Oecologia (2000) 122:479–486

Mitchell T. Allen · Robert W. Pearcy

Stomatal versus biochemical limitations to dynamic photosynthetic performance in four tropical rainforest shrub species

Received: 17 March 1999 / Accepted: 26 October 1999

Abstract Photosynthetic performance under dynamic light regimes was assessed in four different species of tropical shrubs from the family Rubiaceae via field gas exchange measurements conducted on Barro Colorado Island, Panamá. Rates of photosynthetic induction and induction loss were assessed throughout the day in both the wet and dry seasons in order to determine the relative roles of stomata and biochemistry in limiting photosynthetic performance under transient light conditions. A high degree of coordination was observed between stomatal conductance and biochemical capacity for CO_2 assimilation during induction. Rates of biochemical and overall photosynthetic induction sharply decreased when initial stomatal conductance fell below a narrow range of critical values. Time of day or season did not affect rates of biochemical deactivation upon shading, but did influence stomatal closure, which often exerted a significant influence over induction loss in the darkness. In measurements of total assimilation due to a 60-s light pulse, both biochemical activity and stomatal conductance were linearly related to total CO_2 uptake. Only during the mornings of the wet season was stomatal conductance consistently high enough to be non-limiting to dynamic photosynthetic performance. At all other times, stomatal behavior exercised significant influence over induction times, photosynthetic induction loss, and total CO_2 uptake from 60-s light pulses.

Key words Photosynthetic induction · Stomatal conductance · Limitation analysis · *Psychotria* spp. · Barro Colorado Island

Introduction

Photosynthetic responses to changing light intensity are complex, and involve the interaction of several processes operating at different time scales (Pearcy 1994). For plants living in forest understories, utilization of brief high light sunflecks has been shown to contribute a significant percentage of total carbon gain (Pfitsch and Pearcy 1989). Plants that are adapted and/or acclimated to understory life often exhibit an enhanced photosynthetic performance under variable light regimes (Ogren and Sundin 1996; Valladares et al. 1997). Several studies have focused on the regulation of key photosynthetic enzymes and biochemical pools during light transients (Pearcy et al. 1994; Sassenrath-Cole and Pearcy 1994; Woodrow and Mott 1989), while fewer have focused on the influence of stomatal behavior. Previous laboratory investigations have revealed that stomata can exercise significant influence over photosynthetic induction and lightfleck use efficiency (Kirschbaum and Pearcy 1988; Tinoco-Ojanguren and Pearcy 1992; Tinoco-Ojanguren and Pearcy 1993b), but little is known about the relative importance of biochemical versus stomatal limitations to transient photosynthesis under field conditions.

In this study, we measured photosynthetic performance during light transients in four species of tropical shrubs from the family Rubiaceae. Gas exchange measurements were conducted in the field throughout the day, during both the wet and dry seasons. For each species, we measured rates of photosynthetic induction and induction loss, as well as integrated CO_2 uptake in response to 60-s pulses of light. Data from the gas exchange measurements were then used to analyze photosynthetic limitations during induction, induction loss, and 60-s light pulses, and to partition these limitations between stomatal and biochemical components.

Materials and methods

Study site and species

All measurements were made on Barro Colorado Island (BCI), a Smithsonian Tropical Research Institute field station in the Republic of Panamá (9°9'N, 79°51'W). The forest on BCI is classified as a tropical moist forest, with a pronounced dry season typically

M.T. Allen (✉) · R.W. Pearcy
Section of Evolution and Ecology, University of California, Davis, CA 95616, USA
e-mail: mtallen@ucdavis.edu
Tel.: +1-530-7521080, Fax: +1-530-7521449

lasting from mid December to April (Croat 1978). Mean annual rainfall is 2600 mm, while median rainfall for the first 13 weeks of the year is only 84 mm (Windsor 1990). Wet season gas exchange measurements were made during October and November of 1996. Dry season measurements were made in January and February of 1997.

Species used for the study were all shrubs, family Rubiaceae, and included species characteristic of deeply shaded forest understory, forest gaps, and open sites. For further information on the ecology of the species in this study, see Croat (1978), or Mulkey et al. (1993). *Psychotria marginata* Sw., and *P. limonensis* Krause each completes its full life cycle in the shade of the understory. *P. micrantha* H.B.K. is 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. All measurements were made in the field on attached leaves.

Gas exchange measurements

All gas exchange measurements were made in the field with a LI-COR portable photosynthesis system (LI-6400, LI-COR, Lincoln, Neb., USA). In order to improve the signal-to-noise ratio of measurements made at the low photosynthetic and transpiration rates typical of understory plants, the standard leaf chamber was replaced with a custom-built chamber which enclosed roughly twice as much leaf surface area (13.2 cm^2). The system's onboard CO_2 mixer was used to keep CO_2 partial pressures in the chamber close to that of the surrounding air when the leaf was fully induced. For induction experiments, this meant that the CO_2 partial pressure in the chamber was 10–25 µbar bar^{-1} above the ambient level at the beginning of the induction. Relative humidity in the chamber was manually controlled and kept within approximately 10% of ambient humidity. For the wet season, this translated to chamber humidities between 80% and 90%, and for the dry season, between 70% and 80%. The built-in Peltier units provided partial temperature control, resulting in leaf temperatures that varied between approximately 28 and 32°C. Light was provided by a 12-V/21-W metal halide arc lamp light source (Welch Allyn MR16, Skaneateles Falls, N.Y., USA), and the photon flux density (PFD) was adjusted to the desired level using neutral density filters.

Leaves used for induction responses were kept covered by black cloth for at least 6 h prior to beginning the measurements. Leaves of *I. haenkeana* and *P. micrantha* were further shaded with umbrellas to prevent overheating. The leaves were kept darkened while being sealed in the chamber, and once stable rates of transpiration and CO_2 exchange were observed, automatic data recording was initiated at 2-s intervals. After approximately 20 s of darkness, a manual shutter was opened, and the PFD was increased to saturating levels (pre-determined from light curves made for each species). Data was logged at 2-s intervals for the first 5 min, and every 10 s until the maximum assimilation rate (A_{max}) was achieved. Two different measures of induction, previously described by Chazdon and Pearcy (1986) and Tang et al. (1994), were used to compare induction rates among species, seasons, and times of day. Induction state at 60 s (IS_{60}) is the assimilation rate 60 s after the increase in PFD, expressed as a percentage of a given leaf's A_{max}. Time to 50% induction is the length of time required to reach 50% of A_{max}. Time to 90% induction was not used since afternoon values of A_{max} were often less than 90% of morning A_{max}. The maximum value of A_{max} measured for a given leaf and day was used in calculating induction parameters; in almost all cases, this was the morning value. Steady state A/c_i curves were also measured in the morning for all leaves involved in induction measurements. After a leaf was fully induced, it remained in the chamber under saturating light for an additional 20–30 min while A/c_i data were collected using an automatic protocol native to the LI-6400.

Limitations to photosynthetic induction were partitioned into stomatal and biochemical components using the method of I.E. Woodrow and K.A. Mott (Tinoco-Ojanguren and Pearcy 1993b; Woodrow and Mott 1989). Briefly, assimilation rates (A) during induction were recalculated to a constant c_i equal to that at A_{max}. At any given time during induction, the relative difference between this recalculated assimilation rate (A') and A_{max}, or $(A_{max}–A')/A_{max}$, represents the limitation imposed by incomplete biochemical activation. Correspondingly, $(A'–A)/A_{max}$ is the limitation imposed by stomata.

Rates of induction loss were measured by first inducing leaves with saturating PFD from 12-V quartz halogen projector lamps for 50–60 min. After induction, the leaf was then sealed in the chamber under saturating PFD, and once a steady state was achieved, values for A_{max} and maximum g_s were recorded. The leaf was then removed from the chamber and covered with dark cloth for either 20 or 60 min. A few minutes before the end of this dark period, the leaf was again sealed in the cuvette while exposed to only very low PFD (<2 µmol m^{-2} s^{-1}). Automatic data recording at 2-s intervals was initiated 15–20 s before the end of the dark period, at which time the PFD was increased to saturation and data logging continued for 90 s. IS_{60} values were later calculated from the assimilation rate 60 s after the PFD was increased.

Photosynthetic responses to 60-s saturating lightflecks were investigated using the red LED light source for the LICOR 6400 (LICOR 6400–02). We relied on the short duration of these measurements to avoid any effects of the absence of blue light on the stomata. A leaf previously exposed to the natural PFD regime in the understory was sealed in the cuvette and kept in the dark for 15–20 s until steady rates of transpiration and CO_2 exchange were observed. Automatic data acquisition was then initiated at 2-s intervals, and a 60-s pulse of saturating light was given to the leaf. Data logging continued for another 45–60 s after the lightfleck ended in order to record any post illumination CO_2 fixation. For each species, 8–12 leaves were sampled at four different times throughout the day. These measurements were completed in the dry season (17–25 February 1997).

Results

Partitioning limitations to photosynthetic induction

Representative limitation analyses are shown in Fig. 1, which is drawn from two wet season induction time courses for a leaf of *P. marginata*. Figure 1a shows a typical relationship between A_{max}, A', and A, for a leaf with a high initial g_s and a morning induction that subsequently followed an approximately hyperbolic time course to A_{max}. In the afternoon, the same leaf began the induction with a lower g_s and followed a slower sigmoidal time course to reach A_{max}. Figure 1c,d shows the time course of biochemical (L_m) and stomatal (L_s) limitations calculated from the data in Fig. 1a,b. Both the stomatal and biochemical limitations decrease more slowly in the afternoon than in the morning, and never fully dissipate.

Limitation analyses such as that shown in Fig. 1 were performed on all wet and dry season measurements from all four species. In order to condense this information, the time required for the biochemical limitation to relax to 50% (time to L_m50) was chosen as an index of the overall rate of biochemical induction. A comparable value for stomatal limitation could not be obtained due to a lack of a consistent pattern in stomatal responses. Initial g_s, measured before induction commenced, was instead chosen as an index of potential stomatal limitation during induction. For both *I. haenkeana* (Fig. 2a) and *P.*

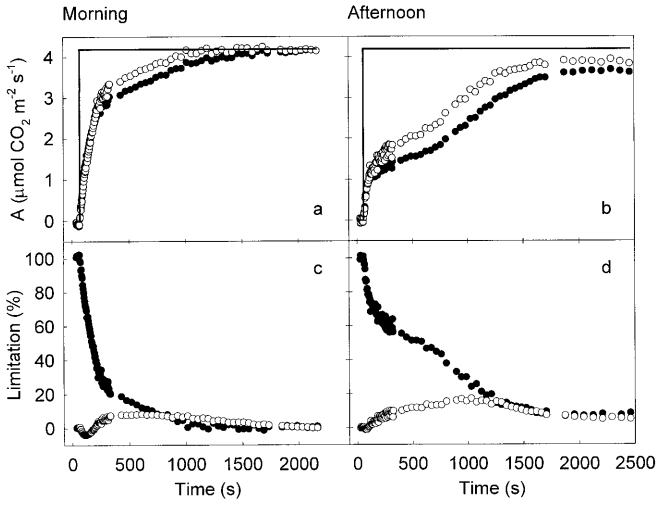

Fig. 1 Examples of **a,c** morning and **b,d** afternoon limitation analysis for a leaf of *Psychotria marginata*. Prior to measurement, leaves were kept completely darkened for at least 6 h. **a,b** Time courses for CO_2 assimilation rate (*closed circles*), assimilation rate corrected to the c_i calculated at A_{max} (*open circles*), and A_{max} (*solid lines*). **c,d** Corresponding graphs of limitation time courses, with *closed circles* representing biochemical limitation, and *open circles* representing stomatal limitation. For visual clarity, only every fourth data point collected is shown

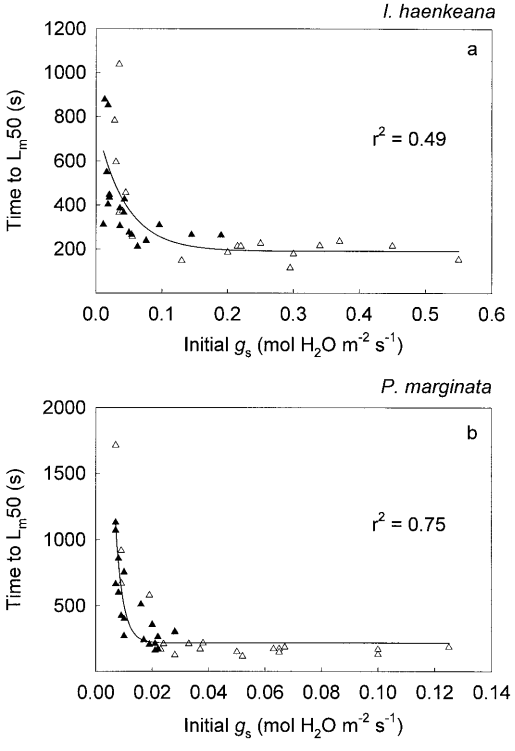

Fig. 2 Relationship between stomatal conductance at the start of photosynthetic induction and time to L_m50 for leaves of **a** *Isertia haenkeana*, and **b** *P. marginata*. *Open symbols* represent data collected during the wet season (both morning and afternoon), and *closed symbols* are dry season values (morning and afternoon). All leaves were kept darkened for at least 6 h prior to the start of induction measurements

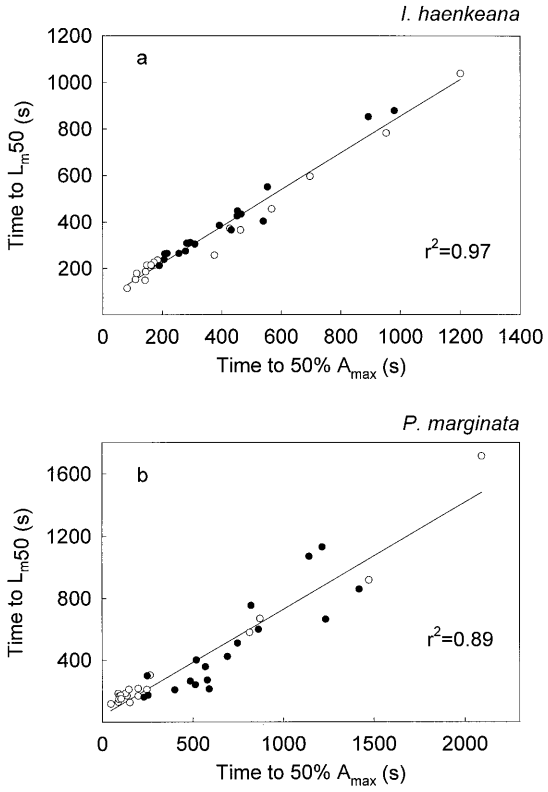

Fig. 3 Relationship between time to 50% of A_{max} and time to L_m50 for leaves of **a** *I. haenkeana*, and **b** *P. marginata*. *Open symbols* represent data collected during the wet season (both morning and afternoon), and *closed symbols* are dry season values (morning and afternoon). All leaves were kept darkened for at least 6 h prior to the start of induction measurements

marginata (Fig. 2b), as initial g_s decreased, time to L_m50 remained low for a wide range of conductances and then increased sharply over a fairly small range of initial g_s. The data sets shown in Fig. 2 were fit with an exponential decay equation using the curve fit function in SigmaPlot (SPSS software, Chicago, Ill., USA). Plots of initial g_s versus time to L_m50 for *P. limonensis* and *P. micrantha* showed similar patterns, and the parameters derived for the curve fits for each of the four species are given in Table 1. The time to L_m50 was linearly related to time to 50% of A_{max} over the entire range of values observed for both *I. haenkeana* (Fig. 3a) and *P. marginata* (Fig. 3b), and plots of intial g_s versus time to 50% of A_{max} were therefore similar to the plots shown in Fig. 2 (see also Allen and Pearcy, 1999). A linear relationship between these two quantities was also seen in the corresponding plots for *P. limonensis* and *P. micrantha* (Table 1).

Analysis of biochemical and stomatal influences on induction loss

The photosynthetic induction state of leaves that were fully induced and then darkened under black cloth de-

Table 1 Equations and r^2 values describing the relationships between: time to 50% of A_{max} and time to L_m50, initial g_s and time to L_m50, gs and CO_2 uptake from a 60-s pulse, and biochemical activation state and CO_2 uptake from a 60-s pulse, for each of the four species studied

Species	Equation	r^2
x=Time to 50% of A_{max} (s); y=time to L_m50 (s)		
Psychotria marginata	y=40.45+0.69x	0.89
P. limonensis	y=37.55+0.67x	0.78
P. micrantha	y=61.95+0.76x	0.93
Isertia haenkeana	y=64.75+0.79x	0.97
x=g_s (mol H_2O $m^{-2}s^{-1}$); y=CO_2 uptake from a 60-s pulse (μmol CO_2 m^{-2})		
P. marginata	y=32.90+2870x	0.86
P. limonensis	y=70.02+1970x	0.60
P. micrantha	y=55.72+4910x	0.89
I. haenkeana	y=101.20+3370x	0.83
x=Biochemical activation state (%); y=CO_2 uptake from a 60-s pulse (μmol CO_2 m^{-2})		
P. marginata	y=-0.74+245.45x	0.88
P. limonensis	y=22.84+255.76x	0.79
P. micrantha	y=41.94+451.29x	0.89
I. haenkeana	y=66.56+695.21x	0.90
x=Initial g_s (mol H_2O m^{-2} s^{-1}); y=time to L_m50 (s)		
P. marginata	y=217.0+13112e^{-383x}	0.75
P. limonensis	y=213.6+4672e^{-272x}	0.52
P. micrantha	y=211.7+441e^{-41x}	0.51
I. haenkeana	y=190.3+583e^{-23x}	0.49

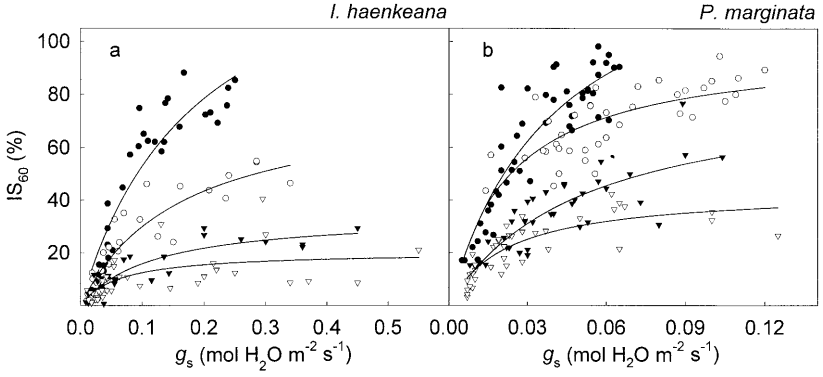

Fig. 4 Relationship between stomatal conductance (g_s) and photosynthetic induction state 60 s into a high-light transient (IS_{60}) for leaves of **a** *I. haenkeana*, and **b** *P. marginata* which were pre-treated in four different ways. *Closed circles* represent leaves from the dry season which were left uncovered prior to measurements. *Open circles* represent leaves that were fully induced and then shaded with black cloth for 20 min prior to measurement, and *closed triangles* are leaves that were fully induced and then covered for 60 min. *Open triangles* represent leaves that were covered with black cloth for at least 6 h prior to measurement. For the treatment represented by the *closed circles*, the lower values of g_s typically occurred later in the day (see Figs. 5, 6 for an example of the daily pattern). For the other three treatments, half of each set was collected during the wet season (this half includes all of the highest g_s values recorded), and the other half during the dry season

creased with time, due to both down-regulation of photosynthetic enzyme activities and stomatal closure. This is manifested as different values of IS_{60} and g_s depending on the time in darkness and the conditions of the experiment. When data from different times of day and seasons were combined, a family of curves resulted, each curve representing a different time in darkness (Fig. 4). In general, the lower values of g_s and IS_{60} in each of the four treatments are from afternoon and/or dry season observations (see Fig. 4 legend for more detail). This pattern suggests that stomata exert a stronger influence on photosynthetic induction loss in the afternoon and during the dry season. In the interest of visual clarity, data points on Fig. 4 are not differentiated by season and time of day. However, the points showing the highest values for g_s were invariably from wet season mornings. Leaves that were darkened longer typically had a lower IS_{60} for a given g_s, indicating a greater loss of biochemical activation due to the longer dark period.

Although Fig. 4a,b are qualitatively similar, some differences in the patterns of induction loss are evident when comparing *I. haenkeana* (Fig. 4a) and *P. marginata* (Fig. 4b). For both the 20- and 60-min darkness treatment, *P. marginata* leaves showed a higher maximum IS_{60} than did *I. haenkeana* leaves, an observation which indicates that the relative rate of photosynthetic enzyme deactivation in darkness is slower for *P. marginata* than for *I. haenkeana*. Patterns of induction loss in *P. limonensis* and *P. micrantha* were qualitatively similar and quantitatively intermediate to those of *P. marginata* and *I. haenkeana* (data not shown).

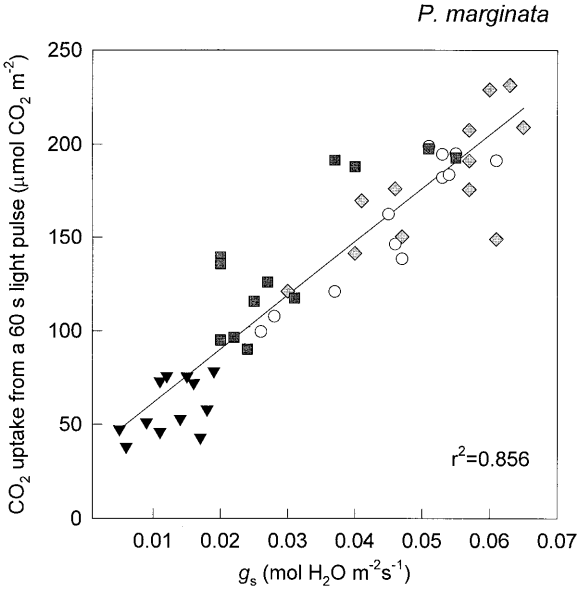

Fig. 5 Relationship between stomatal conductance (g_s) and total CO_2 uptake due to a 60-s pulse of light for leaves of *P. marginata*. Different *symbols* and *shades* represent measurements made during four different time periods throughout the day as follows: *open circles* between 0900 and 1015 hours, *lighter diamonds* between 1025 and 1145 hours, *darker squares* from 1415 to 1530 hours, and *filled triangles* from 1540 to 1700 hours. All measurements were made during the dry season on 17 and 28 February 1997 using leaves which were previously exposed to the natural light regime

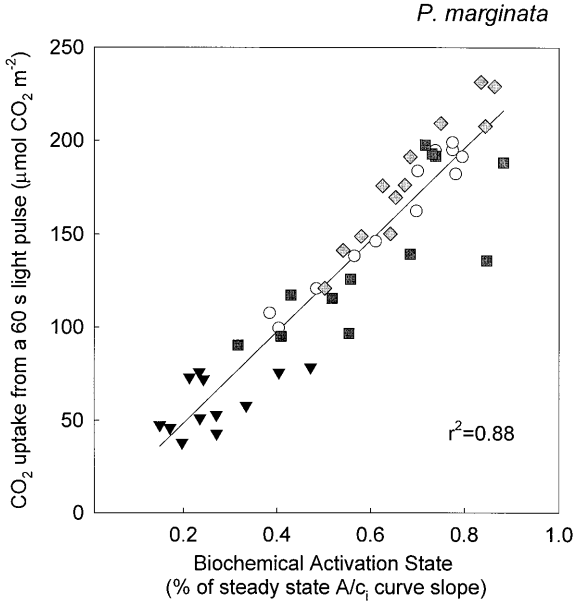

Fig. 6 Relationship between biochemical activation and total CO_2 uptake due to a 60-s pulse of light for leaves of *P. marginata*. Data shown here are from the same set of measurements used in Fig. 5. *Symbols* and *shades* represent measurements made during four different time periods as in Fig. 5

Limitations to photosynthetic performance during 60-s light pulses

The integral CO_2 assimilation resulting from a 60-s light fleck was strongly dependent on both stomatal conductance and the level of biochemical activation exhibited by the leaf at the time of the light pulse (Figs. 5, 6). Total integrated CO_2 assimilation was linearly related to g_s (Fig. 5). Since stomatal conductance did not change during the short measurement period of the 60-s pulse measurements, it is reported as simply g_s, rather than "initial g_s." Biochemical activation state was calculated as the ratio of the slope of an A/c_i curve that would pass through the measured photosynthetic rate and c_i at the end of the light fleck to the slope of the steady-state A/c_i curve. This measure revealed that the total integrated CO_2 assimilation by a leaf during a light fleck was also linearly related to the activation state of the leaf's biochemical machinery (Fig. 6). Although Figs. 5 and 6 show only data from *P. marginata*, the data collected for each species exhibit similar linear relationships (Table 1).

Discussion

After a transition from shade to high light, photosynthetic induction proceeds as the result of several processes, operating at different time scales. Much of the previous work published in this area has suggested that biochemical activation typically exhibits a time constant of 4–5 min, and is essentially complete within the first 10–12 min of photosynthetic induction (Pearcy 1990; Woodrow and Mott 1989). Further increases in photosynthetic rate after 10–12 min have generally been credited solely to subsequent increases in stomatal conductance (Pearcy et al. 1994; Tinoco-Ojanguren and Pearcy 1993a). In contrast, the results of this study indicate that rates of biochemical activation and stomatal opening are highly coordinated, and that complete induction of the biochemistry is potentially much slower than the typically reported times of 10–12 min. Figure 3 illustrates this variability in biochemical induction times; time to L_m50 is not a fixed quantity, but varies linearly with overall time to 50% of A_{max} across the entire range of induction times observed. The relationship between rates of biochemical activation and stomatal conductance can be seen in Fig. 2. Time to L_m50 is at a minimum for a wide range of initial stomatal conductances, but then increases sharply with decreasing initial stomatal conductance once initial g_s falls below a fairly narrow range of critical values. The critical value of g_s increases along an increasing light gradient, with *I. haenkeana* showing the highest value for critical g_s, and *P. marginata* the lowest (Table 1). However, when these critical values for g_s are divided by that species' maximum g_s, the resulting ratio does not vary in a systematic way (Data not shown), suggesting that the relationship between this critical value of g_s and a species' maximum g_s may be consistent among

species. In the cases where initially low stomatal conductances result in a slow relaxation of stomatal limitation to induction, biochemical induction time is apparently slowed down to match. A similar relationship has previously been observed between initial g_s and time to 90% of A_{max} (Valladares et al. 1997), and between initial g_s and time to 50% of A_{max} (Allen and Pearcy, 1999).

Limitation analysis of this kind depends on an accurate estimation of intercellular CO_2 concentration (c_i), and previous theoretical and experimental work has demonstrated that heterogeneous stomatal behavior (stomatal patchiness) may lead to erroneous calculations of c_i (Buckley et al. 1997; Laisk 1983; Mott 1995; Van Kraalingen 1990). To address this issue, we conducted a parallel field investigation into the occurrence and effects of stomatal heterogeneity during induction, utilizing chlorophyll fluorescence imaging in conjunction with gas exchange measurements (M.T. Allen, R.W. Pearcy, T.N. Buckley, and K.A. Mott,unpublished work). Stomatal patchiness was common during photosynthetic induction, and quantitative analysis of the fluorescence images suggested that the observed heterogeneity was capable of generating significant errors in the calculation of c_i. However, the time required to reach L_m50 was seen to be quite insensitive to the effects of stomatal patchiness, even in cases where patchiness led to significant errors in gas-exchange-based estimates of overall biochemical activation time.

Mott and Woodrow (1993) observed a strong dependence of Rubisco activation time on intercellular CO_2 concentration, and Pearcy et al. (1994) suggested that this CO_2 dependence may function to coordinate Rubisco activation with stomatal opening during transient photosynthesis. Coordination of this kind is consistent with the results of this study as well as with previously reported observations that photosynthetic induction often proceeds at a relatively constant c_i (Chazdon and Pearcy 1986; Pearcy et al. 1985; Zipperlen and Press 1997). In a multispecies study of photosynthetic responses to variable light, Ogren and Sundin (1996) reported rates of biochemical activation which appeared to vary with rates of stomatal opening, although they did not provide information about initial stomatal conductances in their summary table. Tinoco-Ojanguren and Pearcy (1993b) reported slower time constants for changes in mesophyll conductance during the first 2–10 min of induction in leaves that were subjected to a high vapour pressure deficit (VPD), and thus exhibited reduced initial stomatal conductance. Although Tinoco-Ojanguren and Pearcy (1993b) suggested that these apparent differences in biochemical activation times might be an artefact of stomatal heterogeneity, the observed effects of VPD could have instead been due to actual changes in the rates of biochemical induction resulting from stomatal influence over c_i.

Loss of induction state is the result of both biochemical deactivation and stomatal closure. Previous studies have shown that Rubisco activation is typically a hysteretic process with slower times for loss than for gain of activation (Sassenrath-Cole and Pearcy 1994; Woodrow and Mott 1989). Stomatal dynamics under variable light regimes has been the subject of a number of studies, and the nature of the stomatal response to light transients has been shown to depend on many factors, including: VPD (Tinoco-Ojanguren and Pearcy 1993a), growth form (Knapp and Smith 1989), and water stress (Knapp and Smith 1988). For the species in this study, stomatal behavior was influenced by both time of day and season. Compared with wet season mornings, g_s for a given species and treatment tended to be lower during the dry season as well as during the afternoon of the wet season (see also Allen and Pearcy, 1999). Rates of biochemical induction loss were not affected by season or time of day, as demonstrated by the fact that for a given darkness treatment all observations followed a consistent relationship between stomatal conductance and IS_{60} (Fig. 4). Figure 4 also illustrates the fact that leaves of both understory and high light species maintain a higher state of biochemical induction if left under their natural light regimes than if fully induced and then darkened for 20 min. As noted above, the observations of leaves under natural light regimes were only performed in the dry season. Based on the extremely high stomatal conductances often observed during wet season mornings, even in leaves which have been kept in the dark, this tight relationship between g_s and IS_{60} for uncovered leaves might be expected to break down during wet season mornings. After 20 min in the dark, leaves of *P. marginata* maintained a higher maximum IS_{60} than did leaves of *I. haenkeana*, indicating that the relative rate of biochemical deactivation was slower for *P. marginata*. Also evident from Fig. 4 is the relatively slow decrease in biochemical activation over time. After 60 min under black cloth, the leaves of both *P. marginata* and *I. haenkeana* showed higher levels of biochemical activation than did completely uninduced leaves (at least 6 h dark pretreatment), with *P. marginata* again exhibiting a slower relative rate of biochemical induction loss. These findings are consistent with the results of previous studies which showed that rates of Rubisco deactivation often differ based on species and treatment (Ernstsen et al. 1997; Pearcy 1990). Taken as a whole, the results presented in Fig. 4 suggest that rates of induction loss will be primarily driven by rates of biochemical deactivation only if stomatal conductance in the shade remains very high. Observations in the afternoon at all times of the year, or during the morning in the dry season generally fall in a region where stomatal conductance is low enough to exercise significant control over IS_{60}.

Dry season measurements of photosynthetic response to 60-s light pulses revealed a striking degree of coordination between stomatal and biochemical regulation. Over the entire range of stomatal conductances observed, CO_2 uptake due to a 60-s pulse of light varied linearly with g_s (Fig. 5). At the same time, total CO_2 uptake was also linearly related to the biochemical activation state of the leaf during the 60-s pulse (Fig. 6). The data presented in these two figures are, however, not entirely independent. In other words, a leaf that exhibited both a high

stomatal conductance *and* a high integrated assimilation of CO_2 in response to a 60-s light pulse must also show a high biochemical activation state. However, it is not inevitable that stomatal conductance and biochemical activity co-vary. For example, it would be possible for a leaf to exhibit a high g_s and a low biochemcial activation state; a condition that would result in a low integrated assimilation of CO_2 in response to a 60-s pulse. Such a combination of high g_s with a low biochemical activation state, or high biochemical activity with low g_s was never observed. Throughout the day, stomatal conductance and biochemical activation state co-varied, presumably in response to the constantly changing light intensities in the understory. Such coordination may be important to photosynthetic performance under fluctuating light. In a case where Rubisco remained active while the stomata closed, a light fleck could result in considerable photorespiratory losses as the c_i was rapidly drawn down and the RuBP pool was depleted as the result of the oxygenase activity of Rubisco. If, on the other hand, Rubisco activity co-varied with stomatal opening, a transition to high light would not result in as large a decrease in c_i, thus preventing a large carbon flow to photorespiration during the time required for the biochemistry and stomata to induce in tandem.

Although difficult to completely quantify, the stomatal behavior of the plants in this study clearly has considerable influence over their photosynthetic responses to transient light. For completely uninduced leaves, a threshold relationship was seen between initial g_s and the time required for 50% of complete light activation of photosynthetic biochemistry (Fig. 2). Initially low stomatal conductances also led to slower rates of overall photosynthetic induction (see also Allen and Pearcy, in press), resulting in tight coordination between biochemical activation time and overall photosynthetic induction time (Fig. 3). The data shown in Figs. 2 and 3 are from leaves that were kept completely darkened prior to measurements. Covering the leaves in this way was done in order to standardize the protocol for plants in both high light and understory environments, and it should be noted that this level of biochemical inactivation would be unlikely during daylight hours among unmanipulated leaves. In leaves that were left exposed to natural light fluctuations, the activation state of leaf biochemistry and stomatal conductance tended to co-vary, resulting in a linear relationship between stomatal conductance and dynamic photosynthetic performance (Fig. 5). During the mornings of the wet season, all species maintained continuously high stomatal conductances (even when kept in the dark), and dynamic photosynthetic performance was determined primarily by biochemical limitations. At all other times, however, stomatal conductance had an important influence on induction times, rates of induction loss, and photosynthetic response to 60-s light pulses.

Acknowledgements This research was supported by Mellon Foundation /Smithsonian Institution Senior and Junior Fellowships to RWP and MTA, respectively and by USDA NRI grant 95-37100-1619.

References

Allen MT, Pearcy RW (2000) Stomatal behavior and photosynthetic performance under dynamic light regimes in a seasonally dry tropical rain forest. Oecologia 122:470–478

Buckley TN, Farquhar GD, Mott KA (1997) Qualitative effects of patchy stomatal conductance distribution features on gas-exchange calculations. Plant Cell Environ 20:867–880

Chazdon RL, Pearcy RW (1986) Photosynthetic responses to light variation in rain forest species. I. Induction under constant and fluctuating light conditions. Oecologia 69:517–523

Croat TB (1978) Flora of Barro Colorado Island. Stanford University Press, Stanford

Ernstsen J, Woodrow IE, Mott KA (1997) Responses of Rubisco activation and deactivation rates to variations in growth-light conditions. Photosynth Res 52:117–125

Kirschbaum MUF, Pearcy RW (1988) Gas exchange analysis of the relative importance of stomatal and biochemical factors in photosynthetic induction in *Alocasia macrorrhiza*. Plant Physiol 86:782–785

Knapp AK, Smith WK (1988) Effect of water stress on stomatal and photosynthetic responses in subalpine plants to cloud patterns. Am J Bot 75:851–858

Knapp AK, Smith WK (1989) Influence of growth form on ecophysiological responses to variable sunlight in subalpine plants. Ecology 70:1069–1082

Laisk A (1983) Calculation of leaf photosynthetic parameters considering the statistical distribution of stomatal apertures. J Exp Bot 34:1627–1635

Mott KA (1995) Effects of patchy stomatal closure on gas exchange measurements following abscisic acid treatment. Plant Cell Environ 18:1291–1300

Mott KA, Woodrow IE (1993) Effects of O_2 and CO_2 on non-steady-state photosynthesis – further evidence for ribulose-1,5-bisphosphate carboxylase oxygenase limitation. Plant Physiol 102:859–866

Mulkey SS, Wright SJ, Smith AP (1993) Comparative physiology and demography of three Neotropical forest shrubs: alternative shade-adaptive character syndromes. Oecologia 96:526–536

Ogren E, Sundin U (1996) Photosynthetic responses to variable light: a comparison of species from contrasting habitats. Oecologia 106:18–27

Pearcy RW (1990) Sunflecks and photosynthesis in plant canopies. Annu Rev Plant Physiol Plant Mol Biol 41:421–453

Pearcy RW (1994) Photosynthetic response to sunflecks and light gaps: mechanisms and constraints. In: Baker NR, Bowyer JR (eds) Photoinhibtion of photosynthesis: from molecular mechanisms to the field. Biosis Scientific, Oxford, pp 255–271

Pearcy RW, Osteryoung K, Calkin HW (1985) Photosynthetic responses to dynamic light environments by Hawaiian trees. The time course of CO_2 uptake and carbon gain during sunflecks. Plant Physiol 79:896–902

Pearcy RW, Chazdon RL, Gross LJ, Mott KA (1994) Photosynthetic utilization of sunflecks, a temporally patchy resource on a time scale of seconds to minutes. In: Caldwell MM, Pearcy RW (eds) Exploitation of environmental heterogeneity by plants: ecophysiological processes above and below ground. Academic Press, San Diego, pp 175–208

Pfitsch WA, Pearcy RW (1989) Daily carbon gain by *Adenocaulon bicolor*, a redwood forest understory herb, in relation to its light environment. Oecologia 80:465–470

Sassenrath-Cole GF, Pearcy RW (1994) Regulation of photosynthetic induction state by the magnitude and duration of low light exposure. Plant Physiol 105:1115–1123

Tang Y-H, Koizumi H, Satoh M, Washitani I (1994) Characteristics of transient photosynthesis in *Quercus serrata* seedlings grown under lightfleck and constant light regimes. Oecologia 100: 463–469

Tinoco-Ojanguren C, Pearcy RW (1992) Dynamic stomatal behavior and its role in carbon gain during lightflecks of a gap phase and an understory *Piper* species acclimated to high and low light. Oecologia 92:222–228

Tinoco-Ojanguren C, Pearcy RW (1993a) Stomatal dynamics and its importance to carbon gain in two rainforest *Piper* species. I. VPD effects on the transient stomatal response to light-flecks. Oecologia 94:388–394

Tinoco-Ojanguren C, Pearcy RW (1993b) Stomatal dynamics and its importance to carbon gain in two rainforest *Piper* species. II. Stomatal versus biochemical limitations during photosynthetic induction. Oecologia 94:395–402

Valladares F, Allen MT, Pearcy RW (1997) Photosynthetic responses to dynamic light under field conditions in six tropical rainforest shrubs occurring along a light gradient. Oecologia 111:505–514

Van Kraalingen DWG (1990) Implications of non-uniform stomatal closure on gas exchange calculations. Plant Cell Environ 13: 1001–1004

Windsor DM (1990) Climate and moisture variability in a tropical forest, long-term records for Barro Colorado Island, Panamá. Smithson Contrib Earth Sci 29:1–145

Woodrow IE, Mott KA (1989) Rate limitation of non-steady-state photosynthesis by ribulose-1,5-bisphosphate carboxylase in spinach. Aust J Plant Physiol 16:487–500

Zipperlen SW, Press MC (1997) Photosynthetic induction and stomatal oscillations in relation to the light environment of two dipterocarp rain forest tree species. J Ecol 85:491–503