RESEARCH ARTICLE

Photosynthetic induction and activity of enzymes related to carbon metabolism: insights into the varying net photosynthesis rates of coffee sun and shade leaves

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Received: May 16, 2013; Accepted: May 21, 2013

ABSTRACT: The shade leaves of coffee (*Coffea arabica* L.) apparently retain a robust photosynthetic machinery that is comparable to that of sun leaves and can fix CO_2 at high rates when subjected to high light intensities. This raises the question of why the coffee plant would construct such a robust photosynthetic machinery despite the low photosynthetic rates achieved by the shade leaves at low light supply. Here, we grew coffee plants at 100% or 10% full sunlight and demonstrated that the shade leaves exhibited faster photosynthetic induction compared with their sun counterparts, in parallel with lower loss of induction states under dim light, and were well protected against short-term sudden increases in light supply (mimicking sunflecks). These findings were linked to similar photosynthetic capacities on a per mass basis (assessed under nonlimiting light), as well as similar extractable activities of some enzymes of the Calvin cycle, including Rubisco, when comparing the shade and sun leaves. On the one hand, these responses might represent an overinvestment of resources given the low photosynthetic rates of the shade leaves when light is limiting; on the other hand, such responses might be associated with a conservative behavior linked to the origin of the species as a shade-dwelling plant, allowing it to maximize the use of the energy from sunflecks and thus ultimately contributing to a positive carbon balance under conditions of intense shading.

KEYWORDS: *Coffea*, photosynthesis, photosynthetic induction kinetics, sun/shade tolerance, sunflecks.

ABBREVIATIONS: BL: biochemical limitation - ETR: electron transport rate - LCP: light compensating point - LSP: light saturating point - PAR: photosynthetically active radiation - SL: stomatal limitation - SLA: specific leaf area.

INTRODUCTION

Light provides the energy required for photosynthesis and plant growth, and plants adjust their growth and developmental processes in response to changes in light intensity to optimize their fitness (Walters 2005, Lusk et al. 2008). The effects of variable light environments on photosynthesis are best understood in the context of sunflecks, in which the duration and frequency of light patches affect carbon assimilation and biomass accumulation via responses by an array of physiological and morphological processes (Wayne and Bazzaz 1993, Pearcy et al. 1994, Valladares and Niinemets 2008). The shade and sun leaves are at the two ends of the continuum of leaf responses to light (Boardman 1977). Shade leaves maximize light capture but reduce the costs of maintaining excess pho-

tosynthetic machinery. Shade leaves are generally thinner and/or lighter and thus have a high specific leaf area (SLA); they also exhibit high chlorophyll concentrations per unit leaf mass and low ATPase activities and Rubisco contents compared with their sun counterparts (Boardman 1977, Walters 2005, Niinemets 2007). Because this relatively low investment in photosynthetic proteins yields a low light-saturated photosynthetic rate, a classic shade leaf is at risk of photoinhibition and damage from the high irradiance of sunflecks, while a classic sun leaf will be ill-suited to shade conditions, overinvesting in photosynthetic proteins that cannot be fully utilized (Boardman 1977, Way and Pearcy 2012).

Coffee is the most important commodity in the international agricultural trade, generating over 90 billion dollars each year, and approximately 500 million people are involved in its processing, from cultivation to final consumption. It evolved as an understory tree and consequently has been assumed to be a shade-demanding species. However, in many situations, modern coffee cultivars grow well and even produce greater yields in the sun than in the shade (DaMatta 2004, DaMatta et al. 2010). At the leaf scale, the question of whether the rate of net CO_2 assimilation rate (A) is higher in sun or shade leaves remains controversial. This controversy seems to be largely related to the environmental conditions prevailing during measurements because these conditions may affect the stomatal conductance (g_s) and thus A. In any case, as reasoned by DaMatta (2004), sun coffee leaves have higher rates of light-saturated A per unit area than their shade counterparts to the extent that stomatal aperture is not limiting. However, due to adjustments in SLA, the differences in light-saturated A per unit mass between shade and sun leaves are remarkably smaller (Matos et al. 2009); such rates can even be higher in shade than in sun leaves, as deduced by Araújo et al. (2008). These findings raise the question of why the coffee plant would construct such a robust photosynthetic machinery despite the low A of the shade leaves under low light. Here, we hypothesized that this apparent incongruence might be reconciled by considering that the anticipated robustness of the photosynthetic apparatus of shade leaves would be an advantage to support fast photosynthetic induction responses to sunflecks, as noted in other studies (e.g., Tausz et al. 2005, Way and Pearcy, 2012). Furthermore, the diurnal intercepted photon irradiance of leaves can differ by a factor of 25 between the deepest shade leaves and the more exposed leaves in the canopy of coffee trees growing under full exposure (Matos et al. 2009); taking advantage of these intermittent light periods may significantly improve carbon gain at a whole tree level.

Given the facts described above, our main goal was to examine whether the anticipated robustness of the photosynthetic machinery in the coffee plant might represent an adaptive strategy used to capture the extra energy provided by sunflecks. To this end, we assessed the activities of several key enzymes of the Calvin cycle and performed a detailed photosynthetic induction kinetic analysis of the response to sudden increases in light supply (mimicking the occurrence of sunflecks) in leaves from coffee plants grown under shade or full sunlight conditions.

MATERIAL AND METHODS

Plant material, growth conditions, and experimental design: The experiment was conducted in Viçosa (20°45'S, 42°54'W, 650 m in altitude) in southeastern Brazil. Uniform seedlings of *Coffea arabica* L. cv "Catuaí Vermelho IAC 44" obtained from seeds were grown in 12 L pots containing a mixture of soil, sand, and composted manure (4:1:1, v/v/v). Plants were grown either under full sunlight conditions (100% light) or under low light in a shaded environment (10% full sunlight). The shade enclosure was constructed using neutral-density black nylon netting, and the plants were kept under these conditions for 12 months before measurements. Throughout the experiment, the plants were grown under naturally fluctuating conditions of temperature and air relative humidity and were fertilized and irrigated as necessary. The pots were randomized periodically to minimize the effects of any variation within each light environment. For all samplings and measurements, the youngest fully expanded leaves, corresponding to the third or fourth pair from the apex of plagiotropic branches, were used.

Photosynthetic measurements: All leaf gas exchange and chlorophyll *a* fluorescence were measured in the morning with an open-flow infrared gas-exchange analyzer system equipped with a leaf chamber fluorometer (LI-6400XT, Li-Cor, Lincoln, NE, USA). The environmental conditions in the leaf chamber were set to achieve a leaf-to-air vapor pressure deficit between 1.2 and 2.0 kPa and a leaf temperature of 25°C.

Photosynthetic light-response curves were produced by increasing photosynthetically active radiation (PAR) stepwise (in 10 steps) from 0 to 1,500 μmol m^{-2} s^{-1} at 25°C. Initially, leaf tissues were exposed to a 5 Pa CO_2 partial pressure for 5 min to allow stomatal aperture; subsequently, A/PAR curves were obtained at a 40 Pa CO_2 partial pressure. The light compensating point (LCP), light saturating point (LSP), light-saturated A, and dark respiration rate (R_D) were determined from these curves. Further details of the methods used have been given elsewhere (Cavatte et al. 2012).

The responses of A to internal CO_2 concentration (A/C_i curve) were determined at 1,000 µmol photons m^{-2} s^{-1} at 25°C under ambient O_2 supply. The first measurements were taken at 40 Pa CO_2, and, once steady-state was achieved, the CO_2 partial pressure was gradually lowered to 5 Pa and then increased stepwise to 200 Pa. The maximum rate of carboxylation (V_{cmax}) and maximum rate of carboxylation limited by electron transport (J_{max}) were estimated by fitting the mechanistic model of CO_2 assimilation proposed by Farquhar et al. (1980).

Photosynthetic light induction curves: The photosynthetic light induction curves were obtained using the timed lamp program of the Li-6400 based on the protocol described by Bai et al. (2008), with several modifications. Leaves were previously dark-adapted for at least 6 h, after which a weak modulated measuring beam (0.03 µmol m^{-2} s^{-1}) was applied to obtain the minimal fluorescence (F_0). The maximum fluorescence emissions (F_m) were measured after applying a saturating white light pulse of 8,000 µmol m^{-2} s^{-1} for 0.8 s. The leaf samples were then subjected to a PAR of 20 µmol m^{-2} s^{-1} for 5 min (initial state) and logged for 2 min using the leaf chamber of the Li-6400. Thereafter, the PAR was increased to 1,500 µmol m^{-2} s^{-1} in one step, after which the rates of gas exchange (A, g_s, and C_i) were logged at 5-s intervals and stored as 1-min averages over 35 min (induction time). During the induction time, the fluorescence signals, that is, the steady-state fluorescence (F_s) under actinic illumination (1,500 µmol m^{-2} s^{-1}), the maximum fluorescence during a light-saturating pulse of *c.* 8,000 µmol m^{-2} s^{-1} (F_m'), and the light-adapted minimal fluorescence (F_0') obtained using a weak far-red illumination, were measured at 60-s intervals. After the induction time, the leaf tissues were subjected to a PAR of 20 µmol m^{-2} s^{-1} for 15 min and then exposed to a PAR of 1,500 µmol m^{-2} s^{-1} for 1 min to estimate the loss of photosynthetic induction. The light source was subsequently turned off for 15 min, after which both F_0 and F_m were measured as described above.

Several parameters were then calculated: initial and maximum A and g_s values; internal CO_2 concentration (C_i); the time to reach 90% A_{max} ($t_{90\% A}$); dark respiration rates (R_D); the induction state; initial and maximum electron transport rates (ETRs), estimated according to Genty et al. (1989); and the variable-to-maximum chlorophyll fluorescence ratio, calculated as $F_v/F_m = [(F_m - F_0)/F_m)]$. Additionally, the energy absorbed by photosystem II, as reflected by three yield components for dissipative processes (Φ_{PSII}, the yield of photochemistry; Φ_{NPQ}, the yield for dissipation by down-regulation; and Φ_{NO}, the yield of other nonphotochemical losses), was calculated as described by Kramer et al. (2004).

The limitations to photosynthesis throughout the induction curves were calculated using the model proposed by Woodrow and Mott (1989). In this model, stomatal limitations (SLs) to photosynthesis are artificially removed via the normalization of photosynthetic rates for a constant C_i. The A values without SLs (A^*) were estimated as:

$$A^* = [(A + R_D)(C_{if} - \Gamma^*)]/(C_i - \Gamma^*) - R_D$$

where C_{if} describes the C_i values at the end of the induction period and Γ^* is the CO_2 compensation point in the absence of mitochondrial respiration. The conservative parameter Γ^* for coffee was taken from Martins et al. (2013). Subsequently, the SLs and biochemical limitations (BLs) were calculated:

$$SL = (A^* - A)/(A_{max} + R_D)$$
$$BL = (A_{max} - A^*)/(A_{max} + R_D)$$

where A_{max} is the maximum A at the end of the induction period. The total limitations to photosynthesis were calculated as the sum of SLs and BLs.

Enzyme activities: Leaf discs were collected around midday on cloudless days, flash frozen in liquid nitrogen, and subsequently stored at -80°C until analysis. Enzyme extracts were prepared from these samples as described by Nunes-Nesi et al. (2007). The activities of the following enzymes were assessed: aldolase, NADP-glyceraldehyde-3-phosphate dehydrogenase, triose-phosphate isomerase (all assayed as detailed in Fernie et al. 2001), and Rubisco (Sulpice et al. 2007).

RESULTS

Compared with shade leaves, sun leaves displayed higher LSP (607 against 340 µmol photons m^{-2} s^{-1}) and higher values of other photosynthetic parameters, such as light-saturated A (9.4 against 6.6 µmol CO_2 m^{-2} s^{-1}) and higher V_{cmax} (41.3 against 30.1 µmol CO_2 m^{-2} s^{-1}) and J_{max} (97.9 against 70.1 µmol e^- m^{-2} s^{-1}), on a per area basis (Table 1). However, due to differences in SLA (14.0 and 22.9 m^2 kg^{-1} in sun and shade leaves, respectively; data not shown), the photosynthetic trait values did not differ significantly between sun and shade leaves on a per mass basis (Table 1).

The photosynthetic induction curves followed a sigmoidal pattern that was more evident in the shade leaves than in the sun leaves (Figure 1). The shade leaves displayed higher A than sun leaves (0.7 against 0.08 µmol m^{-2} s^{-1}) at the beginning of the curve, but there were no significant differences in initial g_s (ranging from 0 to 1.4 mmol H_2O m^{-2} s^{-1}) or ETR between the

Table 1. The light-saturated net CO_2 assimilation rate (A), maximum rate of carboxylation (V_{cmax}), maximum rate of carboxylation limited by electron transport (J_{max}), light compensation point (LCP), and light saturation point (LSP) measured in coffee plants grown under 10% or 100% full sunlight

Parameters	Treatments			
	Area basis (μmol m^{-2} s^{-1})		Mass basis (μmol g^{-1} DW s^{-1})	
	10% sunlight	100% sunlight	10% sunlight	100% sunlight
A	6.6±0.2	9.4±0.5*	0.15±0.01	0.13±0.01^{ns}
V_{cmax}	30.1±0.9	41.3±3.1*	0.69±0.02	0.58±0.04^{ns}
J_{max}	70.1±2.7	97.9±6.1**	1.60±0.06	1.40±0.08^{ns}
LCP	3.8±0.9	18.7±1.7**	–	–
LSP	340±20	607±34**	–	–

The results are expressed on an area or mass basis. n=6±SE. Significance: ^{ns} not significant, *p<0.05, **p<0.01.

two types of leaves (Table 2). In both types of leaves, the photosynthetic saturation (approximately 6.5 μmol CO_2 m^{-2} s^{-1}) was achieved at the same time ($t_{90\% A}$, approximately 30 min), and the g_s and ETR were similar (Figure 1, Table 2), though the time to reach ETR saturation was lower ($t_{90\% ETR}$, approximately 20 min) than that of $t_{90\% A}$ (Table 2). Importantly, the shade leaves exhibited more rapid Rubisco activation in response to illumination (coupled with earlier decreases in C_i) and were better able than their sun counterparts to maintain the photosynthetic activation state (84 against 53%) in parallel to higher g_s upon 10 min under dim light (20 μmol m^{-2} s^{-1}) (Figure 1, Table 2). The limitations to photosynthesis observed during the photosynthetic induction curve suggest that BLs were prevalent in the first 5 min upon illumination. In shade leaves, the SLs were greater than biochemical constraints at 10 min or more of illumination. In sun leaves, the SLs were low (approximately 14%) and inferior to biochemical constraints throughout the photosynthetic induction curve (Figure 2). The total limitations to photosynthesis were, overall, higher in sun leaves than in shade leaves (Figure 2).

Irrespective of treatments, rapid activation of the fluorescence parameters was observed after exposing dark-acclimated leaf tissues to bright illumination, as demonstrated by the dramatic decrease in Φ_{PSII} in parallel with a strong increase in Φ_{NPQ} at the beginning of the photosynthetic induction curve, whereas Φ_{NO} remained essentially unaltered throughout the induction curve (Table 3). The values of these parameters varied minimally, if at all, at 1, 15, or 30 min upon exposure to bright light. Regardless of the light supply, the F_v/F_m was approximately 0.80 in dark-adapted leaves. Notably, this ratio was measured at 15 min of dark acclimation following 30 min of exposure to 1,500 μmol photons m^{-2} s^{-1} and was similar (approximately 0.73) between treatments (Table 3). These findings suggest that there was a slight dynamic photoin-

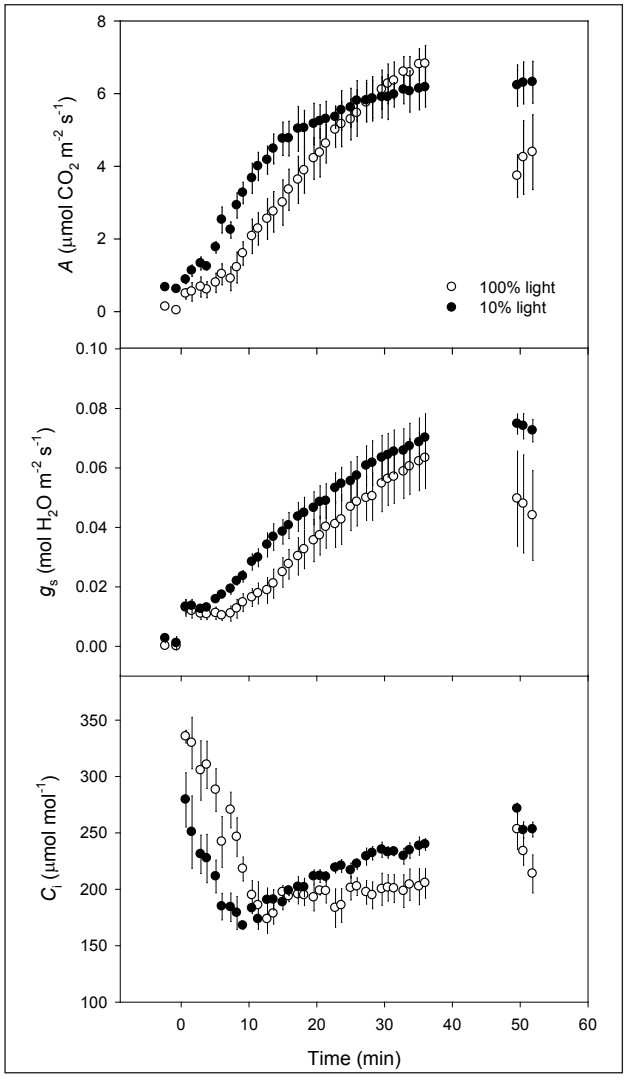

Figure 1. Time course of photosynthetic parameters [net CO_2 assimilation rate (A), stomatal conductance (g_s), and internal CO_2 concentration (C_i)] during induction for coffee plants grown under 10% or 100% full sunlight. n=4±SE.

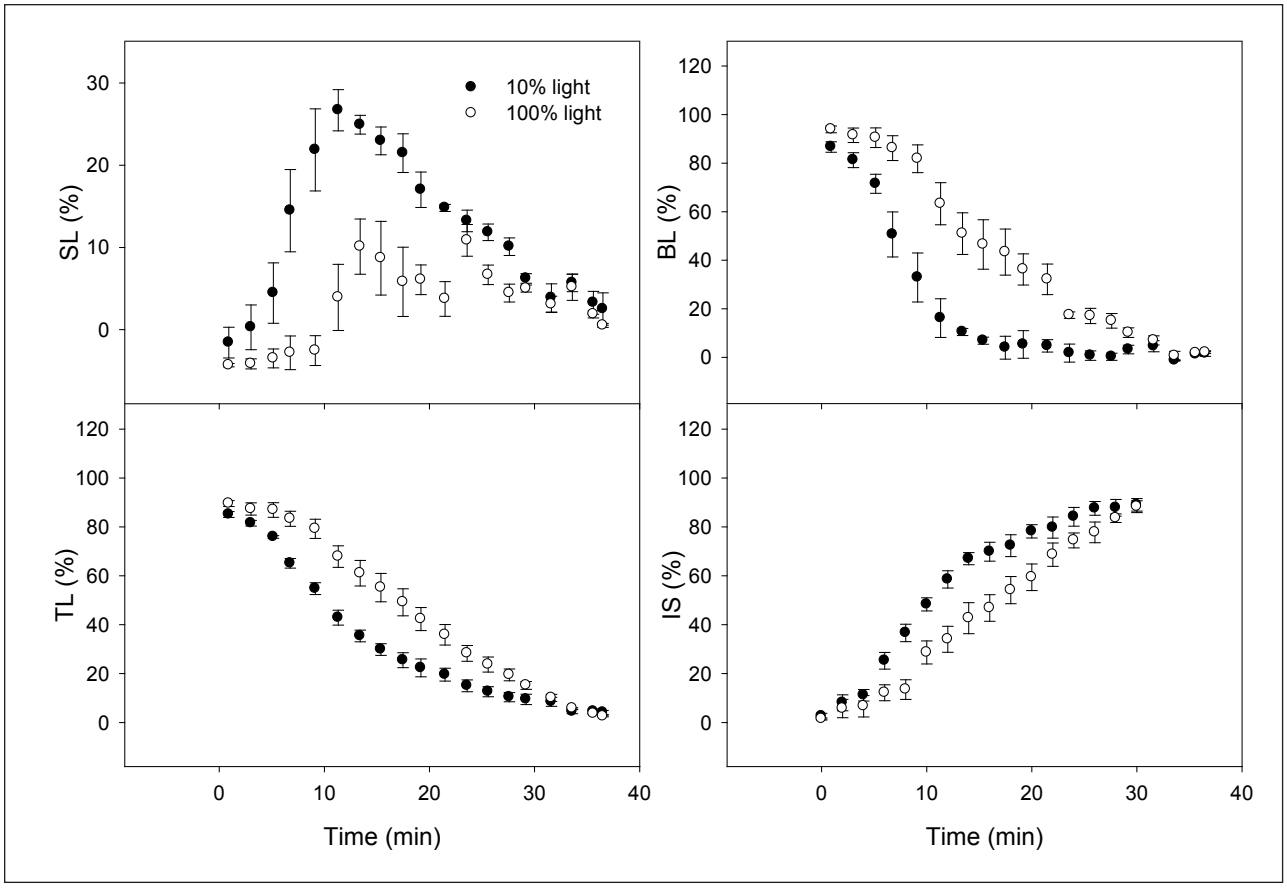

Figure 2. Stomatal (SL), biochemical (BL), and total (TL) limitations driving the photosynthetic rates during the induction curve, along with the induction state (IS) of the photosynthetic apparatus for coffee plants grown under 10% or 100% full sunlight. n=4±SE.

hibition, that is, that the shade leaves could cope with short-term intense light in a similar way as that of sun leaves.

We measured the activities of certain enzymes (expressed on a per mass basis) associated with carbon metabolism (Table 4). Notably, both initial and total Rubisco activities were virtually unaltered by light treatment, as was also the case for other enzymes of the Calvin cycle, such as NADP-glyceraldehyde-3-phosphate dehydrogenase and aldolase. In contrast, the activity of triose-phosphate isomerase, an enzyme that is also involved in the glycolytic pathway, was significantly higher (21%) in sun leaves than in their shade counterparts.

DISCUSSION

The maximum PAR intercepted by shade leaves was approximately 200 μmol m^{-2} s^{-1}, a value lower than their LSP. Nonetheless, these leaves were able to fix CO_2 at high rates, as demonstrated by the comparable photosynthetic capacity on a mass basis (accompanied by similar activities

Table 2. The initial and maximum values of the rate of net CO_2 assimilation (A), stomatal conductance (g_s) and electron transport rates (ETRs), time to reach 90% A_{max} or ETR ($t_{90\% A}$ or $t_{90\% ETR}$, respectively), and the induction state after 10 min of exposure to dim light, as obtained in photosynthetic induction curves for coffee plants grown under 10% or 100% full sunlight

Parameters	Treatments	
	10% sunlight	100% sunlight
Initial A (μmol CO_2 m^{-2} s^{-1})	0.70±0.07	0.08±0.03**
Maximum A (μmol CO_2 m^{-2} s^{-1})	6.5±0.6	6.9±0.6^{ns}
Initial g_s (mmol H_2O m^{-2} s^{-1})	1.4±2.2	0.0±1.0^{ns}
Maximum g_s (mmol H_2O m^{-2} s^{-1})	69±0.1	63±0.1^{ns}
Initial ETR (μmol e^- m^{-2} s^{-1})	7.9±0.2	8.0±0.1^{ns}
Maximum ETR (μmol e^- m^{-2} s^{-1})	56.7±2.5	56.7±2.8^{ns}
$t_{90\% A}$ (min)	27.7±1.9	30.7±0.4^{ns}
$t_{90\% ETR}$ (min)	18.8±1.4	20.7±1.9^{ns}
Induction state (%)	83.5±8.7	52.7±7.9*

The results are expressed on an area or mass basis. n=4±SE. Significance: ^{ns}, not significant, *p<0.05, **p<0.01.

Table 3. The variable-to-maximum fluorescence ratio at the beginning ($F_v/F_{m\ initial}$) of the induction curve and after 15 min of dark adaptation following the end of that curve ($F_v/F_{m\ 15\ min}$), the yield of photochemistry (Φ_{PSII}), the yield for dissipation by down-regulation (Φ_{NPQ}), and the yield of other nonphotochemical losses (Φ_{NO}) as measured at 0, 1, 15, and 30 min of the induction curve for coffee plants grown under 10% or 100% full sunlight

Parameters	Treatments	
	10% sunlight	100% sunlight
$F_v/F_{m\ initial}$	0.80±0.00	0.79±0.01^{ns}
$F_v/F_{m\ 15min}$	0.74±0.00	0.72±0.00*
$\Phi_{PSII\ initial}$	0.66±0.01	0.66±0.01^{ns}
$\Phi_{PSII\ 1min}$	0.05±0.00	0.04±0.01^{ns}
$\Phi_{PSII\ 15min}$	0.08±0.01	0.07±0.00^{ns}
$\Phi_{PSII\ 30min}$	0.09±0.00	0.09±0.00^{ns}
$\Phi_{NPQ\ initial}$	0.08±0.01	0.08±0.00^{ns}
$\Phi_{NPQ\ 1min}$	0.61±0.01	0.60±0.02^{ns}
$\Phi_{NPQ\ 15min}$	0.64±0.00	0.64±0.02^{ns}
$\Phi_{NPQ\ 30min}$	0.63±0.00	0.61±0.02^{ns}
$\Phi_{NO\ initial}$	0.26±0.01	0.26±0.01^{ns}
$\Phi_{NO\ 1min}$	0.34±0.01	0.35±0.02^{ns}
$\Phi_{NO\ 15min}$	0.28±0.01	0.29±0.02^{ns}
$\Phi_{NO\ 30min}$	0.28±0.01	0.30±0.02^{ns}

The results are expressed on an area or mass basis. n=4±SE. Significance: ^{ns} not significant, *p<0.05.

Table 4. The activities of some Calvin cycle enzymes (expressed as μmol min^{-1} g^{-1} FW) in coffee plants grown under 10% or 100% full sunlight

Enzymes	Treatments	
	10% sunlight	100% sunlight
Rubisco total activity	4.92±0.27	4.86±0.36^{ns}
Rubisco initial activity	3.44±0.20	3.13±0.32^{ns}
Rubisco activation state (%)	70.3±3.7	55.8±3.5*
NADP-glyceraldehyde-3-phosphate dehydrogenase	4.01±0.09	3.79±0.02^{ns}
Aldolase	6.75±0.65	6.48±0.69^{ns}
Triose-phosphate isomerase	68.4±1.3	82.8±4.9*

n=6±SE. Significance: ^{ns} not significant, *p<0.05, **p<0.01.

of some key enzymes of the Calvin cycle) relative to sun leaves, provided that light is nonlimiting. Although these large biochemical investments in photosynthetic machinery might seem to represent a wasteful use of resources given the low realizable *A* by the shade leaves at low light supply, these investments could support fast photosynthetic induction responses to sunflecks, thereby supporting our working hypothesis.

The photosynthetic induction curves in both sun and shade leaves were associated with low initial g_s values, which might to a certain extent explain the relatively long time required for photosynthetic saturation ($t_{90\%\ A}$, approximately 30 min) (Way and Pearcy 2012). However, the light induction of ETR was faster than that of CO_2 assimilation, as has been noted in other studies (e.g., Bai et al. 2008, Wong et al. 2012). It is likely that Rubisco is not be fully activated during the early phases of light induction, which may result in greater electron flow partitioning for alternative electron sinks (Makino et al. 2002). In any case, our values of initial g_s and $t_{90\%\ A}$ are similar to those obtained for shade species by Urban et al. (2007) but differ greatly from the average values observed in understory evergreen species by Bai et al. (2008), who reported initial g_s and $t_{90\%\ A}$ of approximately 30 mmol H_2O m^{-2} s^{-1} and 13 min, respectively. The large differences in the initial g_s and $t_{90\%\ A}$ reported in the literature may reflect differences in experimental conditions such as time of exposure to darkness, irradiance intensity used during the photosynthetic induction curves (Urban et al. 2007), leaf age (Urban et al. 2008), species and growth conditions (Kursar and Coley 1993), etc. Indeed, the role of g_s in the dynamics of induction is quite variable; g_s typically exhibits a short lag during induction before increasing to a maximum over another 10–60 min (Way and Pearcy 2012).

The induction curves followed a sigmoidal pattern (Figure 1) that is characteristic of species with low initial g_s, in contrast to a more hyperbolic pattern when initial g_s values are high (Valladares et al. 1997, Way and Pearcy 2012). We showed that *A* increased more rapidly than g_s upon exposure to bright illumination such that the total limitations to photosynthesis were dominated by stomatal rather than biochemical constraints, particularly in shade leaves. Species that are better acclimated to shade conditions usually display a higher activity and/or concentration of Rubisco activase and lower concentrations of inhibitors such as 2-carboxyarabinitol-1-phosphate (Parry et al. 2008) or higher proportions of Rubisco activase relative to Rubisco (Sage et al. 2002). Collectively, these features may be associated with a faster activation of Rubisco (and thus lower BLs), which would be a favorable trait in shade species that can efficiently capture energy from sunflecks (Pearcy, 1990).

The shaded leaves also exhibited lower losses of photosynthetic induction after 10 min under dim irradiance coupled with unaltered g_s, which could translate into a more efficient use of the energy of subsequent sunflecks (Valladares et al. 1997, Way and Pearcy 2012). This feature, together with more

rapid induction, appears to be a general behavior displayed by plants in shady understories or grown under low-light conditions compared with plants grown in more open habitats (Way and Pearcy 2012, and references therein). Nevertheless, the finding of more rapid photosynthetic induction of the shade leaves in this study contrasts with those of several other studies (e.g., Valladares et al. 1997, Wong et al. 2012), in which higher initial g_s played major roles in determining faster inductions when comparing species with contrasting light requirements.

We showed that the shade leaves displayed capabilities similar to those of sun leaves against photoinhibition, as indicated by the strong up-regulation of Φ_{NPQ} upon exposure to bright light, as well as by the discrete decrease of F_v/F_m ratio at the end of the induction curve only (after 15 min of dark adaptation). Moraes et al. (2010) observed pronounced increases in the zeaxanthin pools coupled with increases in the de-epoxidation state of the xanthophyll cycle in coffee seedlings that were transferred from low to high light, suggesting an increased capacity for photoprotection linked to a higher thermal dissipation (Logan et al. 2007). These findings are consistent with the present results (increases in Φ_{NPQ}) and imply that shade leaves are well protected against sudden increases in light availability in the short term.

In summary, we demonstrated that the shade leaves, compared with their sun counterparts, displayed faster photosynthetic induction concomitant with lower loss of induction states under dim light and were well protected against sudden short-term increases in light supply. These findings were linked to similar photosynthetic capacities on a per mass basis as well as to similar extractable activities of some enzymes of the Calvin cycle including Rubisco, regardless of the growth irradiance. Collectively, these data suggest that shade coffee leaves are not only able to efficiently capture and use limiting light but are also able to avoid the potentially damaging effects of excess irradiance when suddenly exposed to bright light. In this regard, shade leaves are believed to contribute positively to whole-plant carbon gain, albeit at the expense of an overinvestment in photosynthetic machinery. This response might represent a conservative behavior linked to the shady origin of the species aimed at maintaining a positive carbon balance under the conditions of intense shading that are typically experienced in its native habitats. This response has also likely contributed to the successful cultivation of coffee trees under high-density plantation conditions, under which the innermost leaves may receive as little as 1.5% of the total incoming irradiance (Matos et al. 2009); in this situation, the efficient use of the energy provided by the sunflecks may assume a central role in allowing those leaves to maintain a positive daily carbon balance.

ACKNOWLEDGMENTS

This research was supported by the Foundation for Research Assistance of Minas Gerais State, Brazil (Fapemig, Grant APQ-01138-12), and by the National Council for Scientific and Technological Development (CNPq) (Grants 302605/2010-0 and 475780/2012-4) to FMD. A PhD scholarship granted by CNPq to SCVM is also gratefully acknowledged.

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