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Patterns of dynamic irradiance affect the photosynthetic capacity and growth of dipterocarp tree seedlings

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Abstract In the deeply shaded understorey of S.E. Asian rain forests the growth and survival of dipterocarp seedlings is limited by their ability to maintain a positive carbon balance. Photosynthesis during sunflecks is an important component of carbon gain in understorey plants. To test the sensitivity of photosynthesis and growth to variation in the pattern of dynamic irradiance, dipterocarp tree seedlings (*Shorea leprosula* and *Hopea nervosa*) were grown for 370 days under shaded forest light treatments of equal total daily photosynthetic photon flux density (~3.3 mol m^{-2} day^{-1}), but characterised by either long flecks (LF) or short flecks (SF). Seedling growth was more than 4-fold greater under LF, compared with SF, in both species. Variation in the relative growth rates (RGR) and light saturated rates of photosynthesis (A_{max}) were strongly positively correlated with the mean duration of sunflecks. Variation in RGR was strongly correlated with greater unit leaf rate growth, indicating that photosynthetic carbon gain per unit leaf area was greater under LF. The accumulation of starch in leaves over the diurnal period was 117% greater in both species under LF, compared with SF. Greater carbon gain in seedlings under LF is likely to have resulted from the combination of (1) greater A_{max} (*S. leprosula* 35%, *H. nervosa* 40%), (2) more efficient dynamic photosynthesis, and (3) greater incident photosynthetic quantum yield, compared with seedlings receiving the SF irradiance treatment. The pattern of dynamic irradiance received by seedlings may significantly impact their growth and survival to a previously unrecognised extent, with important consequences for regeneration processes and hence forest structure and composition.

Keywords Dynamic photosynthesis · Forest understorey · Acclimation · Seedling regeneration · Sunflecks

Introduction

Tree seedling growth and survival under the highly dynamic irradiance patterns in tropical rain forests are primarily determined by light limitation of carbon gain (Chazdon 1988; Fetcher et al. 1994; Press et al. 1996). Sunflecks contribute 10–90% of total daily photosynthetic photon flux density (PPFD) and, as a consequence, drive up to 65% of total daily photosynthesis (Pearcy 1983; Chazdon 1988; Pfitsch and Pearcy 1989). There is marked spatial heterogeneity in total daily PPFD, as well as the pattern (duration, frequency and intensity) of sunflecks, both within and between forest types (Chazdon 1988; Pearcy et al. 1994; Leakey 2002). However, very little is known about the consequences of variation in patterns of dynamic irradiance (defined here as variation in the pattern, or contribution to total daily PPFD, of sunflecks) for the growth of tree seedlings under field conditions, despite its potential importance to forest regeneration processes. Specifically, while plant growth and carbon gain have been correlated with sunfleck availability (Pearcy 1983; Pfitsch and Pearcy 1989), there have been few reports on the impact of patterns of dynamic irradiance that are independent of changes in total daily PPFD.

Under controlled environment conditions, at equal total daily PPFD, growth rates of *Alocasia macrorrhiza* were greatest under uniform irradiance, intermediate under short, high frequency flecks and lowest under long, low frequency flecks (Sims and Pearcy 1993). Growth rates of four Australian understorey plants responded in a species-specific manner to sunflecks versus uniform irradiance, of equal total daily PPFD (Watling et al. 1997). Within the comparable growth forms, species

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responses reflected the paradigm that faster growing, more competitive species utilise favourable growing conditions more productively than slow growing stress tolerators, while these patterns of resource utilisation efficiency are reversed under stressful conditions (Grime 2001).

The efficiency of sunfleck sequences in driving photosynthetic carbon gain varies with different patterns, as each leads to a unique combination of physiological limitations to dynamic photosynthesis (photosynthetic induction gain following increased irradiance, maintenance of induction state after reduced irradiance, and post-irradiance CO_2 metabolism; Pearcy et al. 1994). The efficiency of photosynthesis during sunflecks also varies between species, being typically higher in shade tolerant species (Ögren and Sundin 1996; Valladares et al. 1997).

Variation in the light environment can lead to plasticity in physiology (Strauss-Debenedetti and Bazzaz 1996; Valladares et al. 2000). However, significant differences in light saturated photosynthesis (A_{max}) have not been observed between higher plants that have developed under either different patterns of sunflecks, or sunflecks compared with uniform irradiance (Sims and Pearcy 1993; Watling et al. 1997; Leakey et al. 2002). Nonetheless, the potential for such responses exists, as growth under different patterns of irradiance altered (1) the photosynthetic capacity of algae (Flameling and Kromkamp 1997) and (2) the plasticity of A_{max} in response to variation in total PPFD in birch seedlings (Wayne and Bazzaz 1993).

Seedling competition affects which species later form the canopy and fulfil their reproductive potential. Therefore, if seedlings respond to differences in patterns of dynamic irradiance with changes in photosynthetic carbon gain and growth, it will have important consequences for forest composition and biodiversity (Whitmore 1984; Still 1996). Niche differentiation along axes of resource availability in the regeneration phase has been postulated to maintain high biodiversity in tropical rain forests (Watt 1947; Grubb 1977). Competitive exclusion of sympatric species may be prevented by specialisation of light utilisation, optimising performance to specific irradiance regimes within the heterogeneous light environment (Denslow 1987). Dipterocarp species form a continuum of relative shade tolerance, exhibiting variation in photosynthetic capacity and maximum growth rates (Burgess 1966). However, partitioning sites between species on the basis of total PPFD alone does not provide sufficient differentiation to prevent competitive exclusion with the species diversity that exists (Brown and Whitmore 1992; Zipperlen and Press 1996; examples in Press et al. 1996). If seedling growth responds to variation in dynamic irradiance patterns, the latter could be a further axis of resource availability along which species are differentiated.

The Dipterocarpaceae are the dominant climax trees of lowland rain forest in S.E. Asia and a major source of hardwood timber (Whitmore 1984). Approximately 500 species are found over wide geographical ranges and in diverse species assemblages (Symington 1943). As such they are the primary determinants of forest structure and function. Every 3–8 years the trees fruit gregariously, producing recalcitrant seeds that are dispersed close to the parent tree, leading to dense mixed species seedling banks.

This study aimed to test whether the photosynthesis and growth of dipterocarp tree seedlings can respond to variation in the patterns of dynamic irradiance reaching the forest floor. It reports the photosynthesis and growth of seedlings growing for 370 days under two dynamic irradiance treatments, of equal total PPFD, but characterised by either long flecks (LF; $\geq$10 min) or short flecks (SF; $\leq$10 min). The responses of *Hopea nervosa* King. were compared with those of the less shade tolerant species, *Shorea leprosula* Miq. The following hypotheses were tested: (1) irradiance patterns under LF, compared with SF, will favour more efficient photosynthesis and lead to greater assimilate production; (2) seedling growth will be greater under LF, compared with SF; and (3) the more shade tolerant species, *H. nervosa*, will have greater relative growth rates than *S. leprosula*, but be less responsive to differences in resource availability between irradiance treatments.

Materials and methods

Experimental design

Seedlings of two dipterocarp species varying in shade tolerance were grown under equal total daily PPFD, but with different patterns of dynamic irradiance. The irradiance treatments were characterised by either LF or SF.

The experiment was carried out in primary, lowland dipterocarp rain forest close to the Danum Valley Field Centre, Sabah, E. Malaysia, Borneo (4°58'N, 117°48'E). *S. leprosula* is a light hardwood with faster growth rates and lower shade tolerance than the medium-heavy hardwood, *H. nervosa* (Burgess 1966; Zipperlen and Press 1996). Seeds were collected from the forest, germinated in forest soil and maintained for 2 years in polythene pots in a forest nursery prior to planting (total daily PPFD ~9.0 mol m^{-2} day^{-1}).

Seedlings of similar height (±50 mm) were selected and planted at three pairs of forest sites. Each pair consisted of a closed-canopy site (total daily PPFD $\approx$3.3 mol m^{-2} day^{-1}) and a nearby site in a small gap (total daily PPFD $\approx$5.8 mol m^{-2} day^{-1}). These would form the SF and LF treatments, respectively. At each site, 20 individuals of each species were planted alternately in a chessboard-like matrix so that the species comparison was not compromised by any spatial heterogeneity of irradiance. Seedlings were spaced 0.65 m apart, preventing individuals from shading each other. Throughout the experiment competing natural vegetation was weeded from the experimental plots.

In the small gap sites, an enclosure was constructed with shade cloth forming the top and sides (width × length × height: 4.6×5.2×1.5 m). This reduced the direct PPFD incident upon the seedlings in the small gaps by 40% at all times, without restricting their growth. As a result, the seedlings received an unmodified temporal pattern of sunflecks (typical of a small gap), but a lower than usual total daily PPFD. Therefore, the total daily PPFD in the LF and SF irradiance treatments was equal ($\approx$3.3 mol m^{-2} day^{-1}), but sunfleck patterns varied with the different canopy structures above them. Over a growth period of 370 days leaf litter was removed from the shade structures twice weekly, insuring no unwanted shading.

To test for environmental variation between sites, diurnal courses of temperature and relative humidity were measured simultaneously in pairs of sites for 7 days, both at the beginning and end of the growth period (SKH2011 and Datahog2, Skye Instruments, UK). In addition, cores of the top 20 cm of soil were collected from the corners and centre of each seedling matrix. pH and total conductivity were measured using the method described by Rundel and Jarrell (1989).

Forest light environments

The light environment of each seedling was quantified by analysis of hemispherical photographs taken above each individual. The photographs were taken using a digital camera (Nikon Coolpix 990, Delta-T Devices, UK) with a 180° fish-eye lens fitted to self-levelling gimbals (SLM2, Delta-T Devices, UK) mounted on a monopod. A built-in compass allowed the camera to be aligned facing Magnetic North. Photographs were taken shortly after dawn, when the sky was overcast and the sun was not directly visible in the sky. Images were downloaded, converted into bitmap format and contrast was manually adjusted before analysis using Winphot-Hemiphot software (ver 5.0, ter Steege 1994). Monthly means of the daily patterns of sunshine hours were inputted to allow calibration of PPFD incident above the canopy. Images were re-aligned to True North using a magnetic declination of 0°0.33'W for Danum Valley (altitude 200 m, http://www.ngdc.noaa.gov/cgi-bin/seg/gmag/fldsnth1.pl).

To test for changes in canopy structure and light environment, hemispherical photographs were taken at the beginning and end of the growth period. No significant changes were observed (±5% = maximum variation in total daily PPFD). Final analysis used the photographs from the initial and final time points to calculate the following model output for the first and second halves of the growth period, respectively: (1) mean total daily direct- and diffuse-PPFD, (2) mean daily red to far-red (R:FR) ratio, and (3) diurnal traces of direct- and diffuse-PPFD on 12 evenly spaced days throughout the year, which together represented an even and full distribution of sun tracks. The consistent reduction of PPFD incident upon LF seedlings by the shade cloth was accounted for by reducing the model output for this treatment by 40%. As in previous studies in the dipterocarp forest (Zipperlen and Press 1996; Clearwater 1999), predictions of diurnal traces of PPFD were calibrated and closely matched to data recorded above four seedlings at each site, using PPFD quantum sensors and dataloggers (SKP 215 and Datahog, Skye Instruments, UK) for 2 days both at the beginning and end of the growth period. Predictions of R:FR ratio closely matched data measured above two seedlings at each site, using a R:FR ratio sensor (SKR 110, Skye Instruments, UK).

Diurnal traces of direct-PPFD were analysed to describe the frequency, duration and intensity of patterns of sunflecks. Sunflecks were defined as periods of direct-PPFD separated by diffuse-PPFD. The pattern of sunflecks was very variable between days, depending on the position of the suntracks relative to gaps in the canopy. Therefore, rather than calculate mean daily sunfleck patterns, all sunflecks over the 12 days were grouped and considered as a single population. Frequency relationships were then calculated for classes of sunfleck mean PPFD, maximum PPFD and duration on the basis of the number of sunflecks or the % total sunfleck (direct)-PPFD.

Growth analysis

Growth measurements were made on all individuals immediately before planting (June 2000) and at the end of the growth period (August 2001). Seedling height, branch lengths and leaf dimensions (length and breadth at the widest points of the blade) were recorded. The leaf areas of individual leaves from a subset of seedlings were also calculated from the relative weights of a paper template of each leaf and paper of known area. The relationship between leaf dimensions (length × breadth) and individual leaf area was determined for each species and used to convert leaf dimensions for each seedling into total leaf area (*S. leprosula*: leaf area =0.732×leaf dimensions, r^2=0.99. *H. nervosa*: leaf area =0.751×leaf dimensions, r^2=0.97). Specific leaf area [SLA, dry leaf mass (kg)/leaf area (m^2)] was measured on leaf discs from leaves used for photosynthesis measurements after oven drying for 7 days at 80°C. Relative growth analysis parameters and allometric relationships [relative growth rate (RGR), unit leaf rate (ULR), leaf area ratio (LAR)] were calculated, according to Hunt (1990) but with integrated, total stem and branch length used as an analogue for total biomass.

Photosynthetic gas exchange

All measurements of photosynthesis were made in the field between 0700 and 1200 hours on the same youngest, fully expanded and undamaged leaf of six randomly selected seedlings, per species, at each site. There were no confounding effects of circadian rhythm or midday stomatal closure over the period of data collection (data not shown). Measurements were conducted using an open system IRGA (LCA-4, ADC, Hoddesdon, UK) and a modified 6.25 cm^2 clamp-on leaf chamber (PLC-3, ADC, Hoddesdon, UK), with a light source described by Zipperlen and Press (1996).

Light response curves were constructed within 2 weeks of final biomass measurements and response variables estimated according to the methods of Zipperlen and Press (1996), except relative humidity was maintained at 65±2% and each light level in the stepwise decrease was maintained for 10 min.

Measurements of dynamic photosynthesis were made on the morning 2 days after those for steady state photosynthesis. $A_{\max}$ and the times to reach 50% and 90% of the maxima ($T_{50\%A\max}$ and $T_{90\%A\max}$) were estimated by fitting a sigmoidal curve, as described by Zipperlen and Press (1997). Rates of induction loss, described by induction state (IS%), were measured over shade periods of 10 min, also by the method of Zipperlen and Press (1997).

Leaf chlorophyll, nitrogen, phosphorus and carbohydrate determination

Leaf chlorophyll, nitrogen, phosphorus and carbohydrate contents of each seedling were assessed on the same leaf used for photosynthetic measurements. Chlorophyll content was calculated as the mean of four values measured with a hand held chlorophyll spectrophotometer (Minolta, UK). Meter readings were converted to mg chlorophyll per g leaf dry weight using species specific regression equations (*S. leprosula*: mg chl g^{-1}=7.7×meter reading−9.7, r^2=0.91; *H. nervosa*: mg chl g^{-1}=8.1×meter reading−5.6, r^2=0.91) These were generated from chlorophyll *a* and *b* concentrations determined in leaf discs collected 10 days before the destructive harvest, weighed and ground in 2 ml buffered 80% aqueous acetone (2.5 mM Na_3PO_4, pH 7.8). Chlorophyll concentrations were measured using the spectrophotometric method of Porra et al. (1989).

Leaf discs were taken immediately pre-dawn and 11 h into the photoperiod and immediately stored in 100% ethanol. Concentrations of soluble sugars and starch were determined as described in Scholes et al. (1994).

Foliar nitrogen concentrations were determined, after a micro-Kjeldahl digestion procedure, by a colorometric assay using a flow injection analysis system (Tecator 5042 Detector and 5012 Analyser, Tecator, UK) as described in Bungard et al. (1999).

Statistical analysis

Data from measurements of daily light environment, relative growth analysis, photosynthesis, leaf chlorophyll and nutrient content were analysed using two-way ANOVA and Tukey tests. Carbohydrate concentrations were analysed by three-way ANOVA and Tukey tests. The edaphic and microclimate conditions of forest sites in the irradiance treatments were compared with two-sample *t*-tests. Interactions between seedling photosynthesis, growth and light environment were assessed by testing the significance of Pearson correlation coefficients and fitting linear regression lines. All analyses were performed using Minitab (Minitab 12.0 software, Minitab, Pa., USA).

Results

Light environment

There was no significant difference in the mean total daily PPFD received by seedlings under the two irradiance treatments (Table 1). However, the dynamic nature of irradiance differed markedly between the LF and SF irradiance treatments (Table 1). Under LF, sunflecks contributed 45% of total daily PPFD and were received over 11.7% of the photoperiod. By contrast, under SF, sunflecks contributed a significantly greater fraction of total daily PPFD (61%), but over a smaller fraction of the day (6.1%). Within each irradiance treatment, the light environments of *S. leprosula* and *H. nervosa* seedlings were not significantly different in any respect. Representative diurnal plots of irradiance, measured at leaf level, under LF and SF are shown in Fig. 1.

Sunflecks were defined as periods of direct-PPFD separated by diffuse-PPFD. The pattern of sunflecks under LF and SF differed in duration and intensity. In the SF treatment more than 50% of sunflecks were 10 min or less in duration and all were less than 60 min long. In the LF treatment only 25% had duration shorter than 10 min, while 37% were longer than 60 min (Fig. 2A). The mean PPFD of sunflecks in LF and SF was greater than 200 μmol m^{-2} s^{-1} in 98% and 99% of sunflecks, respectively (Fig. 2B). However, the range of mean PPFD of sunflecks in the LF treatment (maximum PPFD =750 μmol m^{-2} s^{-1}) was considerably smaller compared with the SF treatment (maximum PPFD =1,700 μmol m^{-2} s^{-1}). The duration of shade periods (diffuse-PPFD) intervening sunflecks did not differ significantly between the two treatments (Fig. 2C).

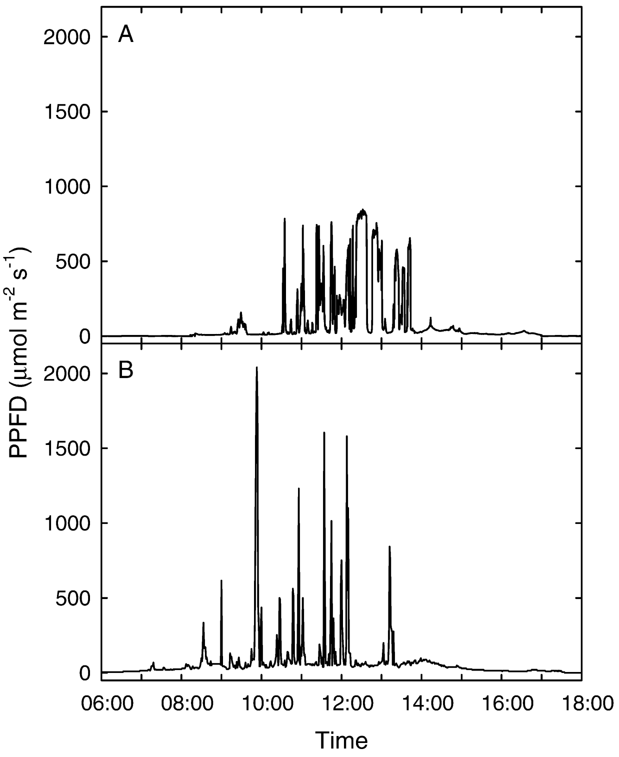


Fig. 1 Representative traces of diurnal PPFD incident on seedlings under (**A**) long fleck and (**B**) short fleck irradiance treatments

Site soil and microclimate

There were no significant differences between the irradiance treatments in site soil pH (mean pH=5.9; *T*=1.07, *df*=4, *P*=0.35) or soil solution conductivity (mean =21.3 μS cm^{-1}; *T*=0.0, *df*=3, *P*=1.00). Diurnal air temperatures were not significantly different between irradiance treatments, with respect to either mean (mean =26.6°C; *T*=2.49, *df*=2, *P*=0.13) or maximum values (mean =27.7°C; *T*=2.2, *df*=3, *P*=0.12). However, while there was no significant difference in mean diurnal relative humidity (mean =89%; *T*=3.41, *df*=2, *P*=0.076), minimum diurnal relative humidity was slightly lower in LF (72%) compared with SF (87%; *T*=22.0, *df*=3, *P*<0.001).

Table 1 Total daily PPFD, sunfleck PPFD, sunfleck duration and R:FR ratio of irradiance incident on *Hopea nervosa* and *Shorea leprosula* seedlings under long fleck (*LF*) or short fleck (*SF*) irradiance treatments. Values are means (±SE), *n*=3 based on three sites per irradiance treatment. Total daily PPFD and R:FR ratio calculated for 365 days of the year, sunfleck PPFD and sunfleck duration calculated for 12 days representative of an even and full distribution of sun tracks across the sky. Data were analysed by 2-way ANOVA; ****P*<0.001, *n.s.* not significant. Where statistical differences occur, means sharing a common letter do not differ significantly (Tukey Test *P*<0.05)

	LF		SF		Flecks	Species	Interaction
	H. nervosa	*S. leprosula*	*H. nervosa*	*S. leprosula*			
Total daily PPFD (mol m^{-2} day^{-1})	3.4±0.2	3.4±0.2	3.3±0.2	3.1±0.2	n.s.	n.s.	n.s.
Sunfleck PPFD (% Total daily PPFD)	45±1^{a}	44±1^{a}	61±2^{b}	61±2^{b}	***	n.s.	n.s.
Sunfleck duration (% photoperiod)	11.2±1.1^{b}	12.2±1.5^{b}	6.0±0.5^{a}	6.1±0.8^{a}	***	n.s.	n.s.
R:FR ratio	0.56±0.03^{b}	0.56±0.02^{b}	0.35±0.01^{a}	0.34±0.01^{a}	***	n.s.	n.s.

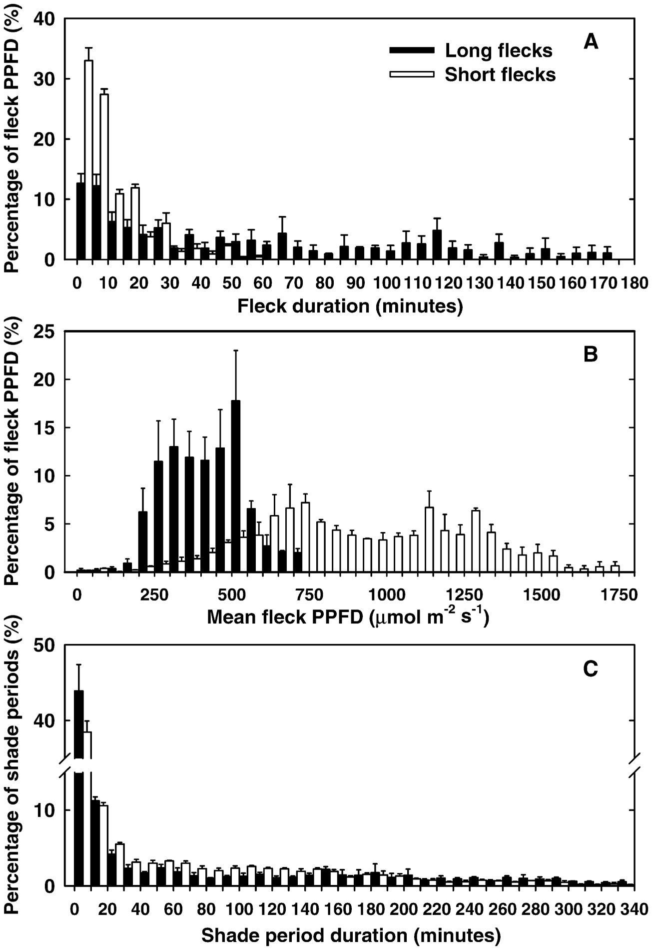


Fig. 2 Frequency histograms of (**A**) the fraction of total sunfleck PPFD contributed by sunflecks of different durations, (**B**) the fraction of total sunfleck PPFD contributed by sunflecks of different mean PPFD and (**C**) the number of shade periods of different durations, incident upon seedlings under short fleck (*filled bars*) or long fleck (*open bars*) irradiance treatments. Values are means (±SE), *n*=3

Growth analysis

Relative growth rates (RGR; total stem and branch length basis) were more than 4-fold greater under LF compared with SF, in both *H. nervosa* and *S. leprosula* (Fig. 3A). There were no significant differences in RGR between the species under either irradiance treatment.

ULR was the most responsive component of RGR to irradiance patterns, being significantly greater under LF, compared with SF, in both species (Fig. 3B). There were also significant light environment effects upon seedling aboveground partitioning (allometry). LAR was significantly greater in both species under LF compared with SF (Fig. 3C). By contrast, there was no difference in SLA between irradiance treatments in either species (Fig. 3D). In *S. leprosula*, the percentage of total stem and branch length contributed by branch length (% branches) was significantly greater under LF (33%) compared with SF (19%; Tukey test: *T*=3.45 *df*=1, *P*<0.05).

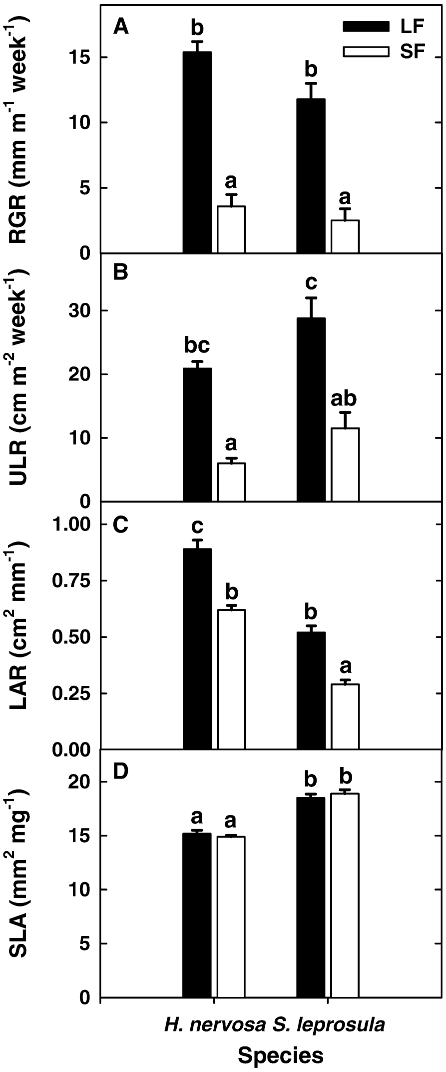


Fig. 3 (**A**) Relative growth rate (*RGR*; total stem and branch length basis), (**B**) unit leaf rate growth (*ULR*), (**C**) leaf area ratio (*LAR*) and (**D**) specific leaf area (*SLA*) of *Hopea nervosa* and *Shorea leprosula* seedlings under long fleck (*filled bars*) or short fleck (*open bars*) irradiance treatments. Values are mean (±SE), *n*=3. Within each graph, bars not sharing a common letter differ significantly (Tukey multiple comparison test *P*<0.05)

Comparing species, *H. nervosa* had significantly higher LAR compared with *S. leprosula* under both LF and SF. This was despite significantly lower SLA in *H. nervosa* under both irradiance treatments, but it was associated with significantly greater % branches in *H. nervosa* under SF (*H. nervosa* 36% versus *S. leprosula* 19%; Tukey test: *T*=4.16, *df*=1, *P*<0.05) and LF (*H. nervosa* 48% versus *S. leprosula* 33%; Tukey test: *T*=3.75, *df*=1, *P*<0.05). The ULR of *S. leprosula* was greater than *H. nervosa* under LF (+42%) and SF (+94%), although this was only significant as a main treatment effect (Fig. 3B, ANOVA: *F*=8.82, *df*=1, 8, *P*<0.05).

Photosynthesis

The photosynthetic capacity (A_{max}) of both species was significantly greater under LF, compared with SF (Fig. 4A). A_{max} of *S. leprosula* was greater than *H. nervosa* under LF (+15%) and SF (+20%), although this was only significant as a main treatment effect (ANOVA: F =6.20, df=1, 8, P<0.05). There were no significant differences in the maximum apparent quantum yield of CO_2 fixation, dark respiration or light compensation point between irradiance treatments in either species (data not shown). Also, there were no significant differences in the PPFD required for 90% of A_{max} ($PPFD_{90\%Amax}$) between either irradiance treatments or species (Table 2).

The times to 50% and 90% of photosynthetic induction ($T_{50\%Amax}$, $T_{90\%Amax}$) were not significantly different between irradiance treatments. Comparing species, the $T_{50\%Amax}$ and $T_{90\%Amax}$ were faster by 45% and 30%, respectively, in *H. nervosa* (Table 2). The rate of photosynthetic induction loss was affected by irradiance treatment only in *S. leprosula* (Table 2). This was measured as a significantly lower photosynthetic induction state after 10 min shade (IS%) in LF compared to SF.

Leaf chlorophyll content and nutrient composition

Irradiance treatment affected chlorophyll content only in *H. nervosa*, with concentrations on a leaf area basis being significantly greater under SF (Fig. 4B). In contrast, only *S. leprosula* responded to the irradiance treatments with changes in nutrient concentration per unit leaf area. Both leaf N and P were significantly greater in *S. leprosula* under LF, compared with SF (Fig. 4C, D). There was no main treatment effect of species on either chlorophyll, N or P content.

Leaf carbohydrates

There was significant accumulation of starch in leaves over the photoperiod under LF, but not SF, with starch content increasing by 1.54 mmol m^{-2} in *H. nervosa* and 1.48 mmol m^{-2} in *S. leprosula* between pre-dawn (AM) and 11 h into the photoperiod (PM) (Fig. 5). There were no significant differences in starch content between

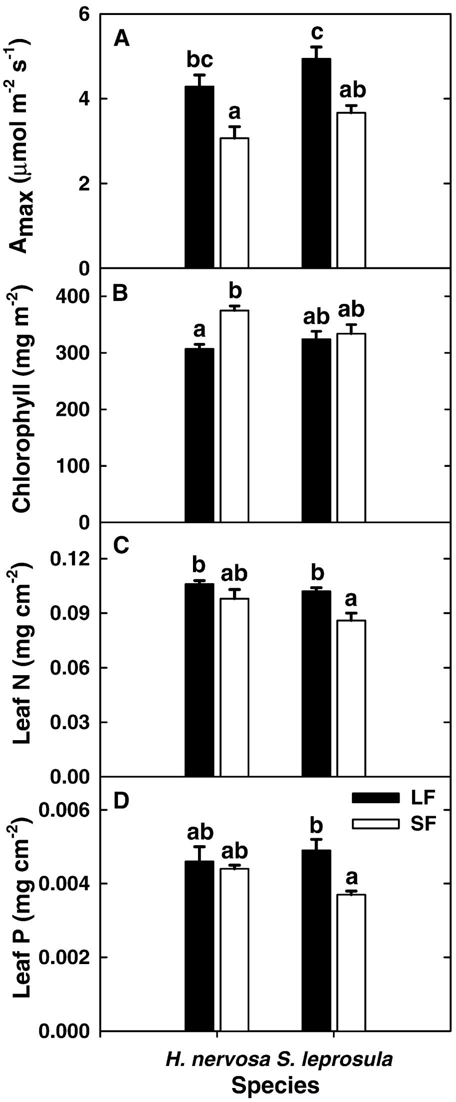


Fig. 4 (**A**) Light-saturated rate of photosynthesis (A_{max}), (**B**) leaf chlorophyll, (**C**) nitrogen and (**D**) phosphorus content of *H. nervosa* and *S. leprosula* seedlings under long fleck (*filled bars*) or short fleck (*open bars*) irradiance treatments. Values are means (±SE), n=3. Within each graph, bars not sharing a common letter differ significantly (Tukey multiple comparison test P<0.05)

Table 2 Time to 50% and 90% of A_{max} ($T_{50\%A}$ and $T_{90\%A}$), photosynthetic induction state after 10 min shade (%) and PPFD required for 90% of A_{max} (PPFD 90% A_{max}) of *H. nervosa* and *S. leprosula* seedlings under long fleck (*LF*) or short fleck (*SF*) irradiance treatments. Values are means (±SE), n=3 based on three sites per irradiance treatment. Measurements of all parameters were made on five seedlings per species, per site. Data were analysed by 2-way ANOVA; *P<0.05, ***P<0.001, *n.s.* not significant. Where statistical differences occur, means sharing a common letter do not differ significantly (Tukey Test P<0.05)

	LF		SF		Flecks	Species	Interaction
	H. nervosa	*S. leprosula*	*H. nervosa*	*S. leprosula*			
PPFD 90% A_{max}	207±33	242±32	200±16	182±22	n.s.	n.s.	n.s.
$T_{50\%\ A}$ (min)	7.1±0.3^{a}	11.6±1.0^{b}	6.3±0.2^{a}	12.6±1.3^{b}	n.s.	***	n.s.
$T_{90\%\ A}$ (min)	17.3±1.7	23.4±2.1	17.6±2.4	21.6 ±1.8	n.s.	*	n.s.
Photosynthetic induction state (%)	72±4^{b}	47±6^{a}	71±5^{b}	71±5^{b}	*	*	*

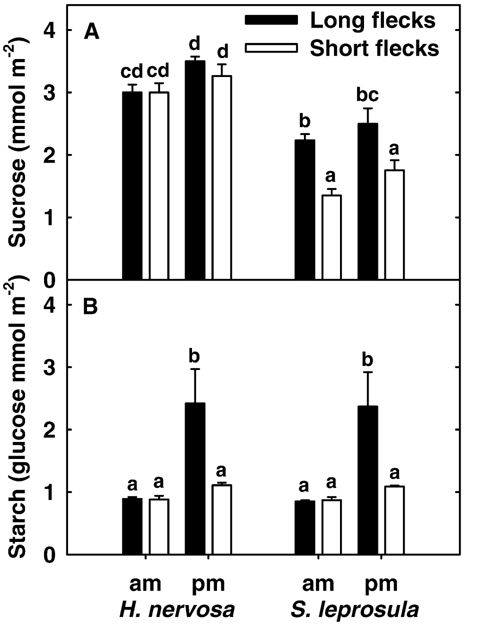


Fig. 5 Sucrose (**A**) and starch (**B**) content of the youngest fully mature leaf of *H. nervosa* and *S. leprosula* seedlings under long fleck (*filled bars*) or short fleck (*open bars*) irradiance treatments. Measurements were made either before the start of the photoperiod (AM) or 11 h into the photoperiod (*PM*). Values are means (±SE), *n*=3. Within each graph, bars not sharing a common letter differ significantly (Tukey multiple comparison test $P<0.05$)

species or between irradiance treatments in the pre-dawn starch concentration.

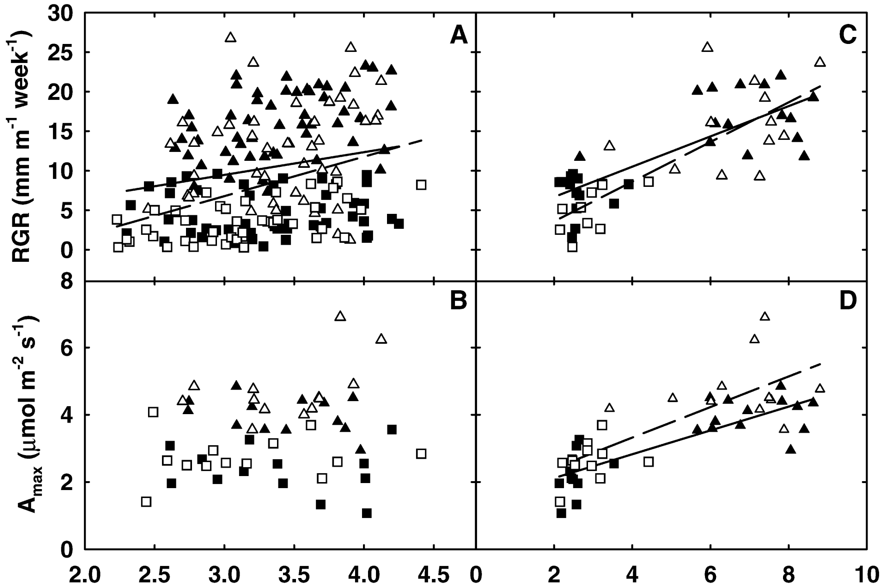
Sucrose concentrations did not significantly increase over the photoperiod in either species, under LF or SF (Fig. 5). However, sucrose concentrations were significantly higher at AM and PM, in *S. leprosula* under LF, compared with SF. By contrast, sucrose concentrations were not significantly different between irradiance treatments in *H. nervosa*. Concentrations of glucose and fructose were very low at all times and did not significantly accumulate over the photoperiod in either species, under LF or SF (data not shown).

Light, photosynthesis and growth interactions

Across the full range of light availability found within tropical rain forests, seedling RGR and $A_{\max}$ are both primarily determined by total daily PPFD (Chazdon 1988; Fetcher et al. 1994). However, across the narrow range of total daily PPFD at sites in this study, there was only a very weak relationship between seedling RGR and total daily PPFD (Fig. 6A) and variation in $A_{\max}$ was not significantly correlated with total daily PPFD in either *S. leprosula* or *H. nervosa* (Fig. 6B). By contrast, mean fleck duration was significantly, positively correlated with both RGR and $A_{\max}$ in *H. nervosa* and *S. leprosula* (Fig. 6C, D). However, sunfleck duration was also significantly, negatively correlated with the percentage of daily total PPFD contributed by sunflecks (r^2=0.777) and significantly, positively correlated with R:FR ratio (r^2=0.911) (data not shown).

Seedling RGR was weakly, positively associated with LAR (r^2=0.250), but strongly, positively associated with ULR (r^2=0.727). RGR was also positively correlated with $A_{\max}$ (r^2=0.461), suggesting that greater seedling growth under LF was primarily associated with changes in photosynthetic physiology.

Fig. 6 Scatterplots of (**A**) relative growth rate (*RGR*; total stem and branch length basis) in relation to total daily PPFD, (**B**) light-saturated photosynthesis ($A_{\max}$) in relation to total daily PPFD, (**C**) RGR (total stem and branch length basis) in relation to mean fleck duration, and (**D**) light-saturated photosynthesis ($A_{\max}$) in relation to mean fleck duration, in *H. nervosa* (*filled symbols*) and *S. leprosula* (*open symbols*) under long fleck (*triangles*) and short fleck (*squares*) irradiance treatments. Regression relationships are indicated for *H. nervosa* (*solid line*) and *S. leprosula* (*dashed line*)



Discussion

Growth analysis

Variation in the patterns of dynamic irradiance (defined here as differences in the pattern, or contribution to total daily PPFD, of sunflecks) resulted in large and significant differences in seedling growth rates, as hypothesised. In both species, RGR was more than 4-fold greater under LF than SF. This was the result of much greater ULRs (*H. nervosa* 248%, *S. leprosula* 150%) accompanied by smaller increases in LAR (*H. nervosa* 44%, *S. leprosula* 79%). RGR was more strongly correlated with ULR and A_{max}, than with LAR. This set of responses indicates that greater seedling growth rates were primarily related to changes in photosynthetic physiology, rather than allometry. This has also been observed in other shade tolerant, tropical forest species in response to variation in total PPFD (Popma and Bongers 1988; Osunkoya and Ash 1991) or patterns of sunflecks (Sims and Pearcy 1993; Watling et al. 1997). Nonetheless, changes in allometry are sometimes important components of seedling responses to variation in total daily PPFD (Popma and Bongers 1988; Osunkoya et al. 1994). However, in this experiment it is not possible to attribute the changes in LAR directly to variation in irradiance patterns. It is possible that, as in previous studies, changes in LAR may have had an ontogenetic basis (Sims and Pearcy 1993). In either case, greater LAR is significant because it interacts with the greater ULR (a stronger and direct response to patterns of dynamic irradiance) to compound increases in RGR under LF. The R:FR ratio was slightly greater under LF (0.56) than SF (0.34). However, this is unlikely to have played a major role in driving the seedling responses reported here as dipterocarps are generally unresponsive, even to much larger variation in R:FR ratio (Lee et al. 1996).

There were no species differences in RGR under either irradiance treatment. However, *H. nervosa* had greater assimilatory surface area relative to seedling size (high LAR), in contrast to the greater physiological capacity (high ULR) of *S. leprosula*. This difference is consistent with the relative success of the species in different light environments; *H. nervosa* achieving greater shade tolerance through allometric adaptation (Burgess 1966).

Photosynthetic plasticity

A_{max} in both species was significantly greater under LF, compared with SF. This photosynthetic plasticity in response to patterns of dynamic irradiance is an unexpected result (compared with: Sims and Pearcy 1993; Watling et al. 1997; Leakey et al. 2002). However, Yin and Johnson (2000) demonstrated the potential of higher plants to acclimate specifically to different sunfleck sequences. Only 7 days after transfer from low uniform irradiance, acclimation was generally greater under long, low frequency flecks compared to short, high frequency flecks. The responses of *S. leprosula* and *H. nervosa* to irradiance patterns are a noteworthy demonstration of the sensitivity of plants to their light environment, in a situation where photosynthetic carbon gain is important to competitive ability. Despite this significance, the overall plasticity of leaf physiology was subtle. There were no differences in SLA, such as occur with morphological adaptations to sun and shade conditions (Field and Mooney 1986). In addition, there were no significant differences in quantum yield, dark respiration, $PPFD_{90\% A max}$ or photosynthetic induction rate.

The extent of plasticity in photosynthesis to sunfleck patterns was similar in *H. nervosa* and *S. leprosula*, but as for growth, the basis of the responses differed between the species. Greater $A_{max,}$ in *S. leprosula* was associated with higher N and P content per unit leaf area, which may reflect greater protein content and sugar-phosphate photosynthetic intermediates, respectively (Evans 1989a; Fredeen et al. 1989). Greater nutrient acquisition and resource utilisation potential are characteristic of competitive species (Grime 2001). By contrast, higher A_{max} in *H. nervosa* was accompanied by an 18% reduction in the chlorophyll content per unit leaf area. Reductions in chlorophyll concentrations at these high levels may have relatively little impact on light absorption (Evans 1989b). This may have been a reallocation of resources within the photosynthetic machinery, which would be typical of a stress tolerator strategy (Grime 2001). Importantly, it is very unlikely that the differences in A_{max} resulted from photoinhibition under SF. Dipterocarps are very resilient to high PPFDs, even when exposed to much more extreme irradiance conditions than seedlings experienced under the SF treatment (Scholes et al. 1997). If photodamage had occurred under SF, reductions in chlorophyll content compared with LF would be expected (Bungard et al. 2000), but these were not observed in seedlings of either species.

Photosynthesis and diurnal carbohydrate accumulation

A_{max} was the only photosynthetic parameter measured that was significantly greater under LF (*H. nervosa* 40%, *S. leprosula* 35%), thus improvements in carbon gain due to physiological plasticity will have been restricted to periods of saturating light (sunflecks). Greater photosynthesis will have occurred during nearly all sunflecks because more than 98% of flecks had a mean PPFD greater than $PPFD_{90\% A max}$ (mean across species =207 μmol m^{-2} s^{-1}), in both irradiance treatments. The total duration of sunflecks across the photoperiod in LF (11.7% of the photoperiod), was approximately double that under SF (6.1% of the photoperiod). Even before accounting for differences in photosynthetic induction limitation, this suggests significantly greater carbon gain could occur under LF. Leaf gas exchange responses to sunflecks of different duration, intensity and frequency have been extensively studied. Generalisations hold true across most species (for review see Pearcy et al. 1994), so the relative efficiency of carbon gain is also likely to have been greater under LF, due to the lower limitation to photosynthetic induction of LF, compared with SF. These benefits to carbon gain will have been partially counteracted in *S. leprosula*, by the additional limitation to photosynthetic carbon gain of faster photosynthetic induction loss in the shade periods between flecks.

The primary difference in photosynthesis between the species was faster photosynthetic induction gain in *H. nervosa* than *S. leprosula*. This specialisation to photosynthesis during sunflecks, typical of shade tolerant species (Ögren and Sundin 1996; Valladares et al. 1997), will have favoured photosynthetic carbon gain and growth under both irradiance treatments. However, this will have been particularly the case under SF where sunflecks were short and, therefore, limitations due to induction gain were high.

The increase in foliar sucrose and starch concentrations across the photoperiod reflect the storage of photosynthetic assimilate in the leaf. As hypothesised, significant concentrations of starch accumulated between AM and PM measurements in *H. nervosa* (117%) and *S. leprosula* (117%), under LF. While assimilate will have been metabolised locally as well as transported out of the leaf, this was consistent with increased photosynthetic carbon gain during the first 11 h of the photoperiod. The significantly greater difference in starch accumulation than $A_{\max}$ (*H. nervosa* 40%, *S. leprosula* 35%), between LF and SF, supports the hypothesis that the efficiency of dynamic photosynthesis was also greater during the LF treatment. The lower mean PPFD of sunflecks and lower fraction of total daily PPFD contributed by sunflecks is also likely to have contributed to greater integrated photosynthesis as a result of higher incident photosynthetic quantum yield.

In summary, lower mean sunfleck PPFD and lower fraction of total daily PPFD contributed by sunflecks under LF will both have favoured increased carbon gain, but only as part of the set of changes dominated by greater $A_{\max}$ and longer fleck duration. Differences in seedling photosynthesis, diurnal carbohydrate accumulations and growth between LF and SF were consistent with each other and indicated that responses to irradiance regimes were primarily driven by changes in photosynthetic physiology.

Implications for seedling ecology

In the experimental treatments, variation in all components of the dynamic irradiance regimes were within the range of those reported for lowland dipterocarp and other tropical rain forest types (Chazdon 1988; Pearcy et al. 1994; Zipperlen 1997; Leakey 2002). Surprisingly, variation in patterns of irradiance altered *S. leprosula* seedling growth by a similar magnitude (167% on a height basis) to variation in total daily PPFD in previous studies. For example, RGR was greater by 200% for *S. leprosula* seedlings receiving total daily PPFD of 7.7 mol m^{-2} day^{-1} compared with 2.2 mol m^{-2} day^{-1} (Zipperlen and Press 1996). This suggests that the pattern of dynamic irradiance may have been overlooked as an important determinant of climax species performance under shaded conditions, which in addition to gap regeneration dynamics, is a key determinant of forest regeneration and the maintenance of high biodiversity (Lieberman et al. 1995; Whitmore and Brown 1996; Hubbell et al. 1999; Schnitzer and Carson 2001).

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