO₂. Gas exchange and chlorophyll fluorescence parameters (A_{max} , g_s , C_i , q_p , Φ_{PSII} , F_v/F_m , F_v'/F_m' and NPQ) were measured during the periods of steady-state gas exchange, after leaf temperatures had been maintained for 30 min at each of seven temperatures between 23 and 43 °C. The value of each parameter and the temperature at the maxima or minima of each response curve were estimated after fitting a second-order polynomial equation. In a separate procedure to determine

whether photodamage had occurred, measurements of F_v/F_m were made 30 min after transfer of a leaf from saturating PFD to darkness at each of the seven temperatures between 23 and 43 °C.

Rates of photorespiration (R_p), across the range of measurement temperatures, were estimated from measured gas exchange parameters using the model equations for C₃ photosynthesis of Farquhar, von Caemmerer & Berry (1980). Re-ordering the equation for net CO₂ assimilation describes photorespiration as:

$$R_p = V_c - R_d - A \quad (1)$$

where V_c is the rate of carboxylation, R_d is the rate of CO₂ evolution from mitochondria in the light and A is the net rate of CO₂ assimilation. Ribulose-1,5-bisphosphate (RuBP) is commonly present in excess in the chloroplast (Perchorowicz, Raynes & Jensen 1981; von Caemmerer & Edmondson 1986). In addition, when varied by altering irradiance, the concentrations of RuBP relative to active Rubisco binding sites were saturating at all but the lowest rates of photosynthesis (Sharkey 1989). Therefore, assuming saturating amounts of RuBP in the chloroplast, V_c is given by:

$$V_c = V_{\text{cmax}} \cdot \frac{C}{C + K_c(1 + O/K_o)} \quad (2)$$

where V_{cmax} is the maximum rate of carboxylation, K_c and K_o are the Michaelis–Menten constants for CO₂ and O₂, respectively, and C and O are the intercellular CO₂ and O₂ concentrations, respectively.

The value of V_{cmax} was estimated for *S. leprosula* after A - c_i curves were constructed at the saturating PFD of 670 $\mu\text{mol m}^{-2} \text{s}^{-1}$. CO₂ supply to the leaf chamber was reduced in a stepwise manner from 600 to 5 $\mu\text{mol mol}^{-1}$, with gas exchange parameters recorded once steady rates of gas exchange were achieved. Analysis of the A - c_i curve for each plant was carried out using the mechanistic model of Farquhar *et al.* (1980). Estimates of apparent maximum carboxylation capacity (V_{cmax}) were calculated using the non-linear regression technique of Wullschlegel (1993) and the temperature corrections of Bernacchi *et al.* (2001). Substituting Eqn 2 into Eqn 1 gives:

$$R_p = \left[V_{\text{cmax}} \cdot \frac{C}{C + K_c(1 + O/K_o)} \right] - R_d - A \quad (3)$$

allowing calculation of R_p from measured gas exchange parameters. Constants were corrected to measurement temperatures, using the generic temperature response equation:

$$\text{Parameter} = \exp(c - \Delta H_a/R \cdot T_k) \quad (4)$$

where R is the molar gas constant (0.0083144 kJ K⁻¹ mol⁻¹) and T_k is the leaf temperature (Tenhunen *et al.* 1976; Harley & Tenhunen 1991). Values for the scaling constant (c) and activation energy (ΔH_a) of the specific parameters relating to Rubisco enzyme kinetics and R_d were taken from recent *in vivo* estimates (Bernacchi *et al.* 2001).

Photosynthesis during a sequence of lightflecks, at 28 versus 38 °C

Gas exchange was measured to compare photosynthesis during a sequence of light flecks at 28 and 38 °C. At each temperature, leaves were exposed to a PFD of 30 $\mu\text{mol m}^{-2} \text{s}^{-1}$, at 350 $\mu\text{mol mol}^{-1}$ CO_2 , until steady-state gas exchange was achieved. They were then subjected to 10, 3-min, flecks of 539 $\mu\text{mol m}^{-2} \text{s}^{-1}$ separated by 1-min low light periods of 30 $\mu\text{mol m}^{-2} \text{s}^{-1}$. From the starting temperatures of either 28 or 38 °C at low photosynthetic photon flux density (PPFD), leaf temperature rose briefly, during each fleck in the sequence, by 0.51 to 0.83 °C (mean at 28 °C = 0.63 °C; mean at 38 °C = 0.69 °C). At the end of the 40-min sequence of flecks leaf temperature at low PPFD had increased by 0.10–0.46 °C (mean at 28 °C = 0.33 °C; mean at 38 °C = 0.23 °C). Vapour pressure deficit was also dynamic during the sequence of flecks but was ≤ 1.2 kPa at all times and did not differ significantly during measurements at 28 °C compared with 38 °C. All gas exchange data were logged every 2 s and after raw output was corrected for the lag of the system response time, parameters were calculated describing the progression of photosynthetic induction gain and increasing stomatal conductance as described in Leakey *et al.* (2002). Lightfleck utilization efficiency during the fleck sequence was calculated by the method of Chazdon & Pearcy (1986).

Loss of photosynthetic induction state in shade, at 28 versus 38 °C

Rates of induction loss after transition of the leaf from saturating light to low PFD (described by induction state, IS% and the g_s as a percentage of values in saturating light), were measured over shade periods of 10 min, following the method of Zipperlen & Press (1997).

Statistical analysis

Differences between the temperature optima of ETR and A_{max} , as well as in photosynthetic parameters during sunflecks at 28 versus 38 °C, were compared by two-sample t -tests. All analyses were performed using Minitab (Minitab 12.0 software, Minitab Inc., State College, PA, USA).

RESULTS

The relationship between leaf temperature and air temperature, for seedlings in the forest understorey

The temperature of a leaf (length \times breadth = 1.25 cm \times 4.4 cm) in the forest understorey was a function of both the surrounding air temperature and the pattern of PFD incident upon it. A representative plot of PFD, air temperature and leaf temperature, measured simultaneously for the youngest fully expanded leaf of a *S. leprosula* seedling, is shown in Fig. 1. The approximate minimum and maximum values of leaf temperature were 24 and

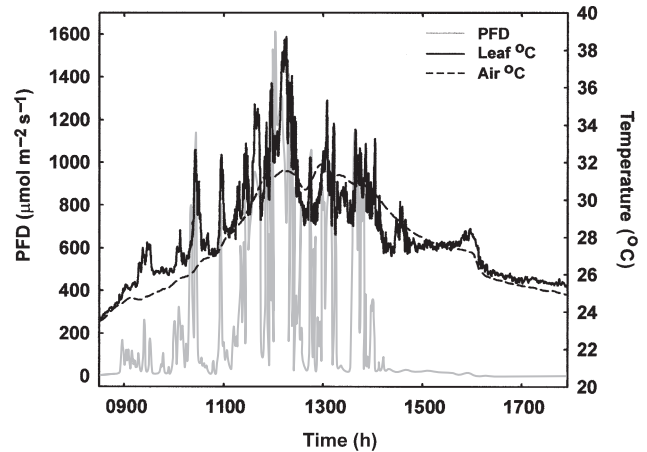


Figure 1. Representative traces over the diurnal period of: leaf temperature (black line) of a *S. leprosula* seedling in a tropical rainforest understorey, PFD incident on the leaf (grey line) and air temperature (dashed line).

38 °C, respectively. Leaf temperature generally tracked air temperature, but uncoupling occurred during sunflecks. During periods with many sunflecks (e.g. from 1030 to 1400 h), leaf temperature was higher than air temperature and highly dynamic. At these times, peaks in leaf temperature were related to the length and intensity of individual sunflecks. 72 and 14% of total daily PFD were received at temperatures, equal to or above 30 and 35 °C, respectively.

The response of steady-state photosynthesis to temperature

Above an optimal temperature of 29.1 °C light-saturated photosynthesis (A_{max}) was progressively inhibited, declining from an optimum of 5.4 $\mu\text{mol m}^{-2} \text{s}^{-1}$ to zero net CO_2 exchange at 43 °C (Fig. 2a). In contrast, calculated rates of photorespiration (R_p) increased rapidly above 30 °C, reaching 6.3 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at 43 °C (Fig. 2a). Stomatal conductance (g_s) decreased progressively at temperatures above 29 °C (Fig. 2b). Changes in A_{max} and g_s resulted in relatively constant intercellular CO_2 concentrations (c_i) of approximately 230 $\mu\text{mol mol}^{-1}$ between 23 and 35 °C, but a marked rise occurred above 37 °C (Fig. 2b).

The temperature optimum for the proportion of absorbed light used for photochemistry (Φ_{PSII}) was 33.4 °C (Fig. 2c). This was significantly higher than the optimal temperature for A_{max} (t -test: $T = 6.1$, d.f. = 5, $P < 0.05$). Although Φ_{PSII} was progressively inhibited above 33.4 °C, significant photochemistry still occurred at 43 °C. Non-photochemical quenching (NPQ) increased significantly above 30.7 °C (Fig. 2c) whereas the intrinsic efficiency of PSII (F_v'/F_m') declined (Fig. 2d). The proportion of open PSII reaction centres (q_p) increased strongly and linearly with rising temperature (Fig. 2d).

Following exposure to saturating PFD at different temperatures, there was no evidence of photodamage, with

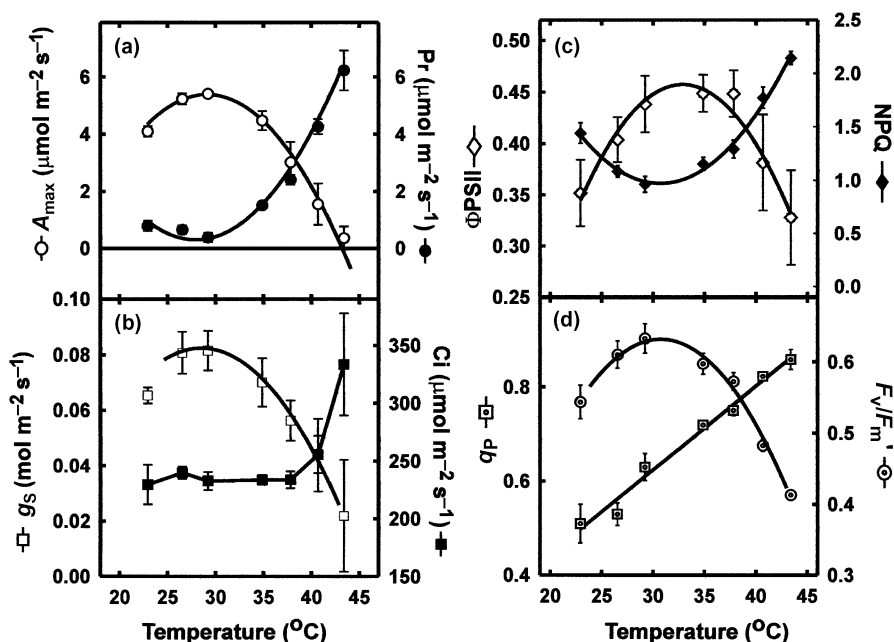


Figure 2. Temperature-dependent responses of: (a) light saturated rate of photosynthesis (A_{max}) and calculated rates of photorespiration (P_r); (b) inter-cellular CO_2 concentration (C_i) and stomatal conductance (g_s); (c) the quantum yield of PSII (Φ_{PSII}) and non-photochemical quenching (NPQ); and (d) the proportion of open PSII (q_p) and intrinsic efficiency of PSII (F_v/F_m') of *S. leprolosula*. Values are means (\pm SE), $n = 5$. Lines indicate: (A_{max} , g_s , P_r , Φ_{PSII} , NPQ , F_v/F_m') mean fitted second order polynomials ($n = 5$); (q_p) mean linear regressions ($n = 5$); (C_i) running mean ($n = 5$).

the dark-adapted maximum quantum efficiency of PSII (F_v/F_m) remaining between 0.79 and 0.81 across the temperature range (data not shown). In addition, measurement of temperature response curves by increasing and then decreasing leaf temperature across the 22–43 °C range indicated no hysteresis in the response of gas exchange or chlorophyll fluorescence parameters (data not shown).

Photosynthesis during a sequence of sunflecks, at 28 versus 38 °C

Both steady-state and dynamic components of photosynthesis during a sequence of lightflecks were significantly different at 28 °C compared with 38 °C. Representative traces of assimilation rates and stomatal conductance are shown in Fig. 3. The initial steady-state photosynthetic rate ($A_{initial}$) in the shade ($30 \mu\text{mol m}^{-2} \text{s}^{-1}$) was significantly lower at 28 °C compared with 38 °C (Table 1). The maximum steady-state rate of photosynthesis ($A_{max-fleck}$) achieved during the fleck sequence was 43% lower at 38 than at 28 °C (Table 1). Stomatal conductance was significantly lower at 38 °C (by 41%), but only after complete photosynthetic induction (Table 1). There were no significant differences in the times for photosynthetic induction or stomatal opening at different temperatures (Table 1). However, lightfleck utilization efficiency (LUE), a measure of the overall efficiency of dynamic photosynthesis during the fleck sequence, was significantly lower (by 14%) at the elevated temperature (Table 1). As a consequence of these combined responses, carbon gain during the sequence of light flecks at 38 °C was significantly lower than at 28 °C (by 59%, Table 1). This reduction in carbon gain was 19% greater than under steady-state, light-saturating conditions (Table 1).

Loss of photosynthetic induction state in shade, at 28 versus 38 °C

The photosynthetic induction state (IS%) remaining 10 min after a step change from saturating PFDF to shade

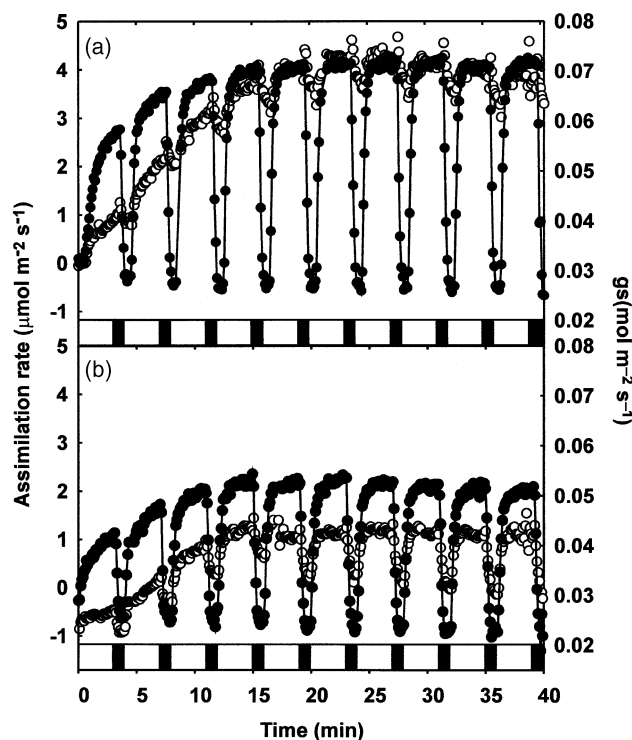


Figure 3. Representative time courses of net photosynthetic rates (closed circles, solid lines) and stomatal conductance (open circles) of *S. leprolosula* measured at (a) 28 and (b) 38 °C, during a simulated sequence of sunflecks. Flecks are of 3 min duration (white bands) separated by 1 min shade periods (dark bands).

Table 1. Steady-state rates of photosynthesis in shade (A_{initial}), maximum photosynthetic rate attained during flecks ($A_{\text{max-fleck}}$), stomatal conductance at steady-state photosynthesis in shade (g_{Sinitial}), maximum stomatal conductance attained during flecks ($g_{\text{Smax-fleck}}$), time to 90% of $A_{\text{max-fleck}}$ ($T_{90\%A}$), time to 90% of $g_{\text{Smax-fleck}}$ ($T_{90\%gS}$), lightfleck utilization efficiency (LUE), and carbon gain during a fleck cluster (carbon gain) of *S. leprosula* measured at either 28 or 38 °C

	28 °C	38 °C	%
A_{initial} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	0.28 ± 0.20	-0.32 ± 0.04	-214%*
$A_{\text{max-fleck}}$ ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	5.27 ± 0.47	3.02 ± 0.16	-43%*
g_{Sinitial} ($\text{mmol m}^{-2} \text{s}^{-1}$)	28 ± 4	23 ± 1	ns
$g_{\text{Smax-fleck}}$ ($\text{mmol m}^{-2} \text{s}^{-1}$)	79 ± 11	47 ± 3	-41%*
$T_{90\%A}$ (min)	15.2 ± 1.5	13.1 ± 0.7	ns
$T_{90\%gS}$ (min)	31.4 ± 3.4	21.8 ± 5.1	ns
LUE (%)	84.8 ± 1.7	73.2 ± 1.3	-14%**
Carbon gain ($\mu\text{mol m}^{-2}$)	7373 ± 763	3013 ± 205	-59%**
A_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	5.36	3.24	-40%

Values are means (± SE), $n = 5$. Significant differences between means indicated by (t -test) * $P < 0.05$, ** $P < 0.01$. % = difference in parameter value between 28 and 38 °C as percentage of mean value at 28 °C. A_{max} were derived from mean fitted temperature response curve for *S. leprosula*, at 28 and 38 °C.

was 81% lower at 38 than at 28 °C (Table 2). Likewise, remaining g_s was significantly lower at 38 °C compared with 28 °C (Table 2).

DISCUSSION

Dipterocarp seedlings in the tropical rain forest understorey experienced high temperatures during periods of sunfleck activity (maximum, approximately 38 °C). These temperatures were significantly higher than those in the few previous reports from sites under closed canopies (Björkman, Ludlow & Morrow 1972; Percy & Calkin 1983). In these cases leaves received fewer, shorter sunflecks, which probably provided less incident radiation. In contrast, *S. leprosula* leaf temperatures were generally lower than those measured in gap sites in a Panamanian tropical forest (Koniger *et al.* 1995) and a south-east Asian rain forest (Clearwater 1997). However, there are often not sharp physical boundaries between gap and non-gap structure in rainforests (Lieberman, Lieberman & Peralta 1989). At

Table 2. Induction state after 10 min shade (IS%), stomatal conductance after 10 min shade as a percentage of values at saturating irradiance (% g_s) of *S. leprosula* measured at either 28 or 38 °C

	28 °C	38 °C	%
IS% (%)	83 ± 2	16 ± 11	-81%**
% g_s	81.7 ± 6.3	40.9 ± 7.8	-50%**

Values are means (± SE), $n = 5$. Significant differences between means indicated by (t -test) * $P < 0.05$, ** $P < 0.01$. % = difference in parameter value between 28 and 38 °C as percentage of mean value at 28 °C.

sites of intermediate canopy structure incident radiation (PFD and infra-red) can be higher than in the stereotypical 'understorey' but still highly dynamic in nature. At such a site in Australian subtropical forest, seedlings also experienced high, dynamic leaf temperatures. Under these conditions, a high proportion of the total daily PFD incident on a representative leaf of *S. leprosula* was received at temperatures that were supra-optimal for photosynthesis (72% at ≥ 30 °C and 14% at ≥ 35 °C).

Steady-state photosynthetic responses to temperature

Light saturated photosynthesis (A_{max}) was progressively inhibited above the temperature optimum of 29.1 °C for *S. leprosula*. The nature of this inhibition can vary between species and with environmental conditions (Berry & Björkman 1980). It was therefore characterized, in detail, to provide a physiological context for high-temperature effects upon photosynthesis during sunflecks and to facilitate comparison with other studies.

Calculated rates of photorespiration (R_p) displayed the inverse response to temperature than that of photosynthesis, with calculated rates increasing significantly at higher temperatures. This is consistent with previous reports of the inhibition of photosynthesis and enhancement of R_p at high temperatures and is most likely due to a reduction in the activation state of Rubisco (determined by Rubisco activase activity; Law & Crafts-Brandner 1999; Crafts-Brandner & Salvucci 2000), together with unfavourable changes in the differential solubility of CO_2 and O_2 (Ku & Edwards 1977; Monson *et al.* 1982) and the kinetic properties of Rubisco (Monson *et al.* 1982; Jordan & Ogren 1984; Sage & Sharkey 1987).

The deleterious effect of high temperatures on photosynthesis in dipterocarp seedlings could be exacerbated if the absorbed light energy no longer used for photochemistry was not dissipated. In this case, photodamage of the thylakoid photosynthetic apparatus would result. However, no change in the maximum quantum efficiency of PSII (F_v/F_m) occurred with temperature, indicating that excess excitation energy was dissipated by photoprotective mechanisms. The proportion of absorbed light used for photochemistry (Φ_{PSII}) was progressively inhibited by high temperatures, but the temperature optimum was significantly higher than that for CO_2 assimilation. This will have maintained electron transport at rates greater than that required for CO_2 fixation at high temperatures, which suggests alternative electron acceptors were active. Photorespiration constitutes a large electron sink, capable of preventing photodamage to the electron transport pathway under stress conditions (Osmond *et al.* 1997; Wingler *et al.* 2000; Ort & Baker 2002). As temperature increased it will have acted as a growing alternative sink for the increasing level of excess excitation energy.

The second mechanism for dissipating excess energy is non-photochemical quenching (NPQ ; Niyogi 2000; Muller, Li & Niyogi 2001), which increased progressively with rising

temperature. Greater NPQ is facilitated by conformational changes in the PSII antennae structure that alter the rate of thermal dissipation of excitation energy within it. This alters the efficiency with which excitation energy is passed to open PSII reaction centres, affecting the intrinsic quantum efficiency of PSII (F_v'/F_m'). The value of F_v'/F_m' decreased at temperatures above the optimum of 30.7 °C in *S. leprosula*. Despite the large increase in NPQ and decrease in F_v'/F_m' , the decline in Φ_{PSII} above 33 °C was only moderate because q_P increased linearly with temperature. An increase in q_P with rising temperature (up to 35 °C) was also seen at low PFD (180 $\mu\text{mol m}^{-2} \text{s}^{-1}$) in a study on the effects of temperature on leaf discs of four species of dipterocarps (Kitao *et al.* 2000). However, in that case q_P and Φ_{PSII} both decreased above 35 °C, and were associated with an increase in F_o' indicative of heat-induced inactivation of PSII (Schreiber & Armond 1978; Yamane *et al.* 1997).

In summary, CO_2 fixation was severely inhibited by high temperatures, but dipterocarp seedlings effectively dissipated the excess absorbed light energy and prevented photodamage, under these conditions. It is noteworthy that this study investigated the effects of high temperatures upon photosynthesis in isolation from the very high PFDs, which can cause photodamage in the field. When very high PFDs (1600 $\mu\text{mol m}^{-2} \text{s}^{-1}$) are incident upon dipterocarp seedlings, photodamage can occur (reduced F_v'/F_m') and is exacerbated by high temperatures (Kitao *et al.* 2000).

Photosynthesis during a sequence of sunflecks at 28 versus 38 °C

To understand how temperature affects the carbon gain of seedlings in the forest it is essential to measure photosynthesis under dynamic irradiance. At 38 °C, compared with 28 °C, differences were seen in both steady-state and dynamic components of photosynthetic performance during a sequence of flecks. The changes in $A_{\text{max-flecks}}$ matched the inhibition seen with temperature on the temperature response curve of A_{max} . This was accompanied by significant reductions in stomatal conductance ($g_{\text{Smax-fleck}}$). However, in contrast to the hypothesized response, there were no significant effects of temperature on the rates of photosynthetic induction gain or stomatal opening, as measured by gas exchange. This implies that increased photorespiration was responsible for the high-temperature inhibition of photosynthesis in the absence of Rubisco activase deactivation.

LUE of photosynthesis during the sequence of flecks was 14% lower at 38 °C compared with 28 °C. This resulted from the greater observed rate of induction loss and possibly also changes in post-irradiance metabolism, during the shade periods between flecks. The flux of intermediates in the photosynthetic and photorespiratory pathways determines the extent of post-irradiance CO_2 fixation and post-irradiance CO_2 burst, respectively (Sharkey, Seemann & Pearcy 1986; Rawsthorne & Hylton 1991). Under steady-state conditions the rate of photosynthesis was lower, whereas the rate of respiration was higher at 38 °C compared with 28 °C. This may have led to lower post-irradi-

ance CO_2 fixation and greater post-irradiance CO_2 burst at 38 °C. Similar responses have been generated by short-term treatments of low CO_2 , high O_2 and high temperatures (Doehrlert, Ku & Edwards 1979; Peterson 1983; Vines *et al.* 1983; Laisk, Kurats & Oja 1984). If reductions occur in post-irradiance carbon gain at high temperatures, they are likely to be greater in the field under natural patterns of short, high-frequency flecks in which post-irradiance metabolism contributes a greater proportion of net carbon gain (Pearcy *et al.* 1990), provided that this coincides with high temperatures.

The inhibition of the dynamic components of photosynthesis (LUE, -14%) appears modest but together with the lower steady-state rates of photosynthesis at elevated CO_2 , resulted in a large decrease in total photosynthetic carbon gain during a cluster of flecks (59%). This inhibition was considerably larger than that predicted from the steady-state temperature response curve for A_{max} (40%), supporting the third hypothesis.

Loss of photosynthetic induction state in the shade, at 28 versus 38 °C

Photosynthetic induction loss was considerably faster at 38 °C, 10 min after the transition from fleck to shade PFD. This was caused, at least in part, by more rapid stomatal closure at 38 °C, but increased de-activation of enzymes responsible for RuBP regeneration or Rubisco may also have occurred in the shade at higher temperatures (Sassenrath-Cole & Pearcy 1992; Ernstsén, Woodrow & Mott 1997). In natural irradiance regimes in which many shade periods may be of this duration (≤ 10 min; Pearcy *et al.* 1994; Leakey 2002) this could significantly decrease photosynthetic carbon gain during subsequent flecks.

Implications for photosynthetic carbon gain and ecology

Current dogma assumes that sunfleck activity, as either long flecks or many short flecks, results in significant photosynthetic carbon gain (Chazdon 1988; Pearcy *et al.* 1994). However, at both this dipterocarp forest site and an Australian rain forest site (J.R. Watling, unpublished results) leaves experienced high temperatures during sunflecks. These temperatures can inhibit photosynthesis, and the inhibition is stronger under sunflecks than under uniform saturating irradiance. Therefore, long sunflecks, or short sunflecks at high frequency, may instead lead to reduced carbon gain, by increasing leaf temperatures. Large reductions in carbon gain during the periods of high PFD, when significant fractions of daily carbon are believed to be fixed, would have significant effects upon seedling carbon balance and therefore probably also on growth and survival (Chazdon 1988; Fetcher *et al.* 1994; Press *et al.* 1996).

The greater impact of high temperatures on photosynthesis during sunflecks, compared with uniform irradiance, also has implications for leaves in the forest canopy. Canopy leaves, at times, experience high-temperature inhibi-

tion of photosynthesis under dynamic irradiance regimes (Roden & Pearcy 1993; Singaas & Sharkey 1998). Inhibition of photosynthesis by short, high-temperature episodes may even be sufficiently deleterious for isoprene emission to have evolved as a counteractive mechanism (Sharkey & Yeh 2001).

The impact of temperature on photosynthesis in these conditions may not have been previously recognized because measurements of diurnal photosynthetic carbon gain by IRGA are likely to have over-estimated carbon gain as a result of leaf chambers buffering the temperatures naturally experienced by the leaf. However, caution should be applied to extrapolating the quantitative nature of the observed responses to field conditions. The magnitude of the inhibition of photosynthesis by high temperatures will be different when leaf temperature is highly dynamic in the field, as opposed to being controlled under laboratory conditions. In addition, it is not known how carbon gain during sunflecks of different patterns will be affected by: (1) the acclimation of photosynthesis to dynamic temperatures during sequences of sunflecks; and (2) the impact of high temperatures on photosynthesis during sunflecks providing excess PFD.

At the variety of sites where dynamic irradiance and high temperatures are experienced by leaves, the effects of high temperatures on photosynthesis, and therefore productivity, are likely to be greater than previously reported (reviewed in Berry & Björkman 1980). This is particularly significant if, as predicted, global climate change results in higher maximum temperatures and more hot days over land areas (IPCC 2001).

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