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Photosynthetic responses to dynamic light under field conditions in six tropical rainforest shrubs occuring along a light gradient

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Abstract We examined in the field the photosynthetic utilization of fluctuating light by six neotropical rainforest shrubs of the family Rubiaceae. They were growing in three different light environments: forest understory, small gaps, and clearings. Gas exchange techniques were used to analyse photosynthetic induction response, induction maintenance during low-light periods, and lightfleck (simulated sunfleck) use efficiency (LUE). Total daily photon flux density (PFD) reaching the plants during the wet season was 37 times higher in clearings than in the understory, with small gaps exhibiting intermediate values. Sunflecks were more frequent, but shorter and of lower intensity in the understory than in clearings. However, sunflecks contributed one-third of the daily PFD in the understory. Maximum rates of net photosynthesis, carboxylation capacity, electron transport, and maximum stomatal conductance were lower in understory species than in species growing in small gaps or clearings, while the reverse was true for the curvature factor of the light response of photosynthesis. No significant differences were found in the apparent quantum yield. The rise of net photosynthesis during induction after transfer from low to high light varied from a hyperbolic shape to a sigmoidal increase. Rates of photosynthetic induction exhibited a negative exponential relationship with stomatal conductance in the shade prior to the increase in PFD. Leaves of understory species showed the most rapid induction and remained induced longer once transferred to the shade than did leaves of medium- or high-light species. LUE decreased rapidly with increasing lightfleck duration and was affected by the induction state of the leaf. Fully induced leaves exhibited LUEs up to 300% for 1-s lightflecks, while LUE was below 100% for 1–80 s lightflecks in uninduced leaves. Both induced and uninduced leaves of understory species exhibited higher LUE than those of species growing in small gaps or clearings. However, most differences disappeared for lightflecks 10 s long or longer. Thus, understory species, which grew in a highly dynamic light environment, had better capacities for utilization of rapidly fluctuating light than species from habitats with higher light availability.

Key words Sunflecks · Photosynthetic induction · Shade plants · Variable light utilization · Stomatal conductance

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Introduction

Light is a very dynamic resource in forest ecosystems, both in the understory and in the outer parts of the canopy, due to a number of factors that range from the rotation of the earth to wind-induced movements of leaves (Pearcy 1990). In tropical forests, light availability affects plant succession and life-history strategies such as those of climax and pioneer species (Bazzaz and Pickett 1980). Both the mean and total daily photon flux densities (PFDs) in the understory of tropical rainforests are very low. However, long periods of low diffuse light are punctuated by bright sunflecks when a direct light beam passes through the openings in the forest canopy. Sunflecks are very short and rather unpredictable, but they can contribute up to 80% of the photosynthetically active PFD available for understory plants (Chazdon 1988). Photosynthetic utilization of sunflecks requires a quick, dynamic physiological response, which is dependent on several regulatory factors, each working at a different time scale and exhibiting remarkable variation among species and individuals grown under different light conditions (Pearcy 1990). These factors influence the following four main features of the photosynthetic response to fluctuating light: (1) photosynthetic induction response to a rise in the irradiance, (2) ability to maintain photosynthetic induction under low-light conditions,

which allows a plant to better exploit the next sunfleck, (3) stomatal response to light intensity, and (4) ability to extend the photosynthetic activity into the shade period immediately following a pulse of high light (post-illumination CO_2 fixation).

The dynamic aspects of photosynthetic responses to fluctuating light are little known for plants growing in the field. Most of our current understanding of photosynthetic use of fluctuating light comes from experiments with seedlings grown under controlled conditions, usually under constant light. In these experiments, shade-acclimated plants have been shown to exhibit higher lightfleck use efficiency (Chazdon and Pearcy 1986a,b; Küppers and Schneider 1993; Yanhong et al. 1994; Ögren and Sundin 1996). However, photosynthetic induction responses of plants of the same species grown in forest gaps or in the understory were indistinguishable in a rainforest field study in Panama (Kursar and Coley 1993). Different results regarding the influence of the light environment on the induction response were obtained for the pioneer species *Cecropia obtusifolia* and for the climax tree *Dypterix panamensis* (Poorter and Oberbauer 1993).

In this study, we examined the field photosynthetic response of six neotropical rainforest plant species to dynamic light. They were growing in three environments of differing light availability: forest understory, small forest gaps and trail edges, and forest edges and clearings. All six species were evergreen shrubs belonging to the family Rubiaceae, with four of the six from the genus *Psychotria*. Each light environment was studied in detail using both photosensors to record diurnal courses of light intensity at 1-s intervals, and hemispherical canopy photographs to obtain a general, integrated value of light availability. Despite some conflicting evidence for and against, we hypothesised that the more shady the habitat was, the more saplings depended on sunflecks to overcome low light limitations of growth and survival. We wanted to determine the effect of the light environment on the four previously enumerated factors that affect photosynthetic utilization of sunflecks.

Since stomatal limitations during fluctuating light might have been underestimated in previous studies due to overestimations of C_i during induction (Kirschbaum and Pearcy 1988), the possible role of stomata in regulating the use of dynamic light has been carefully examined here. Stomatal conductance data were absent or not thoroughly explored in previous field studies of utilization of dynamic light by neotropical rainforest plants (Kursar and Coley 1993; Poorter and Oberbauer 1993).

Materials and methods

Study site and species

All measurements were made on Barro Colorado Island (BCI, 9°9′N, 79°51′W), a field station in the Republic of Panamá of the Smithsonian Tropical Research Insitute. The forest on BCI is classified as a wet tropical forest (Croat 1978). Species used for the study were all shrubs within the family Rubiaceae, and included species characteristic of deep forest understory, forest gaps, and open sites. *Psychotria acuminata* Benth., *P. marginata* Sw., and *P. limonensis* Krause complete their full life cycle in the shade of the understory. *P. micrantha* H.B.K., and *Palicourea guianensis* Aubl. are typically found growing in light gaps in the forest or along trail edges. *Isertia haenkeana* DC. grows primarily in open sites, and the individuals used for this study grew around the margin of the laboratory clearing. For further information on the ecology of the species in this study, see Croat (1978), Wright et al. (1992), and Mulkey et al. (1993).

Most measurements of light environment and gas exchange were carried out during the wet season of 1995. The wet season on BCI typically lasts from April through December. However, gas exchange measurements on *P. micrantha* were conducted in January and February of 1996, months that were as wet as typical wet season months, even though they normally fall in the dry season.

Characterization of light environments

Light in each of the three different environments (deep forest understory, forest gaps, and open sites) was quantified using two separate methods. First, hemispherical canopy photographs were taken at the sites where the individuals in the study were growing. For each species, three individual plants were chosen as representative for the purposes of the canopy photos. The photos were subsequently analyzed using a computerized image analysis sytem and the software program CANOPY (Rich 1989), in order to calculate the proportion of light received annually at a given site as diffuse light (indirect site factor), and the proportion of light received as direct light (direct site factor). Calculations from each of the individual photographs were combined into means for each of the three light environments.

For a more complete characterization of each light environment, light dynamics within the three environments was studied from diurnal courses of light intensities recorded with dataloggers during the wet season. For each environment, small gallium arsenide photosensors (GaAsP model G1118, Hanamatsu, Japan) were mounted on wooden dowels and installed horizontally and pointing to the zenith. Photosynthetic photon flux density (PFD, μmol photons m^{-2} s^{-1}) values were then recorded for each sensor at 1-s intervals for 7 days, in the case of the open site, and for 15 days for the understory and gap sites. In total, eight sensors were used in the understory and open sites, and four sensors were used in the gap site. Sensors were connected to a CR21X datalogger, and data was recorded using a CSM1 card storage module (Campbell Scientific, Inc., Logan, Utah, USA). Data from each site was then compiled and averaged, and descriptive characteristics of the three light environments such as total daily PFD, number and relative contribution of sunflecks (all increases in the background understory PFD above 50 μmol photons m^{-2} s^{-1} were considered to be sunflecks), mean sunfleck length and other parameters were calculated with the program HISTO (R.W. Pearcy, unpublished work).

Gas exchange measurements

Measurements of dynamic gas exchange responses were made using a custom-built, transportable system optimized for resolving transient photosynthetic responses in the field. CO_2 and H_2O concentrations in reference and analysis lines were measured with a LI-6262 infrared gas analyzer (IRGA; LICOR, Lincoln, Neb., USA). A low-volume, one-sided chamber, combined with high flow rates through the system allowed measurements of rapid CO_2 and H_2O transients during induction and lightfleck responses. Flow rates through the chamber were maintained at approximately 4 l min^{-1}, except for the experiments on *P. micrantha*, which exhibited rapid stomatal closure when placed in the chamber under such conditions. To avoid

this artifact, and still include *P. micrantha* in the study, gas exchange measurements for this species were performed at a flow rate of approximately 1 l min^{-1}; thus allowing sufficient time response for the system, but not causing experimentally induced stomatal closure. The relatively large leaf surface area enclosed in the chamber (22.4 cm^2) provided for a high signal-to-noise ratio at the low photosynthetic rates commonly measured in the understory. Chamber temperatures were controlled with a Peltier unit, together with a water jacketed chamber and a continuously re-circulating buffer volume of water. Light was provided to the leaves with a 21-V, 150-W quartz-halogen slide projector lamp (General Electric Multimirror model ELD/EJN), using neutral density filters to adjust the PFD. An electro-mechanical shutter (Uniblitz Model 225L, Vincent Associates, Rochester, N.Y., USA) coupled with a darkroom timer allowed us to accurately administer lightflecks as short as 1 s. Signals from the IRGA, the mass flowmeter, the chamber PFD sensor, and the chamber thermocouples were logged at 1-s intervals by a CR21X datalogger, which was connected to a laptop computer. This arrangement allowed real-time calculation of gas exchange parameters.

Leaves to be used for dynamic gas exchange measurements were selected on the evening prior to the measurements, and shaded with an opaque umbrella to prevent photosynthetic induction. Measurements were made between 6 a.m. and 1 p.m. Due to the relatively bulky nature of the gas exchange system, it could not be readily moved from plant to plant. This necessitated working with detached shoots that could be transported to the equipment. A branch with the desired leaf was cut from the plant, and the cut end immediately re-cut under water to remove xylem embolisms. The shoot was then carried to the tent for subsequent measurements, with care taken to shade the leaf during transport. In September 1996, a commercially-available portable gas exchange system (model LI-6400, LiCor) was used to repeat induction measurements for each of the species in order to confirm that the cutting procedure did not introduce any artifacts into the measurements of gas exchange parameters.

For induction response experiments, the leaf was sealed in the cuvette, and after reaching steady state gas exchange readings at low light (5–10 μmol photons m^{-2} s^{-1}), data collection began at 1-s intervals. 30–60 s of shade readings were collected, and then the shutter was opened, beginning the induction measurements. For approximately the first 5 min, data was collected at 1-s intervals; after this point, the sampling time was changed to every 10 s. Data recording continued until several minutes after a steady state maximum net photosynthetic rate (A_{max}) was reached. Stomatal conductance was recorded simultaneously and initial and maximal stomatal conductance ($g_{initial}$ and g_{max} respectively) obtained in these induction experiments were used in statistical comparisons of species. Two parameters of induction, which have been previously described (Chazdon and Pearcy 1986a; Yanhong et al. 1994), were calculated: time to 90% induction (which is the length of time taken to achieve 90% of A_{max}), and induction state at 60 s (which is the assimilation rate at 60 s into the induction response as a percentage of that leaf's A_{max}). For each species, 8–12 leaves (from 3–5 individual plants) were measured for induction responses.

Leaves to be used in measurements of induction loss rate were first pretreated by illuminating them with saturating light from 12-V halogen bulbs for approximately 45 min. These lamps provided 600–1700 μmol photons m^{-2} s^{-1} depending on the working distance, which was sufficient to fully induce leaves from the three different light environments studied. After the leaf was fully induced, it was placed in the chamber under saturating light, and A_{max} was measured. The leaf was then returned to low light (5–10 μmol photons m^{-2} s^{-1}) for 5, 10, 20, 40, or 60 min. After the shade period, the shutter was opened to again expose the leaf to saturating light. The assimilation rate 60 s after opening the shutter was recorded, and the induction state at 60 s was subsequently calculated as the ratio of the 60-s value to A_{max}. For each species, and at each value of shade time (5, 10, 20, 40, or 60 min), four or five leaves were subjected to this procedure; thus 20–25 leaves were required per species, and these were collected from three to five individual plants.

If the leaf was to be used for measurements of lightfleck use efficiency (LUE), it was sealed in the chamber under low light as described above, and data collection began when steady state readings were reached. While all gas exchange parameters were saved at 1-s intervals, a series of saturating lighflecks (simulated sunflecks) of increasing length were given to the leaf (1, 2, 5, 10, 20, 40, and 80 s), separated by shade periods of approximately 60–90 s. The leaf was then exposed to continuous saturating light until full induction (i.e., maximum assimilation rate) was reached, after which the shutter was closed for approximately 90 s, and the previously described lightfleck procedure was repeated on the photosynthetically induced leaf. Lightfleck use efficiency (LUE, Eq. 1) is calculated by integrating the CO_2 fixation that resulted from a lightfleck, and dividing this amount by the assimilation that would result if the leaf were to photosynthesize at its maximum rate for the duration of the sunfleck.

$$\text{LUE}=[\text{integrated assimilation due to lightfleck}/(A_{max}\times\text{length of lightfleck})]\times 100 \quad (1)$$

LUE compares actual carbon gain during the fleck to the carbon that would be gained by a leaf that showed an instantaneous response to illumination (Chazdon and Pearcy 1986b; Pearcy 1990). Sample size for each species was 8–12 leaves, which came from three to five individual plants.

Photosynthetic response to PFD and CO_2 was measured in the wet season of 1995 in all of the species in the study using a CIRAS-1 portable photosynthesis system (PP systems, UK), and again in September 1996 using the LI-6400 portable photosynthesis system. No consistent bias was seen when he measurements made with the two systems were compared, so all data were used in statistical analyses. In total, four to five light response curves and four to five CO_2 response curves were made for each species, with each curve representing a different leaf (and generally a different plant). Light response curves were fitted to a rectangular hyperbola according to the model in Thornley (1976). The following parameters were obtained from these fitted curves: rate of dark respiration, quantum yield for CO_2 assimilation, and curvature factor, the factor that determines the transition from light limitation to light saturation. Assuming the Farquhar-von Caemmerer-Berry model of C3 photosynthesis (Farquhar et al. 1980), we calculated the maximum rate of electron transport (V_{jmax}) and of carboxylation (V_{cmax}) from the CO_2 response curves.

Statistical analysis

Physiological parameters were determined from measured PFD or CO_2 photosynthetic response curves by a least-squares fit to corresponding models using the nonlinear regression (Marquardt-Levenberg algorithm) routines in SigmaPlot (Jandel Scientific, Calif., USA). The predictive value and the associated level of confidence of the regression function obtained were tested using SigmaStat (Jandel Scientific, Calif., USA). Species or light environment differences in the parameters studied were analyzed by one-way ANOVA using routines in SigmaStat. Data sets were tested for normality and equal variance and a log transformation was applied when significant discrepancies from normality were found. Multiple comparisons among species or light environments were carried out by Student-Newman-Keuls tests of paired comparisons.

Results

Light environments of the study species

The three light environments studied were very different in terms of both the daily total irradiance and the dynamic nature of the available light. The percentage of irradiance potentially received as direct sunlight in relation to

Table 1 Properties of the light environments during the wet season in the understory, small gaps and trail edges, and clearings and forest edges sites in which the study species occured. The values are means±SD for eight light sensors for 7–15 days. Light environments that do not share the same letter for a certain parameter were significantly different (ANOVA, $P<0.05$)

	Forest understory	Small gaps and trail edges	Clearings and forest edges
Direct site factor (%)	5.54± 3.1 a	8.0 ±2.4 b	32.21±11.02 b
Indirect site factor (%)	0.87±0.61 a	2.25±0.85 a	63.10±12.08 b
Daily integral of PFD (mol m^{-2})	0.33±0.17 a	4.30±2.27 b	12.08±5.20 c
Number of high PFD periods per day (>50 μmol m^{-2} s^{-1})	77.1 ±53.9 a	73.5 ±40.1 a	22.2 ±21.2 b
Percent of daily integral irradiance received during high PFD periods	33.2 ±14.0 a	86.1 ±8.5 b	93.9 ±8.3 b
Mean duration of high PFD periods (s)	11.1 ±6.1 a	229.6 ±275.2 b	2332 ±1983 c
Time between high PFD periods (% of cases >4 min)	26.4 ±23.5 a	19.7 ±14.5 a	34.2 ±25.8 a
Percent of high PFD periods with 50–100 μmol m^{-2} s^{-1} maxima	71.3 ±10.4 a	77.2 ±12.5 a	43.8 ±35.3 b
Percent of high PFD periods with >1500 μmol m^{-2} s^{-1} maxima	0.8 ±0.9 a	6.9 ±7.1 a	56.9 ±38.2 b

that available in the open as determined by canopy closure (DSF) was 6 times larger in the clearings and forest edges than in the understory (Table 1). The difference in diffuse light transmission to the plants as indicated by the ISF was 70 times larger in the clearings than in the understory. These differences translated to an average daily PFD reaching the plants during the wet season which was 37 times higher in the clearings than in the understory with the small gaps and trail edges having intermediate values (Table 1). Irradiance in the forest understory and in the small gaps was more dynamic than in the clearings, since a relatively large amount was derived from the large number of relatively brief sunflecks (Table 1). These sunflecks however had relatively low PFDs as compared to the period of direct PFD in the gap or the PFD at the open site. Despite their brevity and low maximum PFD, sunflecks contributed 33% of the total daily PFD in the understory (Table 1). Periods of high PFD in the gap and in the open contributed the vast majority of light available for photosynthesis. In all three environments, 20–30% of the shade periods were long enough (>4 min) for significant induction loss to occur. However, sunflecks were both shorter and of lower intensity in the understory than in the clearings, which explains the decreased relative contribution of sunflecks to the daily total PFD in the understory compared to the clearings. Nevertheless, sunflecks contributed one-third of the daily total PFD in the understory (Table 1). The time between sunflecks was similar in the different light environments studied. Around one-fourth of them were more than 4 min apart (Table 1).

Species differences in steady-state gas exchange parameters

The three understory species (*Psychotria marginata, P. limonensis* and *P. acuminata*) exhibited lower values for most of the steady-state gas exchange parameters studied than the species found in small gaps and trail edges (*Psychotria micrantha* and *Palicourea guianensis*) or in the clearings (*Isertia haenkeana*). Average A_{max} was 4 times larger and average dark respiration was almost 3 times larger in *I. haenkeana* than in the understory species (Table 2). Both initial and maximal stomatal conductances were *c*. 10 times larger in *I. haenkeana* than in the understory species. Differences in maximum rates of carboxylation and electron transport of *I. haenkeana* in comparison to the understory species were not as pronounced, but were still significant (Table 2). The quantum yield for CO_2 assimilation did not differ significantly among species, and the curvature factor was larger in the understory species than in the high light species. *Psychotria micrantha* exhibited values intermediate between the other species of small gaps, *Palicourea guianensis*, and the three understory species.

Time course of photosynthetic induction and stomatal effects

The photosynthetic response of the leaves to a sudden increase in PFD (from 5 to 600–1700 μmol m^{-2} s^{-1} depending on the species) showed an induction period of 20–40 min before steady-state photosynthetic rates (A_{max}) were reached. The time course of induction varied from hyperbolic in shape to a sigmoidal rise of net photosynthesis (Fig. 1a and d respectively). In the exponential response, photosynthesis quickly rose to 60–80% of A_{max} and the intercellular CO_2 concentration (C_i) exhibited a slight drop of less than 20% of the initial values (Fig. 1b). In the sigmoidal response, photosynthetic induction occured in two phases, an initial rapid phase followed by a slow, gradual rise to the steady-state rate. For leaves exhibiting a sigmoidal induction response, C_i dropped abruptly to values well

Table 2 Stomatal and photosynthetic parameters for the six species studied (for abbreviations see Materials and methods; *Dark R* dark respiration). Light environment of each species is indicated within parentheses. Values are averages of 6–12 leaves from different individuals ±SD. Species that do not share the same letter for a certain parameter were significantly different (ANOVA, $P<0.05$)

	Psychotria marginata (Understory)	*P. limonensis* (Understory)	*P. acuminata* (Understory)	*P. micrantha* (Gaps)	*Palicourea guianensis* (Gaps)	*Isertia haenkeana* (Clearings)
$g_{initial}$ (mmol H_2O m^{-2} s^{-1})	63.0 ±42.5 a	50.5 ±22.8 a	43.6 ±32.9 a	53.9 ±28.9 a	174.8 ±165.0 b	900.0 ±686.5 c
g_{max} (mmol H_2O m^{-2} s^{-1})	94.8 ±39.3 a	88.1 ±24.6 a	66.3 ±29.6 a	250.0 ±88.5 b	215.4 ±127.1 b	1018.3 ±642.5 c
A_{max} (μmol CO_2 m^{-2} s^{-1})	4.8 ±1.3 a	4.9 ±1.1 a	3.9 ±0.9 a	10.8 ±1.3 b	9.5 ±1.1 b	19.5 ±2.1 c
Dark R (μmol CO_2 m^{-2} s^{-1})	0.35 ±0.05 a	0.41 ±0.11 a	0.45 ±0.13 a	0.75 ±0.18 a, b	0.65±0.15 a	0.87 ±0.3 b
Quantum yield	0.057±0.023 a	0.048±0.008 a	0.055±0.011 a	0.049±0.010 a	0.061±0.022 a	0.066±0.035 a
Curvature factor	0.75 ±0.22 a	0.80 ±0.05 a	0.73 ±0.15 a	0.79 ±0.11 a	0.55±0.15 b	0.51 ±0.24 b
V_{jmax} (μmol CO_2 m^{-2} s^{-1})	97.2 ±35.9 a,c	57.9 ±5.4 b	65.6 ±8.8 b	150.1 ±22.0 c	86.9 ±28.2 c,a	300.2 ±51.6 d
V_{cmax} (μmol CO_2 m^{-2} s^{-1})	69.7 ±37.7 a	54.4 ±5.7 a	58.3 ±7.6 a	114.5 ±13.7 b	74.3 ±18.3 a,b	96.2 ±16.9 b

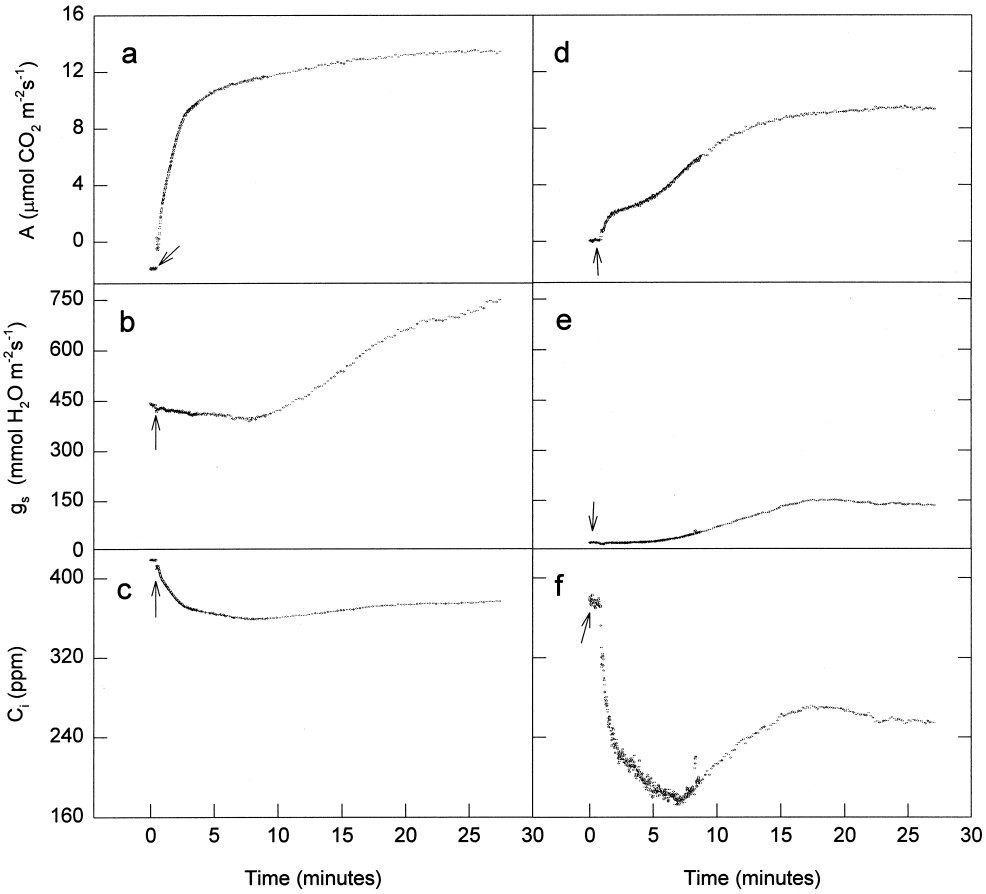

Fig. 1 a–f Time course of **a, d** net assimilation, **b, e** stomatal conductance, and **c, f** intercellular CO_2 concentration during photosynthetic induction of a leaf of *Isertia haenkeana* exhibiting a hyperbolic in shape type of induction (**a, b, c**) and a leaf of *Psychotria micrantha* exhibiting a sigmoidal type of induction (**d, e, f**). Parameters were recorded every 1 s during the first 8 min, and every 10 s afterwards. The *arrows* indicate when light was increased from low to saturating PFD

below 250 ppm, while C_i was never below 310 ppm in leaves exhibiting a hyperbolic in shape induction response (Fig. 1). When the steady-state photosynthetic response to C_i was plotted together with the dynamic photosynthetic response to C_i during induction, the two types of induction responses produced different plots. Sigmoidal responses exhibited two phases in the assimilation-C_i response (a sharp decrease in C_i as light activates enzymes before the stomata have fully opened, followed by a gradual increase in C_i as the stomata open), while hyperbolic induction responses exhibited a rather constant albeit gradual decline of C_i as the photosynthetic rate increased and then attained a steady state (Fig. 2).

The rise in stomatal conductance always lagged behind the increase in CO_2 assimilation. Exponential photosynthetic induction responses were usually associated with relatively high initial stomatal conductances ($g_{initial}$), while sigmoidal induction responses were more frequently found in leaves exhibiting very low $g_{initial}$. Actually, the speed of the induction process was affected by $g_{initial}$. We observed a significant exponential decrease in

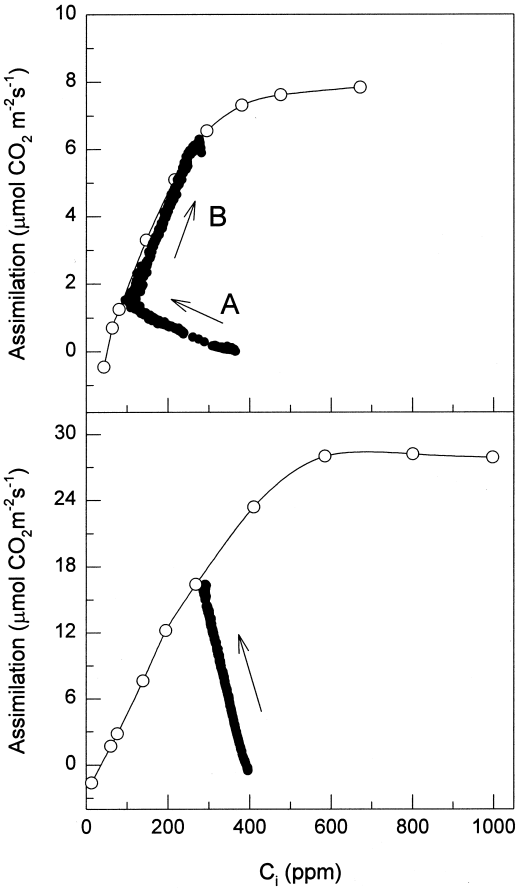

Fig. 2 Relationship between photosynthetic rate and intercellular CO_2 concentration measured every 1 s (every 10 s after the first 8 min) during photosynthetic induction at ambient CO_2 concentration (380–400 ppm, *solid circles*), and at steady-state at various CO_2 concentrations (*open symbols* and *curves*). *Arrows* indicate time evolution of induction. Data in the *upper graph* are from a *Psychotria micrantha* leaf exhibiting a sigmoidal type of induction with two phases in the evolution of C_i (an initial, abrupt decrease, *A*, followed by a more gradual increase, *B*). Data in the *lower graph* are from an *Isertia haenkeana* leaf exhibiting a type of induction hyperbolic in shape

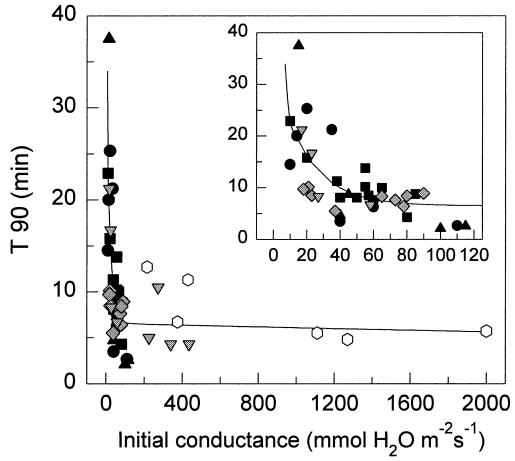

Fig. 3 Time required to reach 90% of A_{max} after exposure to saturating light as a function of the stomatal conductance of the leaf under low light prior to initiation of induction following an increase in PFD from low to saturating values. *Solid symbols* are for understory species, *grey symbols* are for small gap species and *open symbols* are for the species growing in clearings. The *fitted line* is given by the equation $y=27.5e^{(-0.0521x)}+6.56\ e^{(-0.0000767x)}$. The function fitted equally well all data points together and data points from the three light environments separately ($r^2=0.71$, $P<0.001$). The *inset* shows an expanded scale for initial stomatal conductance at low values (*filled triangles Psychotria marginata, filled circles P.acuminata, filled squares P. limonensis, grey diamonds P. micrantha, grey inverted triangles Palicourea guianensis, open hexagons Isertia haenkiana*)

the time required to reach 90% of A_{max} after transfer from low to high light (T 90) with increasing $g_{initial}$ (Fig. 3). There was no evidence of any differences between species from the three different environments in this dependence of induction time on $g_{initial}$.

Species differences in induction and induction loss

Understory species showed the most rapid induction, with 90% of A_{max} being reached within 4–8 min of exposure to saturating light. Induction state after 60 s exposure to saturating light (IS_{60}) was significantly higher in understory species (around 50%) than in the species found in clearings or small gaps (around 30%, Fig. 4). *Psychotria marginata* exhibited the fastest and *Isertia haenkeana* the slowest induction response.

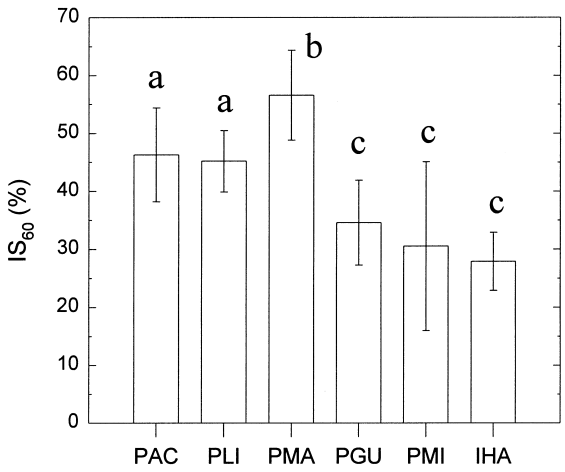

Fig. 4 Induction state after 60 s exposure to saturating light (IS_{60}) of leaves of the 6 species studied. Leaves were kept under low light for a minimum of 14 h. Abbreviations of species are *Psychotria marginata* PMA; *P. acuminata* PAC; *P. micrantha* PMI, *P. limonensis* PLI; *Palicourea guianensis* PGU; *Isertia haenkeana* IHA. Values are means ±SD of 8–12 leaves (from 3–5 individual plants). *Letter code* indicates groups that were significantly different (ANOVA, $P<0.05$)

The loss of induction in low light followed a negative exponential function in five of the six species studied. Only in *P. limonensis* was induction loss close to a linear function of time (Fig. 5). After 60 min in low light, leaves of all species dropped below 50% relative induc-

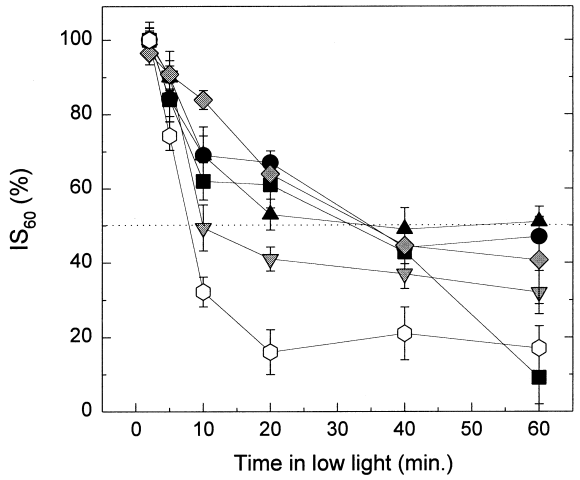

Fig. 5 The loss of induction state (IS_{60}) as a function of time in low light. The leaves were initially brought to full induction prior to shading. *Solid symbols* are for understory species, *grey symbols* are for small gap species and *open symbols* are for the species growing in clearings (symbols for species as in Fig. 3). Values are means ±SD of 8–12 leaves (from 3–5 individual plants)

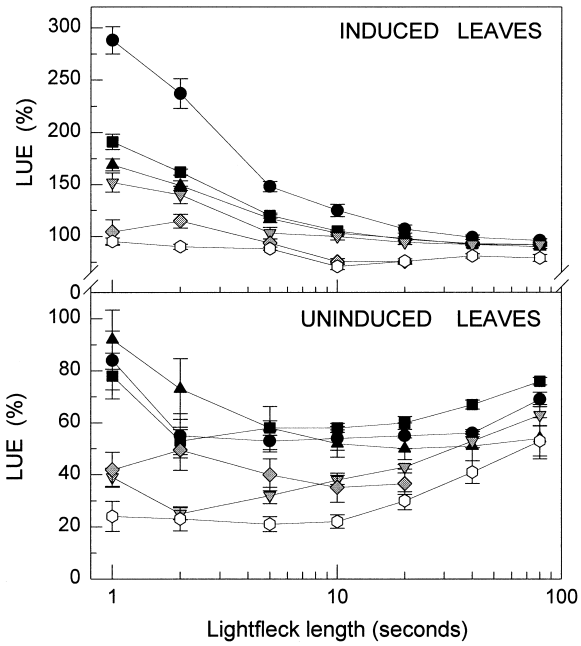

Fig. 6 Lightfleck use efficiency (LUE) as a function of lightfleck duration (logarithmic scale) of induced (*upper graph*) and uninduced leaves (*lower graph*) of the 6 species studied. *Solid symbols* are for understory species, *grey symbols* are for small gap species and *open symbols* are for the species growing in clearings (symbols for species as in Fig. 3).Values are means ±SD of 8–12 leaves (from 3–5 individual plants). Effects of species and of lightfleck duration on LUE were significant both for induced and uninduced leaves (two-way ANOVA, $P<0.001$)

tion. The rate of induction decay during the first 5 min was similar for all species, but after 10–20 min substantial species differences were evident (Fig. 5). A clear influence of the light environment was observed in the rate of induction loss. After 30 min in low light IS_{60} was above 50% in understory species but below 50% in high-light species, indicating that understory species lost induction more slowly when shaded. The species growing in clearings, *I. haenkeana*, showed the fastest induction loss.

Species differences in photosynthetic utilization of lightflecks

Although assimilation cannot respond instantaneously to a step increase in light intensity, rapidly fluctuating light can enhance the overall photosynthetic efficiency because CO_2 assimilation continues briefly into the low-light periods following a light pulse. A standard way of assessing the influence of the initial photosynthetic lag time combined with the post-illumination CO_2 fixation is by calculating the lightfleck use efficiency (LUE, see Materials and methods). LUE decreased rapidly with increasing lightfleck duration and was affected by the induction state of the leaf. Fully induced leaves exhibited LUE as high as 300% for 1-s lightflecks, while for uninduced leaves LUEs were always less than 100% for all lightfleck durations (Fig. 6). For short ligtflecks, the species-light environment affected LUE for short lightflecks significantly. Both induced and uninduced leaves of understory species exhibited higher LUE for 1–5 s lightflecks than did species growing in small gaps or clearings (Fig. 6). However, most differences disappeared for lightflecks 10 s long or longer.

Discussion

Photosynthetic induction occuring upon a sudden increase in PFD has two main components based on the time scales of the processes involved. The so-called fast-inducing component, important in the earliest phases of induction, is due mostly to an increase in ribulose-1,5-bisphosphate regeneration capacity and the buildup of Calvin-cycle metabolite levels (Sassenrath-Cole and Pearcy 1992). Then a slower increase in assimilation occurs because of light regulation of Rubisco and light-driven stomatal opening (Pearcy 1990). An unresolved issue has been the role of stomatal opening versus light activation of Rubisco as limitations during induction, since some studies have shown evidence for a role for stomata while others indicate that most of the limitation is biochemical. The relatively modest changes in the calculated C_i during induction have been taken to suggest little stomatal and hence mostly biochemical limitation. However, when stomatal conductances are low, failure to account for cuticular conductance can cause an underestimation of the role of stomata (Kirschbaum and Pearcy 1988). In this study, we found a significant correlation between the rate of induction and initial stomatal conductance in low light prior to the induction in all the species studied, regardless of native light environment (Fig. 3). This result,

which agrees with previous studies of other neotropical shrubs (Tinoco-Ojanguren and Pearcy 1993), points to an important role of stomata that deserves further investigation. The initial conductances were high enough that it is unlikely that including cuticular conductance would make any difference in the calculated C_i. However, the calculated C_i during induction could be overestimated if stomata open non-uniformly (Terashima et al. 1988), so patchy stomatal behavior during induction could mask the importance of stomatal conductance in controlling the use of fluctuating light. Recently, Eckstein et al. (1996) have reported that stomatal conductance can be quite patchy in low PFD. Moreover, increases in PFD initially increased patchiness before a more uniform stomatal conductance was achieved. The role of stomata in limiting the use of fluctuating light is likely to vary among species and with environmental conditions due to the observed variability in the light response of stomata and in the rate of stomatal opening in high light and closing after the leaf is again in the shade (see discussion in Pearcy and Sims 1994).

The time course of the induction response in the plants studied here varied from an increase in photosynthetic rate upon exposure to high light that was hyperbolic in shape to a sigmoidal increase of net photosynthesis (Figs. 1 and 2). These two types of response have been previously found in two rainforest species, *Alocasia macrorrhiza* and *Omalanthus novoguinensis* (Watling and Woodrow 1993). In that study, sigmoidal responses were more evident following very low PFD in shade whereas exponential (or hyperbolic) time courses occurred following higher shade PFD. They interpreted the shift as incomplete versus complete activation of Rubisco activase, which activates at very low PFD. Our results suggest a possible role of stomata in the transition from sigmoidal to hyperbolic induction responses, or, at least an effect on the slow phase of induction. This is consistent with results from simulations that show a similar dichotomy of sigmoidal versus hyperbolic shape with high versus low initial stomatal conductance (Pearcy et al. 1994). Unfortunately, no data regarding stomatal conductance were given by Watling and Woodrow (1993). In a field study of shade-tolerant neotropical plants, differences in the final phase of induction did not disappear at high CO_2 concentrations, which led to the suggestion that slow induction did not result from stomatal limitation (Kursar and Coley 1993). However, the absence of stomatal conductance measurements in this latter study limits the conclusions which can be drawn from it regarding the mechanisms affecting induction.

Shade-acclimated plants have been shown to exhibit higher LUE in a number of comparative studies (Chazdon and Pearcy 1986a,b; Küppers and Schneider 1993; Yanhong et al. 1994; Ögren and Sundin 1996). However, most of our current understanding of photosynthetic use of fluctuating light comes from experiments with seedlings grown under controlled conditions, where the light treatment usually resembled total PFD in natural sun and shade environments, but without sunflecks. Even though different constant PFD growth regimes can affect dynamic stomatal responses to light transients in certain plant species (Tinoco-Ojanguren and Pearcy 1992), a similar lightfleck use efficiency has been reported in plants grown under constant and fluctuating light regimes equal in total daily PFD (Sims and Pearcy 1993; Yanhong et al. 1994), suggesting that the results of the laboratory experiments on light acclimation and use of fluctuating light can be extrapolated to natural populations. In the present study, we have found an inverse relationship between total PFD available in the native growth environment and three characteristics that enhance the use of fluctuating light (rapid induction response, slow induction loss, and high LUE), confirming the expectations from laboratory experiments. Ögren and Sundin (1996) suggested that increased lightfleck use efficiency in low-light acclimated plants is due to the increased relative capacity for electron transport apparently involved in low-light acclimation (Ögren 1993). This suggestion is based on the fact that an overshoot of electron transport during a lightfleck leads to a build-up of metabolites that can be later used for post-illumination CO_2 fixation (Sharkey et al. 1986).

Differences in the three light environments studied (forest understory, small gaps, and clearings) included not only the total PFD available for the plants, but also the frequency, length and relative importance of sunflecks, illustrating the very dynamic nature of the forest understory light environment (Table 1). Characteristics of sunflecks depend on canopy attributes such as leaf area distribution and height of the canopy trees, and the forest understory of BCI seems particularly poor in sunflecks. We have measured an average of 77 sunflecks per day during the wet season on BCI, while values of more than 200 day^{-1} have been reported from a redwood forest understory (Pearcy and Pfitsch 1994), and of more than 300 day^{-1} have been observed in a lowland rainforest in Mexico (Chazdon and Pearcy 1991). Although the contribution of sunflecks to the total PFD in the forest understory on BCI was smaller than in the other two habitats (33% in comparison to 69% in the redwood forest and 52% in the Mexican rainforest), sunflecks contributed significantly to total daily PFD, and should be even more important during the dry season when cloud cover is reduced. As previously observed in understories of other well-developed forests, sunfleck length in the BCI understory was very short, lasting on average 11 s. When sunflecks are very short and their intensity is well below the full solar beam PFD due to penumbral effects, post-illumination CO_2 fixation makes only a small contribution to daily carbon gain and is at least partially offset by induction limitations (Pearcy and Pfitsch 1994).

Plant adaptation to a specific light environment can be viewed in terms of benefits (e.g., photosynthetic carbon gain) and costs of various traits, and adaptation is expected to result in a situation where the ratio of benefits to costs is maximized (Björkman 1981). In shade envi-

ronments, there is little return on an investment in increasing the capacity of the photosynthetic reactions, and resources are better invested in light harvesting. The reverse is true in high light environments where electron transport, carboxylation capacity, and stomatal conductance tend to be maximized (Pearcy and Sims 1994). These contrasting steady-state photosynthetic characteristics have been frequently observed in ecophysiological studies dealing with sun and shade plants (e.g., Evans et al. 1988). In this study, understory species were more efficient than species from medium- to high-light environments in photosynthetic utilization of fluctuating light. There are almost no data available to address the biochemical and physiological adaptations to the dynamic aspects of the light environment in terms of costs-benefits (Pearcy and Sims 1994). Why high-light species or sun-acclimated plants are not as efficient as their low-light counterparts in terms of photosynthetically utilizing variable light has not been fully explained. To keep the stomata open in the understory should cost little in terms of transpiration, and allow faster photosynthetic responses to unpredictable sunflecks. In the open, where humidities are often lower, the high transpirational costs of keeping the stomata open often force plants to coordinate stomatal conductance with PFD (Knapp and Smith 1990). This has been suggested as an explanation for differences in the stomatal components of the induction response and of the induction loss between sun and shade plants (Pearcy 1990). However, there is no hypothesis to explain some of the biochemical differences found in the ability to use fluctuating light between high- and low-light plants. The results of this study contribute to the increasing evidence for the existence of a trade-off between photosynthetic capacity and sunfleck utilization, and a complete understanding of this trade-off has not yet been reached.

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