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Stomatal behavior and photosynthetic performance under dynamic light regimes in a seasonally dry tropical rain forest

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Abstract Rates of photosynthetic induction upon exposure to high light and rates of induction loss after darkening the leaf were measured in the field for four species of tropical shrubs in the family Rubiaceae. During wet season mornings, stomatal conductance (g_s) in the shade prior to induction was generally high enough so that the time course of induction was determined primarily by rates of activation of biochemical processes. During wet season afternoons, however, g_s values in the shade tended to be considerably lower and photosynthetic induction following a light increase exhibited a slower time course. In the afternoon, the time course of induction was determined by a combination of stomatal opening time and the rates of activation of light regulated enzymes. Stomatal behavior was also correlated with patterns of induction loss following a transfer from high light to darkness. In the afternoon, maximum g_s was lower for all species, and for a given time in the darkness, leaves showed a greater loss of induction in the afternoon than in the morning. During the dry season, maximum g_s and average values for g_s in the shade were reduced in all species. Along with these shifts in stomatal behavior, reduced rates of photosynthetic induction were observed. In the high-light species, the lower maximum g_s values observed during the dry season were also correlated with increased induction loss for a given time in the darkness. For all species, stomatal behavior was affected by season and time of day and, with the exception of wet season mornings, stomata appeared to exert significant control over rates of induction and patterns of induction loss. The results of simulation modeling suggest that the observed seasonal and diurnal changes in rates of induction and induction loss can have significant consequences on sunfleck carbon gain under a dynamic light regime.

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Introduction

The understory of a tropical forest is one of the darkest environments habitable by vascular plants. In such a light-limited environment, the photosynthetic exploitation of brief high-light periods caused by sunflecks is critical for growth and reproduction (Pfitsch and Pearcy 1992). Previous work has shown that understory plants typically exhibit photosynthetic adaptation and acclimation that allows them to maximize carbon gain under such dynamic light regimes (Chazdon and Pearcy 1986a,b; Ogren and Sundin 1996; Valladares et al. 1997). Utilization efficiency of light transients is influenced by a number of dynamic physiological factors, including activation state of ribulose 1,6-bisphosphate carboxylase/oxygenase (Rubisco), current pool sizes of Calvin cycle intermediates, and degree of stomatal opening (Pearcy 1990). Most previous work on sunfleck utilization and induction of photosynthesis has been done in laboratory settings, and has provided a good understanding of the process of biochemical induction and subsequent relaxation once exposure to high light ends (Pearcy and Seemann 1990; Sassenrath-Cole and Pearcy 1994; Sassenrath-Cole et al. 1994; Sharkey et al. 1986; Woodrow and Mott 1989). Opinions regarding the importance of stomatal control over photosynthetic induction and utilization of transient light have varied, and some researchers have argued that stomata exercise little to no control over photosynthetic performance in dynamic light (Usuda and Edwards 1984; Walker 1981). However, a number of laboratory and field studies have revealed a potentially important role for stomata in controlling the utilization efficiency of dynamic light (Fay and Knapp 1993; Kirschbaum and Pearcy 1988; Knapp 1992; Ogren and Sundin 1996; Tinoco-Ojanguren and Pearcy 1993).

In this study, we examined the influence of stomatal behavior on the dynamic photosynthetic performance of four species, ranging from shaded-understory shrubs to open-site shrubs, native to a tropical moist forest that experiences a pronounced yearly dry season. The dry season represents an important resource in terms of available light. Cloud cover is reduced and the photon flux density (PFD) contributed by sunflecks increases by about 40–50% (R.W. Pearcy, unpublished work), which could potentially allow a substantial increase in carbon gain. The ability of understory plants to fully utilize this light will partially depend on how the dry season affects their stomatal behavior. We hypothesized that plants experiencing water stress may regulate stomatal behavior in a manner that minimizes water loss, even at the cost of reducing photosynthetic performance during sunflecks. Correspondingly, plants abundantly supplied with water during the wet season might be expected to exhibit stomatal behavior that maximizes carbon gain even at the possible expense of greater rates of water loss. Previous support for this hypothesis comes from A.K. Knapp and W.K. Smith, who worked in subalpine systems, and observed differences in dynamic stomatal response among different growth forms, and within herbaceous species subjected to different degrees of water stress (Knapp and Smith 1989, 1990). In an effort to quantify the potential impact of seasonal and diurnal changes in rates of induction and induction loss, total carbon gain under various light regimes was simulated using a previously described dynamic model (Pearcy et al. 1997).

Materials and methods

All measurements were made in the field at the Smithsonian Tropical Research Institute field station on Barro Colorado Island (BCI), Panamá (9°9'N, 79°51'W). The forest on BCI is classified as a tropical moist forest, with a pronounced dry season typically lasting from mid-December to April (Croat 1978). Mean annual rainfall is 2600 mm, with only 84 mm falling between January and March (Windsor 1990).

We selected for study four shrub species in the family Rubiaceae that represented a gradient of habitat preferences in terms of light availability. *Psychotria marginata* Sw., and *P. limonensis* Krause are highly shade-tolerant species capable of persisting and reproducing in the understory. *P. micrantha* H.B.K. is typically found in light gaps or forest edges, while *Isertia haenkeana* DC. is found primarily in open sites. The individuals of *I. haenkeana* used for this study grew around the margin of the laboratory clearing. For further information on the ecology of these species, see Croat (1978) or Mulkey et al. (1993).

A portable photosynthesis system (model LI-6400, LI-COR, Lincoln, Neb., USA) was used for all gas exchange measurements. This system was fitted with a custom leaf chamber that enclosed roughly twice the surface area of the stock chamber (13.2 cm^2), thus increasing the signal-to-noise ratio and improving the system's resolution for the low gas exchange rates of these understory plants. Air was supplied at a constant flow rate and CO_2 concentration was controlled by the mixer in the LI-COR system console. We set the CO_2 concentration entering the chamber to be 10–25 µbar bar^{-1} above the CO_2 concentration in the understory (typically 370 µbar bar^{-1}) so that when the leaf was fully induced under light saturation the chamber CO_2 concentration was close to 370 µmol mol^{-1}. Humidity in the chamber was adjusted manually with the bypass valve on the dehumidifier so that the chamber relative humidities were approximately 10% less than the ambient humidities in the understory. This translated to chamber humidities of 80–90% and 70–80% in the wet and dry season, respectively. We used the system's Peltier units to keep the chamber wall temperature at 2–3°C above air temperatures in the understory, which with the radiation load on the leaf resulted in 28–32°C leaf temperatures. Light was provided by a 12-V/21-W metal-halide arc lamp (Model MR16, Welch Allyn, Skaneateles Falls, N.Y., USA) whose beam was reflected onto the leaf via a cold mirror that removed wavelengths longer than 700 nm. The PFD at the leaf surface was adjusted to the desired level with neutral density filters.

Leaves used for measuring photosynthetic induction responses were kept covered by black cloth for at least 6 h prior to the measurements. For the high-light species *I. haenkeana* and *P. micrantha*, we further shaded the black cloth and leaves with an umbrella to prevent overheating. Leaves were kept darkened while they were sealed in the chamber, and once stable rates of transpiration and CO_2 assimilation (A) were observed, logging of the data at 2-s intervals was initiated. While the leaf was still darkened, initial values of A and stomatal conductance (g_s) were recorded for 20 s, after which the shutter was removed and the PFD increased to saturating levels (pre-determined from light curves made for each species). After 5 min of logging at 2-s intervals, the logging interval was increased to 10 s for the remainder of the time required to reach the maximum assimilation rate (A_{max}).

The time courses of A and g_s were used to calculate the induction state of the leaf 60 s after the light increase (IS_{60}) and the time required to reach 50% induction. The ratio of A to A_{max} at any time during induction, expressed as a percentage, is the induction state (IS) of the leaf. The induction state 60 s after a light increase is indicated by the abbreviation IS_{60}. Time to 50% induction is the length of time required to reach an IS of 50%. These two measures, used previously by Chazdon and Pearcy (1986a) and Tang et al. (1994), allow for comparisons among species or within species at different conditions.

Steady-state responses of assimilation to the intercellular CO_2 concentration (A/c_i curves) were measured in the morning after completion of the induction response using the automatic A/c_i program in the LI-COR 6400.

In order to measure rates of induction loss, leaves were first induced for 50–60 min *in situ* with light from 12-V, 50-W quartz-halogen projector lamps with built in cold mirror reflectors. We varied the distance between the lamp and the leaf to give saturating PFD at the surface, confirming the PFD with a quantum sensor. After induction, the chamber was clamped onto the leaf and the steady state values of A and g_s were recorded. These values were taken to be A_{max} and maximum g_s, respectively. The chamber was then removed and the leaf covered with black cloth for either 20 or 60 min. A few minutes prior to the end of the dark period, the cuvette was clamped onto the still darkened leaf, and data logging at 2-s intervals was initiated 15–20 s before the end of the dark period. At the end of the dark period, the PFD was increased to saturating levels, and data logging continued for approximately another 90 s. IS_{60} values were later calculated using A at 60 s into the time course.

Pre-dawn and mid-day leaf water potentials were determined using the pressure chamber technique on five to ten leaves from the same plants that were used for gas exchange. Water potential measurements were not possible with *I. haenkeana* because its leaves exuded clear phloem sap that obscured the end point.

Simulations of photosynthetic carbon gain were run using a previously described dynamic model (Pearcy et al. 1997), and light data collected in the forest on BCI. Briefly, the model requires 22 input parameters such as time constants for light activation/deactivation of enzymes and stomatal opening and closing, and parameters that determine the light and CO_2 dependence of photosynthesis. It runs with the input of a time series of PFD, and outputs simulated dynamic and steady-state photosynthesis. The steady-state output assumes that all dynamic elements in the model attain instantaneous equilibration with the light environment as it changes. Thus, if a steady state is attained in the dynamic simu-

lation (such as after a long period of constant light), its output will be identical to the output of the steady-state model.

Input for the simulations was obtained from PFD records from the 1995 dry season, recorded using gallium arsinide photosensors (GaAsP, model G1118, Hanamatsu, Japan) connected to dataloggers (model CR21X, Campbell Scientific, Logan, Uah, USA). Eight sensors mounted vertically on wooden stakes were connected to each datalogger and the outputs were logged at 1-s intervals. The dataloggers and sensors were moved every 3 days so that a wide range of microsites, including understory microsites where *P. marginata* and *P. limonensis* occurred, as well as nearby gaps, could be sampled. We selected records from five sensors and days representative of a range of sunfleck and gap light conditions for input to the model.

The model was parameterized for four conditions: the induction behavior of *P. marginata* in the wet season morning and the dry season afternoon, and the induction behavior of *P. micrantha* under the same conditions. The modeled response was made to undergo an induction response by subjecting it to a step change in PFD from darkness. The input parameters were then varied to find a parameter set that gave an induction response that matched those measured in the field, while also matching the observed induction losses at 20 and 60 min of darkness. We focused on getting good agreement between the observed and modeled induction and induction loss patterns for assimilation, and between the observed and modeled maximum assimilation rates at full induction. The values for dark respiration rate used in the model were means of the respiration rates measured for each species in the field.

Once parameterized, the model was used to simulate the total carbon gain expected under the five representative time courses of PFD for each of the two species, given the induction characteristics for the morning and afternoon of the wet and dry season, respectively. We separated the carbon gain into that occurring in the shade, and in response to sunflecks (PFD≥15 µmol photons m^{-2} s^{-1}), for both the dynamic and steady state simulations and then calculated a dynamic limitation L_{dyn} as:

$$L_{dyn} = 100 \times \frac{\sum A_{ss} - \sum A_{dyn}}{\sum A_{ss}}$$

where ΣA_{ss} and ΣA_{dyn} are the summed assimilation rates occurring during sunflecks for the steady-state and dynamic simulations at 1-s intervals in the time series. L_{dyn} thus provides a measure of the relative limitations imposed by the dynamic elements in the photosynthetic response under a given light regime.

Results

Representative time courses for photosynthetic induction in *P. limonensis* are shown in Fig. 1a–f. In wet-season mornings, the initial g_s measured just prior to the increase in PFD was generally high, and the time course of assimilation rate typically exhibited a hyperbolic rise (Fig. 1a open circles). In contrast, in wet season afternoons and in both dry season mornings and afternoons, initial g_s values were lower and assimilation generally followed a more sigmoidal pattern of increase (Fig. 1a closed circles and Fig. 1d, open and closed circles). These leaves with lower initial g_s and sigmoidal time courses of induction required a longer time for stomatal opening and for steady-state A to be achieved, reaching maximum values more slowly than when the same leaves were measured in the wet season mornings. Comparison between time of day and season shows that both the initial and maximum g_s were higher in wet season mornings than afternoons and that g_s was generally higher in the wet than in the dry season.

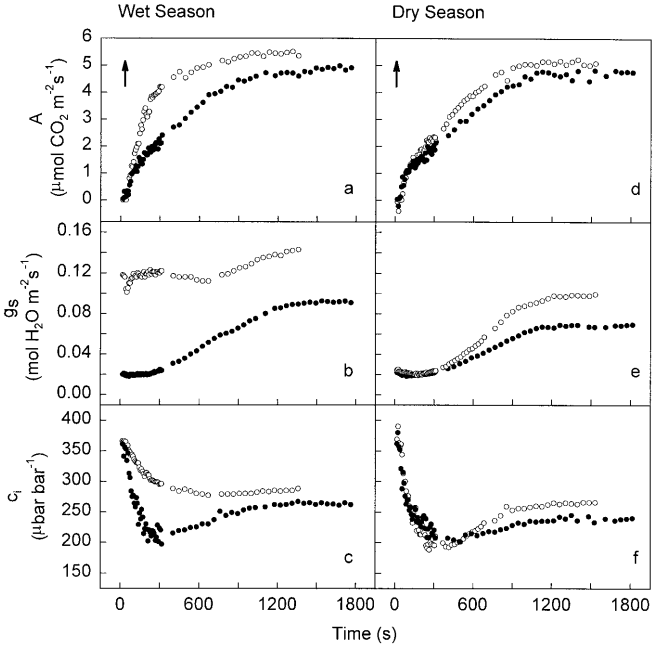

Fig. 1 Time course of **a,d** net assimilation, **b,e** stomatal conductance, and **c,f** intercellular CO_2 concentration during photosynthetic induction. Data are shown for two different leaves of *Psychotria limonensis*: **a-c** one measured in the wet season, **d-f** the other measured in the dry season. *Open circles* indicate morning measurements and *closed circles* are afternoon values for the same leaf. Data were recorded at 1 Hz for the first 5 min and at 0.1 Hz thereafter. *Arrows* indicate when illumination of the leaf began. For visual clarity, only every fourth data point collected is plotted

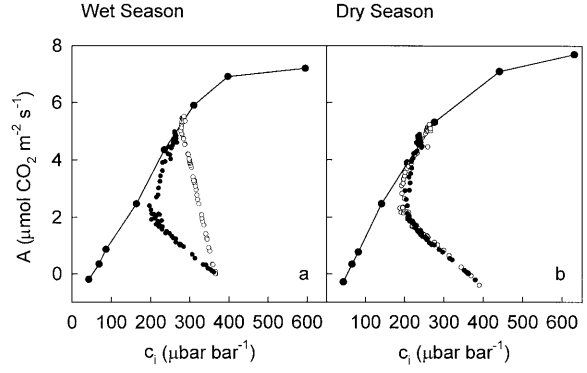

Fig. 2a-b Relationship between photosynthetic rate and intercellular CO_2 concentration during photosynthetic induction (*open circles*), plotted with steady state A/c_i curves (*closed circles* connected by *line*). Induction data presented in **a** are the data presented in Fig. 1a,c, while induction data in **b** correspond to data presented in Fig. 1d,e. *Open circles* indicate morning measurements and *smaller closed circles* (not connected by lines) are afternoon values for the same leaf. For the induction data, only every fourth data point has been plotted

In the cases where initial stomatal conductance was low, c_i reached a minimum value after roughly 5 min of high PFD, and then increased as g_s increased (Fig. 1c, closed circles, and Fig. 1f). During wet season mornings, c_i instead hyperbolically approached a minimum value that occurred at the end of induction (Fig. 1c, open cir-

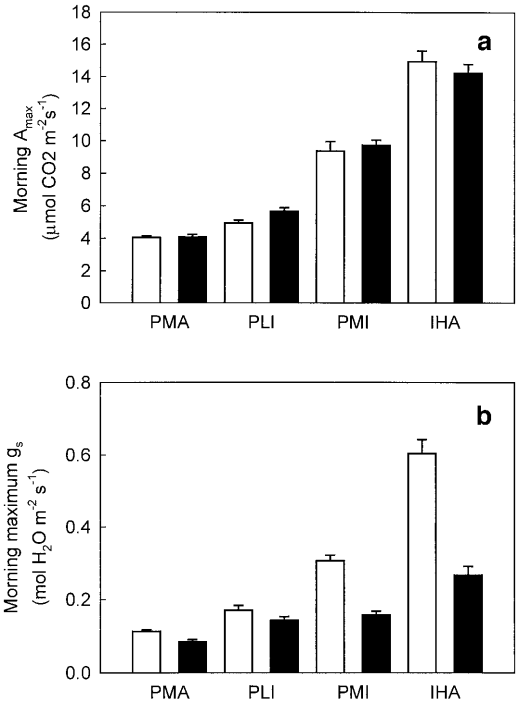

Fig. 3 Steady state values of **a** A_{max} and **b** maximum g_s for each species, in both wet and dry seasons. Each *bar* represents a mean of 8–12 leaves ±SE. *Open bars* represent wet season measurements, *closed bars* are dry season measurements. All values are morning maximums. Abbreviations of species: *PMA Psychotria marginata, PLI P. limonensis, PMI P. micrantha, IHA Isertia haenkeana*

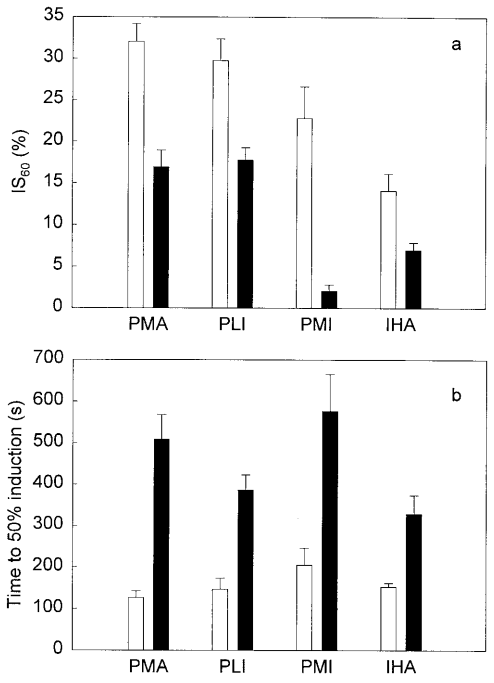

Fig. 4a,b Morning wet season and dry season rates of induction for all four species. **a** Induction state at 60 s and **b** time to 50% of A_{max}. Each *bar* represents a mean of 8–12 leaves ±SE. *Open bars* represent wet season measurements and *closed bars* show dry season values. Abbreviations of species: *PMA Psychotria marginata, PLI P. limonensis, PMI P. micrantha, IHA Isertia haenkeana*

cles). The consequences of the different magnitudes and dynamics of g_s to induction can be seen in plots of A and c_i during induction in relation to the steady state A/c_i curve (Fig. 2). In the wet season morning example, A_{max} was attained with only a small decrease in c_i and the trajectory of A versus c_i during induction intersects the A/c_i curve near A_{max} (Fig. 2a, open circles). In the other examples, when initial g_s was smaller, there was a larger decrease in c_i and the trajectory of A versus c_i approached the steady-state A/c_i curve at a lower point on the curve. Thereafter, the A versus c_i values during induction generally moved along the steady-state A/c_i curve (Fig. 2a, closed circles, Fig. 2b).

Maximum morning values for stomatal conductance were lower for all species in the dry season (Fig. 3b), but values of A_{max} were not significantly different (Fig. 3a). When wet and dry season A/c_i curves were compared, no systematic effect of season was seen (data not shown). Although season did not significantly affect A_{max}, it did have a strong effect on the rate of induction as indicated by the time to 50% induction. In all four species, rates of induction in the morning were significantly faster in the wet than the dry season (Fig. 4a,b). This difference was associated with the higher g_s values typically exhibited by leaves during wet season mornings. When all data were plotted together, however, induction time for a given species was seen to be unrelated to initial stomatal conductance if the initial conductance exceeded a threshold value. Below the threshold for each species, the time required for induction increased dramatically (Fig. 5). The g_s thresholds were higher in the high-light species than in understory species. There was no evidence that this threshold, or the effect of initial g_s values below this threshold, differed between morning and afternoon, or between the wet and dry season. Since these measurements were made early in the dry season, the plants experienced only modest decreases in either pre-dawn or midday water potentials (Table 1).

Table 1 Values of pre-dawn and midday leaf water potential for three *Psychotria* species during the early dry season of 1997. Values shown are means of 5–10 leaves ±SE

Species	Day/month	Water potential, MPa	
		Pre-dawn	Mid-day
P. marginata	8/1	–0.05±0.026	–0.31±0.111
P. limonensis	22/1	–0.05±0.017	–0.51±0.119
P. marginata	25/1	–0.12±0.041	–0.53±0.211
P. micrantha	2/2	–0.30±0.132	–1.36±0.019

Season and time of day had varying effects on induction loss, depending on the species (Fig. 6). Time of day had a stronger effect on induction loss than season, and in the case of the understory species, season had little effect on induction loss. Most of the effects of season or time of day on induction loss were seen in the form of lower maximum IS_{60} values in the afternoon than in the

morning. This downward offset in maximum IS_{60} was the direct result of lower maximum values for g_s and A in the afternoon. The rates of induction loss, as indicated by the slopes of the lines in Fig. 6a–d, differed much less with season or time of day. Stomatal conductance, expressed as a percentage of the maximum morning g_s, was affected by time in the darkness in much the same way as IS_{60} (Fig. 6e–h). As with IS_{60}, most of the effects of season or time of day on g_s were in the form of lower maximum g_s values in the afternoon than in the morning, and not as differences in the relative rates of g_s loss in the darkness. Among species, the rate of decrease of relative g_s in the darkness was greater in the high-light species, *P. micrantha* and especially *I. haenkeana*, than in the shade species. Since maximum g_s was considerably higher in the high-light than in the understory species, absolute rates of decrease in g_s for *P. micrantha* and *I. haenkeana* are even faster in comparison to those of *P. marginata* and *P. limonensis* than indicated by the relative values.

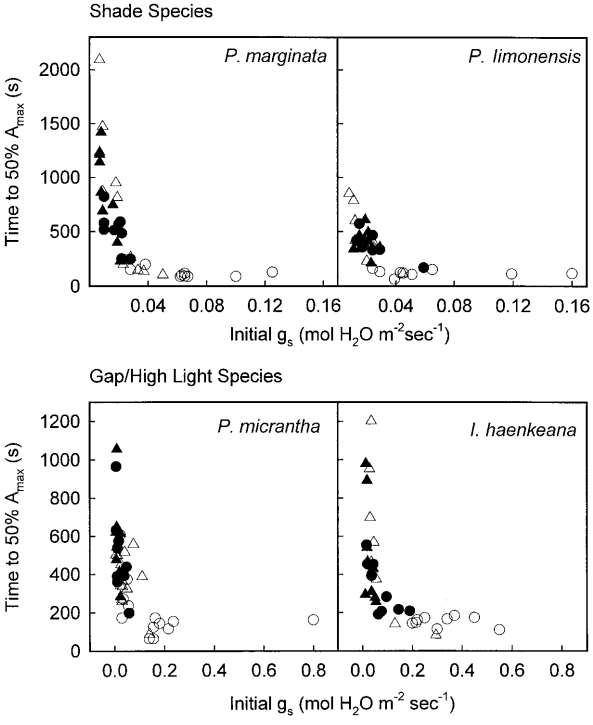

Fig. 5 Relationship between initial stomatal conductance (prior to the beginning of induction) and time to 50% of A_{max}. *Circles* represent measurements made in the morning, while *triangles* show afternoon measurements. *Open symbols* represent wet season values, and *closed symbols* are values from the dry season

The simulated induction and induction loss responses for each species and time period (either wet season morning, or dry season afternoon) were good approximations of the corresponding measured responses with respect to both differences between species and between wet season morning and dry season afternoon responses (Table 2). In the case of the induction responses, the model was fit to the data recorded for individual *P. marginata* or *P. micrantha* induction responses that were chosen as representative for the species and season/time of day. The induction loss responses were modeled to fit the mean responses shown in Fig. 6. This convention was adopted due to the protocol used to collect the field data. Specifically, since an individual leaf was not used for both the 20- and 60-min-darkness experiments, mean responses were used in the place of a characteristic individual leaf response when modeling photosynthetic induction loss.

The effects of seasonal and diurnal changes in rates of induction and induction loss on integrated carbon gain were dependent on the nature of the light environment used in the simulation. Not surprisingly, total carbon gain for a given species/season combination was linearly related to the total PFD over the course of the simulation. The total carbon gain values also revealed the effects of dark respiration rate and A_{max} on the simulations. In the lowest light regimes (time series A and B) *P. marginata* showed higher simulated total carbon gain than *P. micrantha*, due to *P. marginata*'s lower respiration rate. On the other hand, in the simulations with more total light (time series C and E), *P. micrantha* showed higher simulated total carbon gains than *P. marginata*, due to *P. micrantha*'s higher A_{max} (Table 3).

The degree to which carbon gain was limited by rates of induction and induction loss in a simulation was more closely related to the sunfleck frequency and

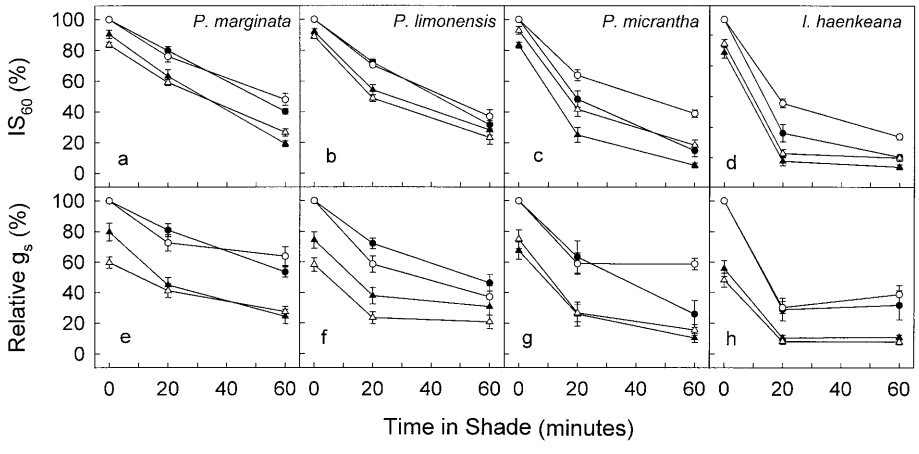

Fig. 6 a-d Rates of induction loss and **e-h** rates of stomatal closure following a transfer from saturating light to darkness. Symbols represent the mean of 8–12 leaves ±SE. *Circles* represent measurements made in the morning, while *triangles* show afternoon measurements. *Open symbols* represent wet season values, and *closed symbols* are values from the dry season

Table 2 Observed versus modeled induction and induction loss responses for *P. marginata* and *P. micrantha* during both wet season mornings and dry season afternoons

	P. marginata		*P. micrantha*	
	Observed	Modeled	Observed	Modeled
Wet season (morning)				
A_{max} (μmol CO_2 m^{-2} s^{-1})	4.19	4.15	12.3	12.5
IS_{60} (%)	36.5	38.4	12.4	22.9
Time to 50% A_{max} (s)	101	120	172	179
Time to 90% A_{max} (s)	890	837	542	665
IS_{60} after 20 min darkness (%)	76.2	65.2	63.8	59.0
IS_{60} after 60 min darkness (%)	48.0	49.9	39.0	36.7
Dry season (afternoon)				
A_{max} (μ mol CO_2 m^{-2} s^{-1})	4.7	4.6	10.7	10.6
IS_{60} (%)	14.5	14.9	7.8	8.3
Time to 50% A_{max} (s)	747	829	413	421
Time to 90% A_{max} (s)	2585	2401	2038	1917
IS_{60} after 20 min darkness (%)	59.2	56.6	25.0	27.1
IS_{60} after 60 min darkness (%)	26.6	31.7	5.3	8.62

Table 3 Characteristics of the sunfleck light regimes and modeled CO_2 uptake and the dynamic limitations to sunfleck utilization (L_{dyn}) for *P. marginata* and *P. micrantha* when the dynamic model was parameterized to optimize fit to either wet season morning or dry season afternoon induction responses

PFD time series	A	B	C	D	E
Total PFD (mol photons m^{-2})	0.23	0.49	0.95	0.92	6.02
Number of sunflecks	391	248	98	26	5
% Total PFD received as sunflecks (%)	56.3	73.6	88.7	94.9	99.9
Average sunfleck length (s)	5.6	13.6	36.2	586	3722
Total length of PFD time series (min)	274	417	240	331	331
P. marginata – with wet season morning parameters					
L_{dyn} (%)	26.1	24.4	8.2	1.3	0.2
Total modeled CO_2 uptake (mol CO_2)	4.17	7.16	8.40	18.96	49.40
P. marginata – with dry season afternoon parameters					
L_{dyn} (%)	39.1	40.8	26.1	4.8	1.0
Total modeled CO_2 uptake (mol CO_2)	3.91	6.56	7.93	19.14	55.35
P. micrantha – with wet season morning parameters					
L_{dyn} (%)	20.3	20.6	5.2	2.0	–0.1
Total modeled CO_2 uptake (mol CO_2)	0.03	2.10	11.18	17.43	83.88
P. micrantha – with dry season afternoon parameters					
L_{dyn} (%)	34.0	38.6	12.8	4.5	0.4
Total modeled CO_2 uptake (mol CO_2)	–0.35	0.66	8.99	16.34	76.17

duration in a particular time series than to total PFD (Table 3). When the available light was divided into many short sunflecks, the dynamic limitation to photosynthesis was increased over the cases where the sunflecks were fewer and longer. For example, time series C and D are similar in terms of total PFD, but different in terms of number and length of sunflecks. When comparing the dynamic limitation to photosynthesis (L_{dyn}) in these two cases, this limitation is consistently higher, regardless of season or species, for the simulations with time series C. Since the dynamic limitation to photosynthesis is greater for the simulations with many short sunflecks, the effects of season and time of day are also most pronounced in these cases (Table 3). The changes in rates of induction and induction loss between the morning of the wet season and the afternoon of the dry season had similar effects on the dynamic limitations to photosynthesis for *P. marginata* and *P. micrantha* Table 3).

Discussion

Time courses for assimilation during photosynthetic induction varied from a hyperbolic pattern when initial g_s was high, to a sigmoidal pattern when initial g_s was low (Fig. 1). These contrasting patterns have been noted previously by Watling and Woodrow (1993), but credited to the activation state of Rubisco prior to the beginning of induction. In the present study, it is unlikely that there were differences in Rubisco activation among leaves, since all leaves were kept darkened for at least 6 h prior to the beginning of measurements. Rather, the results indicate that the initial g_s at the beginning of the induction controls the shape of the induction response, a conclusion consistent with the results of Valladares et al. (1997) and simulations by Pearcy et al. (1994). Small differences in measured rates of induction and induction loss between this study and that of Valladares et al. (1997) can be attributed to the fact that leaves in the earlier

study were shaded, but not totally darkened, prior to induction or during induction loss experiments. The slightly different treatment used in the study of Valladares et al. (1997) resulted in consistently faster induction times and slower rates of induction loss, most likely due to maintenance of some photosynthetic induction by the low shade light.

In all four species studied, maximum rates of induction gain and minimum rates of induction loss occurred during the wet season mornings. In all cases, changes in rates of induction or patterns of induction loss between morning and afternoon, or between wet and dry seasons were correlated with changes in stomatal behavior. These results, combined with the results of Valladares et al. (1997) and Allen and Pearcy (1999), are consistent with the idea that differences in stomatal behavior are the primary factor generating the differences in patterns of induction and induction loss observed in this study.

During the wet season, high initial g_s in the morning resulted in fast induction times, since only the relatively rapid light activation of photosynthetic carbon reduction enzymes limited the rise to A_{max}. In the afternoon, however, lower initial g_s values were accompanied by slower induction times, where A_{max} was reached only after complete stomatal opening had occurred. These results are consistent with the results of Poorter and Oberbauer (1993), who measured induction responses in two rainforest tree species, and also found slower induction times in the afternoon. Also, Pfitsch and Pearcy (1989) observed considerably higher stomatal conductances in the morning than the afternoon for the redwood forest understory herb, *Adenocaulon bicolor*. During the dry season on BCI, initial g_s of leaves used for induction measurements were generally low both in the morning and afternoon, and induction times were subsequently increased. The only time when stomata were consistently and obviously non-limiting to induction was in the mornings during the wet season.

Time of day, and to a lesser degree season, also affected the ability of leaves to maintain induction status once removed from bright light. For all species, IS_{60} was lower at either 20 or 60 min of darkness in the afternoon than in the morning, and for *P. micrantha* and *I. haenkeana*, it was also lower in the dry as compared to the wet season. In all cases, time of day affected IS_{60} more than season. Stomatal closure closely covaried with induction loss, suggesting that the differences in IS_{60} between morning and afternoon (or seasons in the case of the high-light species) were due to differences in stomatal behavior, primarily seasonal and diurnal changes in maximum g_s. The transition to the dry season did not significantly affect induction loss in the two shade species but it did in the high-light species.

The shift in stomatal behavior observed between wet and dry season mornings is consistent with an increased priority on water conservation. As water supply becomes more limited, stomatal closure in the shade could conserve water during low light periods when assimilation rates are low and high values of g_s are not necessary. The cost of this water conservation is poorer utilization of sunflecks because of slower induction times and greater induction loss. Knapp and Smith (1990) observed a similar shift in the stomatal behavior of the subalpine herb *Helianthella quinquenervis* in response to periodic shade periods superimposed on a background of high light as seasonal water stress developed. The measurements reported here are from early in the dry season, and leaf water potentials were higher than those measured later in a severe dry season (Wright et al. 1992). Either these relatively small decreases in water potential, or some other environmental cue were apparently sufficient to trigger significant differences in stomatal behavior. Mulkey et al. (1991) measured leaf gas exchange of *P. limonensis* in irrigated and non-irrigated plots on BCI, and found that g_s values decreased significantly in non-irrigated plants as the dry season progressed, a trend that should impose even greater limitations on photosynthetic induction later in the dry season.

It is less clear why the stomata of understory plants exhibit such a dramatic afternoon closure in the wet season. Rainfall is an almost daily occurrence at this time of year, and the soil profile is saturated. Moreover, the vapor pressure deficit and leaf radiation loads are low in the shaded understory. Nevertheless, stomata were slower to open and maximum g_s values were lower during wet season afternoons, and photosynthetic performance during light transients was reduced. Time of day exerted as strong an influence on induction times as did season, and in the case of rates of induction loss, time of day had a stronger effect. Wet-season afternoons are often rainy and quite dark due to cloudiness, and under these circumstances, a reduction in the ability to utilize sunflecks would be less important than in the morning when skies are often clear. On the other hand, the transpirational costs of keeping the stomata open under these conditions should also be small, and might yield a significant improvement in carbon gain during any sunflecks that might occur.

It is clear that this daily cycle of stomatal opening and closing is not primarily regulated by light; stomata opened in the morning even under black cloth, and tended to close in the afternoon even if left exposed to ambient shade light (data not shown). Light availability also fails to explain the pattern of afternoon stomatal closure observed by Pfitsch and Pearcy (1989) in *Adenocaulon bicolor*, which grows in the redwood forest understory where the mornings are often more overcast than the afternoons. It is possible that the pattern is the result of a circadian rhythm, which have been shown to exert a significant influence on stomata in some species under controlled laboratory conditions (Gorton et al. 1989; Hennessey and Field 1991; Meidner and Willmer 1993). However, Williams and Gorton (1998) have recently reported that the effects of circadian rhythms on leaf gas exchange under field conditions in *Saururus cernuus* are insignificant. Alternatively, even modest decreases in leaf water potential may trigger stomatal closing in these species. Wright et al. (1992) observed up to a 0.5 MPa

depression of mid-day leaf water potentials in understory *Psychotria* shrubs during the wet season. Even at the very low transpiration rates characteristic of these understory shrubs, their hydraulic conductance is apparently low enough to cause the development of a significant water potential gradient between the leaf and the soil. Whether these significant, but still modest water potential gradients (compared with soil to leaf water potential gradients seen in species from higher light environments) are sufficient to result in stomatal closure is not known. Further work will be needed to understand the causal mechanisms resulting in the lower afternoon g_s.

In all four species included in this study, time of day and season each affected photosynthetic performance under dynamic light conditions, apparently through an effect on stomatal behavior. The simulation results suggest that the resulting diurnal and seasonal changes in dynamic photosynthetic behavior will significantly affect sunfleck utilization. The extent of this limitation will also depend on the nature of the sunfleck regime for the day and the microsite. During mostly clear days sunflecks have been shown to contribute 30–70% of the available PFD in forest understories (Chazdon 1988; Pearcy et al. 1994), and the forest on BCI is no exception to this general rule. Because the overall light availability in the understory is so low, induction limitations on sunfleck utilization could be expected to significantly impact carbon balance. In an experimental study, Sims and Pearcy (1993) found significantly reduced carbon gain and growth rates under a sunfleck regime that caused a greater induction limitation than under a sunfleck regime where induction limitations were minimized. On BCI, sunfleck availability is especially high in the dry season when clear skies prevail, but at this time the stomatal constraints on induction may limit their utilization, especially in the afternoon. Thus while the opportunity for carbon gain is high, induction limitations may constrain realized carbon gain and growth, a constraint that will likely increase as the dry season progresses. The stomatal limitations to sunfleck utilization will be reduced in the wet season, when the clearest skies and hence most sunflecks occur in the morning, but biochemical limitations will still be present.

The slightly higher L_{dyn} values for *P. marginata* as compared to *P. micrantha* may at first seem surprising, since the former is the understory species and could be expected to be more dependent on sunflecks for carbon gain. This difference results from the somewhat faster rates of induction gain and loss in *P. micrantha* than *P. marginata*. However, a comparison of these two species based only on the L_{dyn} values is misleading, since the actual carbon gain will also depend on the two species' steady-state photosynthetic characteristics. Indeed, the simulations show that in comparison to *P. marginata*, *P. micrantha* would have a somewhat greater higher carbon gain during to sunflecks due to its threefold greater A_{max}. However, this increased sunfleck carbon gain comes at a substantial respiratory cost, and therefore the total simulated carbon gain (including shade periods) for *P. micrantha* is actually lower than that of *P. marginata* in simulations with low total PFD (Table 3, time series A and B), or where the PFD time series contains very long low light periods (Table 3, time series C). Rapid stomatal responses to changing PFD in *P. micrantha* may function to increase water use efficiency in the gap environment where transpirational demands can be quite high.

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