Physiologia Plantarum
An International Journal for Plant Biology

Physiologia Plantarum 149: 45–55. 2013

Physiological and biochemical abilities of robusta coffee leaves for acclimation to cope with temporal changes in light availability

Nelson F. Rodríguez-López†, Paulo C. Cavatte, Paulo E. M. Silva, Samuel C. V. Martins, Leandro E. Morais, Eduardo F. Medina and Fábio M. DaMatta*

Departamento de Biologia Vegetal, Universidade Federal de Viçosa, 36570-000 Viçosa, MG, Brazil

Correspondence
*Corresponding author,
e-mail: fdamatta@ufv.br

Received 13 August 2012;
revised 30 October 2012

doi:10.1111/ppl.12010

The effects of varying intensities of light on plants depend on when they occur, even if the total amount of light received is kept constant. We designed an experiment using two clones of robusta coffee (*Coffea canephora*) intercropped with shelter trees in such a way that allowed us to compare coffee bushes shaded in the morning (SM) with those shaded in the afternoon (SA), and then confronting both with bushes receiving full sunlight over the course of the day (FS). The SM bushes displayed better gas-exchange performance than their SA and FS counterparts, in which the capacity for CO_2 fixation was mainly constrained by stomatal (SA bushes) and biochemical (FS bushes) factors. Physiological traits associated with light capture were more responsive to temporal fluctuations of light rather than to the amount of light received, although this behavior could be a clone-specific response. The activity of key antioxidant enzymes differed minimally when comparing the SM and SA clones, but was much larger in FS clones. No signs of photoinhibition or cell damage were found regardless of the light treatments. Acclimations to varying light supplies had no apparent additional cost for constructing and maintaining the leaves regardless of the light supply. Both the SM and SA individuals displayed higher return in terms of revenue streams (e.g. higher mass-based light-saturated photosynthetic rates, photosynthetic nitrogen use efficiencies and long-term water use efficiencies) than their FS counterparts. In conclusion, shading may improve the physiological performance of coffee bushes growing in harsh, tropical environments.

Abbreviations – $\Delta^{13}C$, carbon isotopic discrimination; Ψ_w, water potential; A, net photosynthetic rate; A_{max}, light-saturated A; APX, ascorbate peroxidase; Car, carotenoids; CAT, catalase; CC, construction costs; Chl, chlorophylls; C_i, internal CO_2 concentration; C_i/C_a, internal-to-ambient CO_2 concentration; DM, dry matter; FM, fresh matter; FS, bushes receiving full sunlight during most of the day; F_v/F_m, variable-to-maximum Chl fluorescence ratio; GR, glutathione reductase; g_s, stomatal conductance; J_{max}, maximum rate of carboxylation limited by electron transport; LCP, light compensating point; LSP, light saturation point; MC, maintenance costs; MDA, malondialdehyde; N, nitrogen; PAR, photosynthetically active radiation; PNUE, photosynthetic nitrogen use efficiency; R_d, dark respiration rate; SA, bushes shaded in the afternoon and exposed to full sunlight during the morning; SE, standard error; SM, bushes shaded in the morning and exposed to full sunlight in the afternoon; SLA, specific leaf area; SOD, superoxide dismutase; V_{cmax}, maximum rate of carboxylation; VPD, vapor pressure deficit; WUE, water use efficiency.

†Present address: Escuela de Biología, Universidad Industrial de Santander, Bucaramanga, Santander, Colombia.

Introduction

The effects of variable light environments on plant growth and photosynthesis are a classic topic in plant ecology and forest biology. They are best understood in the case of sunflecks, in which the duration and frequency of light patches affects 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). In crop plants, the effects of light environments have often been examined by comparing plants grown entirely at high light against individuals grown under fixed shade (e.g. using nettings with varying light transmittance), or in agroforestry systems with more or less homogeneous ground cover, varying from sparse to deep shade, depending on the attributes and management (e.g. crown architecture, spacing, pruning) of the shelter trees. In any case, local photosynthetically active radiation (PAR) flux to which individual leaves are exposed vary tremendously throughout the canopy of a tree (Niinemets 2007, Prieto et al. 2012). Furthermore, the effects of variable light environments are also influenced by the temporal scale of the diurnal fluctuations of the light environments, even when the total amount of PAR received is kept constant (Sims and Pearcy 1993, Wayne and Bazzaz 1993). To the best of our knowledge, little, if any, efforts have been undertaken in field conditions to examine the effects of the temporal scale of diurnal fluctuations of light availability in crop species.

Acclimation to sun and shade conditions at the scale of the leaf, via morpho-anatomical and physiological adjustments, has been well characterized in a wide range of species (Boardman 1977, Evans and Poorter 2001, Lusk et al. 2008). Leaves developed in high light are generally thicker and/or heavier with a higher nitrogen (N) concentration per leaf area, less chlorophyll (Chl) per unit leaf mass with a reduction of Chl *b*, altered chemical composition and construction costs, higher rates of dark respiration (R_d) and light-saturated photosynthesis (A_{max}), increased photoprotective pigments as well as decreased susceptibility to photoinhibition of photosynthesis compared with their low-light counterparts (Walters 2005, Niinemets 2007, Cavatte et al. 2012b). Whenever the absorbed light energy exceeds the capacity of leaves to use the trapped energy through photosynthesis or to dissipate it as heat, damage to photosystem II may occur. Protection against excess energy may be achieved by downregulation of photochemical efficiency via the xanthophyll cycle or by maintenance of the electron flux involving alternative pathways such as photorespiration and the Mehler-peroxidase reaction (Ort and Baker 2002, Logan et al. 2006).

Among agricultural commodities, coffee, an evergreen tropical shrub crop, has a monetary value surpassed only by oil. Of approximately 100 species of the *Coffea* genus, only *Coffea arabica* (arabica coffee) and *Coffea canephora* (robusta coffee) are economically important worldwide. These species have been cultivated in open fields in many tropical countries worldwide despite their origin in shaded habitats (DaMatta 2004). Presently, there is a growing interest in the cultivation of coffee bushes intercropped with shelter trees, especially due to the benefits to shaded plantations, including the conservation of natural resources, increased biodiversity and the stability of coffee production in addition to financial benefits, e.g. shelter trees increase cash income from fruits, timber or latex. In robusta coffee, however, virtually nothing is known on the effects of light supply on its ecophysiology, most likely because this species has been cultivated in open fields since its relatively recent introduction (1960s–1970s) in countries such as Brazil.

Under full sunlight, most carbon in robusta coffee is fixed in the morning when the stomatal aperture is higher, paralleling milder vapor pressure deficit (VPD) and temperature conditions (DaMatta et al. 2010). Given this fact, we hypothesized that the physiological performance of robusta could be improved by attenuating the radiation inputs (and temperature) in the afternoon. This could translate into a better local environment for longer stomatal aperture and improved photosynthetic rates. We further hypothesized that leaves subjected to varying diurnal light supplies should adjust themselves, both morphologically and physiologically, to optimize their photosynthetic performance according to the prevailing temporal scales of diurnal variations of light received by the leaves. To test these hypotheses, we designed an experiment using clones of robusta coffee intercropped with shelter trees in such a way that allowed us to compare coffee bushes mostly shaded in the morning with those mostly shaded in the afternoon, and then confronting both with bushes receiving full sunlight over the course of the day. We aimed to examine physiological and biochemical abilities to cope with temporal changes in light supply. Specifically, the carbon gain, the expression of the antioxidant system and the chemical composition, construction and maintenance costs of leaves were assessed under real plantation conditions using two clones of robusta coffee with contrasting photosynthetic rates.

Materials and methods

Site description, experimental design and growth conditions

The study site is located in the Experimental Station of Sooretama (19°24′S, 40°31′W, 30 m elevation), Espírito

Santo State, south-eastern Brazil. The soil at the site is a flat, deep, red-yellowish latosol. The site receives an average annual rainfall of 1200 mm mainly distributed from September/October to March/April (the growing season). The average annual temperature is 23.5°C.

The experiment was established in 1999 in an alley cropping system, composed of staggered north–south-oriented rows of rubber trees (*Hevea brasiliensis* cv. 'RRIM 600' – two lines of trees per tree row with a rectangular space of 3.0 m between the lines and 2.5 m between the trees in each line; the tree rows were spaced 40 m from each other) with open land (40 m wide alleys) for coffee (*C. canephora*) bushes with 2.5 × 1.0 m spacing in east–west-oriented hedgerows. As the sun crosses the sky from east to west over the course of the day, thus perpendicularly crossing the rows of the rubber trees, the shade on the coffee crop migrates accordingly (see Fig. 1). The coffee bushes were evaluated in the following three relative positions within the alley: bushes facing the east rubber tree rows, which were shaded in the morning and were exposed to full sunlight in the afternoon; plants located in the middle of the alley, which received full sunlight during most of the day and bushes facing the west rows, which were exposed to full sunlight during the morning and were shaded in the afternoon. In summary, the following three light treatments were established: bushes shaded in the morning (SM), bushes under full sunlight (FS) and bushes shaded in the afternoon (SA) (Fig. 1).

The coffee bushes were trained with three orthotropic heads (main stems). Both the rubber trees and coffee bushes were submitted to routine agricultural practices, including hoeing, fertilization and chemical control of insect and pathogen attacks. No supplemental irrigation was provided, but there was abundant rain during the growing season. Sampling and measurements were carried out on cloudless days in January 2010 (the rubber trees were approximately 8 m tall, and the coffee bushes were approximately 2 m tall). Two clones with contrasting photosynthetic performance, i.e. clones 03 and 120 displaying relatively higher and lower photosynthetic rates, respectively (Silva et al. 2012), were analyzed. The evaluations were performed using three bushes per clone per light treatment. In the SM and SA treatments, the bushes near the rubber tree rows [up to 4.0 m from the rows; the coffee bush nearest (1.0 m apart) to the tree rows was considered to be the border] were used; the three most central bushes in the alley gave the FS treatment (Fig. 1). Two plagiotropic (lateral) branches per bush (each from a distinct orthotropic stem), one facing north and another facing south, were evaluated. The experimental plot consisted of one orthotropic stem per bush so that six orthotropic stems per treatment combination served as conditional replicates. All physiological measurements and leaf samples were taken from the youngest, fully expanded leaves, corresponding to the third or fourth leaf pair from the apex of the branches in the middle third of the coffee bushes.

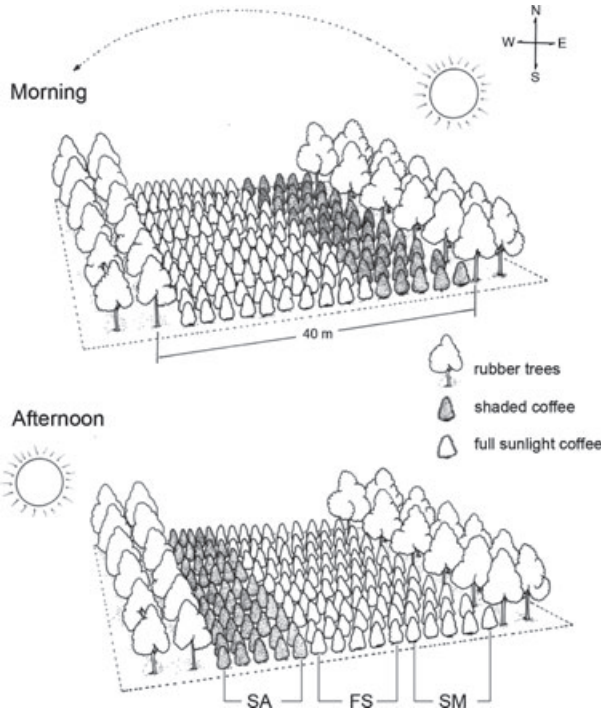

Fig. 1. Schematic representation showing an alley cropping system, composed of north–south-oriented rows of rubber trees (two lines of trees per row; the tree rows were spaced 40 m from each other) with open land (40 m wide alleys) for coffee bushes growing in east–west-oriented hedgerows. As the sun crosses the sky from east to west over the course of the day, thus perpendicularly crossing the rows of the rubber trees, the shade on the coffee crop migrates accordingly. Three light treatments were established, defined as follows: bushes facing the east rubber tree rows, which were shaded in the morning and exposed to full sunlight in the afternoon (SM); plants located in the middle of the alley, receiving full sunlight during most of the day (FS); and bushes facing the west rows, which were exposed to full sunlight during the morning and shaded in the afternoon (SA).

Environmental parameters

The total daily PAR over January 2010 was measured using LI-190SA quantum sensors (LI-COR, Lincoln, NE) positioned 1 m above the coffee bushes in each relative position in the alley. Each sensor was precisely positioned above the central bush from the three plants that were analyzed in each light treatment. Air temperature and relative humidity were also monitored. All of the sensors were connected to an LI-1400 data

logger (LI-COR), which acquired data from the sensors every minute and stored them as 5-min averages. The leaf-to-air VPD was estimated as described in Chaves et al. (2008).

Photosynthetic parameters

The net rate of carbon assimilation (A), stomatal conductance (g_s), internal-to-ambient CO_2 concentration ratio (C_i/C_a) and transpiration rate (E) were measured in an open system under both ambient temperature and CO_2 partial pressure using an infrared gas analyzer (LI-6400, LI-COR, Lincoln, NE). Instantaneous water use efficiency (WUE) was estimated from the A/E ratio. The variable-to-maximum Chl *a* fluorescence ratio (F_v/F_m) in dark-adapted (30 min) leaves was estimated immediately after gas-exchange analyses using a portable fluorometer (MINI-PAM, Walz, Effeltrich, Germany) as described in Araújo et al. (2008). Measurements were made during the following three periods throughout the day: 08:00–10:00 h, 11:00–13:00 h and 14:00–16:00 h (solar time), under artificial PAR, i.e. 250 µmol photons $m^{-2}\,s^{-1}$ [for the SM and SA treatments in the morning (08:00–10:00 h) and afternoon (14:00–16:00 h), respectively] and 1250 µmol photons $m^{-2}\,s^{-1}$ (for the SM and SA treatments in the afternoon and morning, respectively, as well as for FS treatments regardless of time point) at the leaf level. These PAR intensities corresponded approximately to the ambient irradiance intercepted by the sampled leaves (in their natural angles) for each light treatment in each time point. After fitting the leaf tissue in the leaf chamber, the rates of gas exchange were typically settled within 3–4 min, nearly paralleling the stabilization for internal CO_2 values. The measurements were repeated on three separate days (for each leaf within each time point), such that the gas-exchange parameters for each replicate were computed as the average values obtained over the measurement days.

Photosynthetic light-response curves (A/PAR) were produced by increasing PAR in 10 steps from 0 to 1500 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 40 Pa CO_2 partial pressure. Dark respiration rates (R_d), light compensating point (LCP), light saturating point (LSP) and light-saturated A (A_{max}) were determined from these curves. Further details have been given elsewhere (Cavatte et al. 2012a). The responses of A to internal CO_2 partial pressure (A/C_i curve) were determined at 1000 µmol (photons) $m^{-2}\,s^{-1}$, at 25°C. Measurements started at 35 Pa CO_2 partial pressure and once the steady state was reached, CO_2 partial pressure was gradually lowered to 5 Pa and then increased stepwise up to 160 Pa. The maximum rate of carboxylation (V_{cmax}) and the maximum rate of carboxylation limited by electron transport (J_{max}) were estimated from these curves, as detailed by Araújo et al. (2008). Measurements were made in early morning using leaves from branches detached at about 06:00 h, recut under water to prevent xylemic embolism, and immediately brought to the laboratory with their bases immersed in water.

Chemical composition, construction and maintenance costs of leaf tissues

Leaf tissues were collected at midday, frozen in liquid nitrogen, freeze-dried, grounded in a ball mill to allow passage through a 0.080 mm sieve and oven-dried at 60°C for 48 h. A 10 mg sample was used to measure the C and N contents with an elemental analyzer (Carlo Erba, Milan, Italy), as well as the relative abundances of ^{13}C and ^{12}C using a mass spectrometer (ANCA-GSL 20-20, Sercon, Crewe, UK). From these values, the carbon isotope composition ratio was estimated, after which the carbon isotope discrimination ($\Delta^{13}C$) was calculated. Further details on this procedure have been reported previously (DaMatta et al. 2003). The proximate chemical composition of the leaves (starch, total soluble sugars, structural carbohydrates plus lignin, lipids, proteins, organic acids, amino acids, minerals, total phenolics and total methylxanthine alkaloids) was determined as described in Poorter and Villar (1997) with the modifications detailed in Cavatte et al. (2012b), with the exception that total structural carbohydrates (cellulose and hemicellulose) and lignin were quantified together.

The leaf construction costs (CC), defined by the amount of glucose used for constructing 1 g of biomass, were estimated using the microbomb calorimeter technique proposed by Williams et al. (1987) and described in detail elsewhere (Cavatte et al. 2012b). The leaf costs of maintenance (MC) per unit dry mass, which are associated with the energy required to maintain processes that are unrelated to biomass gain, were determined following the procedure reported by Penning de Vries et al. (1974), using the maintenance coefficients reported by Merino et al. (1984).

Biochemical assays

Leaf discs, collected at about midday, were flash frozen in liquid nitrogen and stored at −80°C until analysis. Total Chl and total carotenoids (Car) were extracted using 80% (v/v) aqueous acetone and quantified according to the procedure reported in Lichtenthaler

(1987). Key antioxidant enzymes, including superoxide dismutase (SOD; EC 1.15.1.1), ascorbate peroxidase (APX; EC 1.11.1.11), catalase (CAT; EC 1.11.1.6) and glutathione reductase (GR; EC 1.6.4.2), were extracted by grinding with a cold mortar and pestle with polyvinylpolypyrrolidone and appropriate extraction buffers as described in Pinheiro et al. (2004). Total SOD activity was determined by measuring its ability to inhibit the photochemical reduction of *p*-nitro-blue-tetrazolium chloride at 560 nm. The activity of CAT was estimated by measuring the rate of decomposition of H_2O_2 at 240 nm; total APX activity was estimated by monitoring the decline in absorbance at 290 nm, and GR activity was assessed by measuring the rate of NADPH oxidation at 340 nm. Further details have been reported previously (Pinheiro et al. 2004). Cellular damage was analyzed through malondialdehyde (MDA) accumulation, estimated as the content of total 2-thiobarbituric acid-reactive substances, as detailed in Lima et al. (2002).

Other measurements

The leaf water potential (Ψ_w) was measured before dawn (04:30–05:30 h) and at midday using a Scholander-type pressure chamber (model 1000, PMS Instruments, Albany, NY). The specific leaf area (SLA; leaf area per unit leaf dry mass) was estimated using 20 leaf discs (each 14 mm in diameter). Indirect estimates of photosynthetic N use efficiency (PNUE) were computed as the ratio between mass-based A_{max} and total N in the leaves.

Statistics

The experiment was conducted following a completely randomized design and was analyzed in a factorial (two clones and three-light treatments) scheme. The data were analyzed by two-way ANOVA, and the means were compared using the Newman–Keuls and *t*-tests at $P \leq 0.05$. All of the statistical analyses were performed using the SAEG SYSTEM version 9.1 (SAEG, 2007).

Results

Environment

As can be deduced from Fig. 2, the total daily PAR over the coffee canopies was 54.0 mol m^{-2} day^{-1} in the FS treatment and decreased by 27 and 29% in SM and SA treatments, respectively. From sunrise to midday, the SM plants received 11.4 mol photons m^{-2} (44% less than in SA plants), whereas from midday to sunset the SA plants received 12.1 mol photons m^{-2} (43% less than their SM counterparts). For the gas-exchange measurements, the shade provided by the rubber trees was translated into milder microclimatic conditions [lower air (up to 3.0°C) and leaf (up to 5.7°C) temperatures and lower leaf-to-air VPD (up to 48%)] than in FS environments (Table 1). Leaf temperature reached values as high as 42.8°C, paralleling the elevated leaf-to-air VPD as high as 5.9 kPa, found in clone 120 during the measurements conducted at 11:00–13:00 h. Notably, in these measurements, and independent of the clone, both the leaf temperature and leaf-to-air VPD were significantly higher in FS clones than in either SM or SA clones (Table 1).

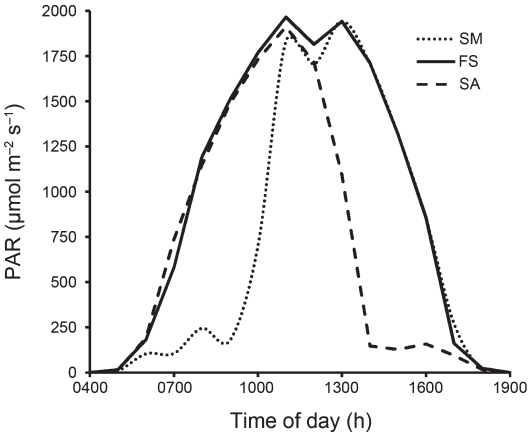

Fig. 2. The time course of the photosynthetically active radiation (PAR) over the coffee canopies. Three PAR treatments were established, defined as follows: coffee bushes shaded in the morning and exposed to full sunlight in the afternoon (SM); bushes receiving full sunlight during most of the day (FS); and bushes exposed to full sunlight during the morning and shaded in the afternoon (SA).

Leaf water potential and specific leaf area

Independent of the clones and the light treatments studied, the Ψ_w before dawn was greater than −0.20 MPa, whereas the Ψ_w at midday was significantly less negative in SM plants than in their FS and SA counterparts, which did not differ from one another in either clone (Table 2).

In clone 03, the SLAs were significantly lower (8%) in FS plants than in their SM and SA counterparts, which did not differ from one another, whereas in clone 120 the SLAs of SM and FS plants were similar and lower than in SA plants (Table 2).

Gas exchanges

Differences in the magnitude of gas exchange were particularly evident in the morning when clone 03 displayed higher g_s, *E* and *A* than clone 120 (Table 1).

Table 1. The air (T_A; °C) and leaf (T_L; °C) temperatures, leaf-to-air vapor pressure deficit (VPD; kPa), net CO_2 assimilation rate (A; μmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s; mmol H_2O m^{-2} s^{-1}), transpiration rate (mmol H_2O m^{-2} s^{-1}), internal-to-ambient CO_2 concentration ratio (C_i/C_a; μmol CO_2 mol^{-1} air) and variable-to-maximum Chl fluorescence ratio (F_v/F_m), as measured in three time points at approximately 08:00–10:00 h, 11:00–13:00 h and 14:00–16:00 h in two clones of robusta coffee subjected to three light treatments, defined as follows: clones shaded in the morning and exposed to full sunlight in the afternoon (SM); clones receiving full sunlight during most of the day (FS); and clones exposed to full sunlight during the morning and shaded in the afternoon (SA). Within each clone, capital letters denote significant differences among light treatments; within each light treatment, small letters denote significant differences between clones ($P \leq 0.05$, Newman–Keuls' and t-tests). $n = 6 \pm$ SE.

	Clone 03			Clone 120		
Parameters	SM	FS	SA	SM	FS	SA
08:00–10:00 h						
T_A	35.4 ± 0.6^{Ba}	37.4 ± 0.8^{Aa}	38.5 ± 0.1^{Aa}	35.4 ± 0.6^{Ba}	37.4 ± 0.8^{Aa}	38.5 ± 0.1^{Aa}
T_L	33.9 ± 0.5^{Cb}	37.8 ± 0.5^{Cb}	39.6 ± 0.1^{Aa}	35.5 ± 0.4^{Ba}	39.6 ± 0.3^{Aa}	39.9 ± 0.1^{Aa}
VPD	2.1 ± 0.2^{Cb}	3.4 ± 0.2^{Bb}	4.0 ± 0.0^{Aa}	2.9 ± 0.2^{Ba}	4.2 ± 0.1^{Aa}	4.2 ± 0.1^{Aa}
A	6.3 ± 0.1^{Aa}	6.1 ± 0.1^{Aa}	5.8 ± 0.2^{Aa}	5.2 ± 0.2^{Ab}	2.9 ± 0.1^{Cb}	3.7 ± 0.1^{Bb}
g_s	160 ± 8^{Aa}	96 ± 3^{Ba}	80 ± 4^{Ba}	122 ± 13^{Ab}	40 ± 2^{Bb}	52 ± 3^{Bb}
E	3.5 ± 0.2^{Aa}	3.3 ± 0.2^{Aa}	3.2 ± 0.1^{Aa}	2.9 ± 0.0^{Aa}	1.7 ± 0.0^{Cb}	2.3 ± 0.1^{Bb}
C_i/C_a	0.77 ± 0.01^{Aa}	0.66 ± 0.01^{Ba}	0.62 ± 0.01^{Ba}	0.74 ± 0.01^{Aa}	0.63 ± 0.01^{Ba}	0.63 ± 0.01^{Ba}
F_v/F_m	0.83 ± 0.00^{Aa}	0.81 ± 0.01^{ABa}	0.80 ± 0.01^{Ba}	0.81 ± 0.01^{Aa}	0.77 ± 0.02^{Aa}	0.80 ± 0.01^{Aa}
11:00–13:00 h						
T_A	37.9 ± 0.1^{Ca}	40.4 ± 0.1^{Aa}	39.6 ± 0.1^{Ba}	37.9 ± 0.1^{Ca}	40.4 ± 0.1^{Aa}	39.6 ± 0.1^{Ba}
T_L	39.9 ± 0.2^{Ba}	41.6 ± 0.1^{Ab}	38.8 ± 0.1^{Cb}	38.9 ± 0.2^{Cb}	42.8 ± 0.3^{Aa}	40.7 ± 0.2^{Ba}
VPD	4.5 ± 0.1^{Ba}	5.3 ± 0.0^{Ab}	4.5 ± 0.0^{Bb}	4.2 ± 0.2^{Ca}	5.9 ± 0.1^{Aa}	5.0 ± 0.1^{Ba}
A	5.2 ± 0.1^{Aa}	2.5 ± 0.2^{Ba}	1.7 ± 0.1^{Cb}	4.9 ± 0.9^{Aa}	1.9 ± 0.1^{Cb}	3.0 ± 0.2^{Ba}
g_s	65 ± 2^{Aa}	31 ± 2^{Ba}	19 ± 1^{Cb}	63 ± 5^{Aa}	24 ± 1^{Cb}	37 ± 2^{Ba}
E	2.9 ± 0.0^{Aa}	1.7 ± 0.5^{Ba}	0.9 ± 0.0^{Cb}	2.6 ± 0.1^{Ab}	1.6 ± 0.1^{Ba}	1.8 ± 0.1^{Ba}
C_i/C_a	0.59 ± 0.01^{Aa}	0.59 ± 0.00^{Aa}	0.56 ± 0.01^{Ab}	0.61 ± 0.00^{Aa}	0.61 ± 0.01^{Aa}	0.61 ± 0.01^{Aa}
F_v/F_m	0.82 ± 0.00^{Aa}	0.81 ± 0.00^{Aa}	0.82 ± 0.00^{Aa}	0.82 ± 0.00^{Aa}	0.79 ± 0.01^{Ba}	0.82 ± 0.00^{Aa}
14:00–16:00 h						
T_A	35.8 ± 0.5^{Cb}	34.3 ± 0.4^{Cb}	32.5 ± 0.4^{Aa}	35.8 ± 0.5^{Cb}	34.3 ± 0.4^{Cb}	32.5 ± 0.4^{Aa}
T_L	37.4 ± 0.6^{Aa}	36.1 ± 0.4^{Ab}	32.1 ± 0.0^{Bb}	37.4 ± 0.0^{Aa}	34.6 ± 0.8^{Bb}	33.3 ± 0.0^{Ca}
VPD	4.1 ± 0.2^{Aa}	3.6 ± 0.1^{Ba}	2.4 ± 0.0^{Ca}	4.0 ± 0.0^{Aa}	3.2 ± 0.3^{Ba}	2.7 ± 0.0^{Ca}
A	1.4 ± 0.2^{Aa}	1.0 ± 0.1^{Aa}	0.5 ± 0.1^{Ba}	1.5 ± 0.6^{Aa}	0.9 ± 0.1^{Ba}	0.8 ± 0.1^{Ba}
g_s	16 ± 1.9^{Aa}	16 ± 0.0^{Aa}	8 ± 0.7^{Bb}	22 ± 0.1^{Aa}	17 ± 0.2^{Ba}	12 ± 0.2^{Ca}
E	0.8 ± 0.1^{Aa}	0.6 ± 0.1^{Aa}	0.2 ± 0.0^{Ba}	0.9 ± 0.1^{Aa}	0.6 ± 0.1^{Ba}	0.3 ± 0.0^{Ca}
C_i/C_a	0.78 ± 0.10^{Aa}	0.69 ± 0.01^{Aa}	0.70 ± 0.01^{Aa}	0.81 ± 0.06^{Aa}	0.71 ± 0.01^{Aa}	0.68 ± 0.01^{Aa}
F_v/F_m	0.79 ± 0.03^{Aa}	0.76 ± 0.02^{Aa}	0.81 ± 0.01^{Aa}	0.79 ± 0.05^{Aa}	0.79 ± 0.01^{Aa}	0.81 ± 0.01^{Aa}

Table 2. The leaf water potential at predawn (Ψ_{pd}; MPa) and midday (Ψ_{md}; MPa) and the specific leaf area (SLA; m^2 kg^{-1}) in two clones of robusta coffee subjected to three light treatments, defined as follows: clones shaded in the morning and exposed to full sunlight in the afternoon (SM); clones receiving full sunlight during most of the day (FS); and clones exposed to full sunlight during the morning and shaded in the afternoon (SA). Statistics are defined as in Table 1.

	Clone 03			Clone 120		
Parameters	SM	FS	SA	SM	FS	SA
Ψ_{pd}	-0.14 ± 0.02^{Ab}	-0.14 ± 0.03^{Aa}	-0.17 ± 0.03^{Aa}	-0.07 ± 0.01^{Aa}	-0.14 ± 0.03^{Ba}	-0.19 ± 0.04^{Ba}
Ψ_{md}	-0.84 ± 0.06^{Aa}	-1.23 ± 0.08^{Ba}	-1.17 ± 0.05^{Ba}	-0.92 ± 0.07^{Aa}	-1.32 ± 0.05^{Ba}	-1.19 ± 0.05^{Ba}
SLA	12.8 ± 0.3^{Aa}	11.7 ± 0.2^{Ba}	12.7 ± 0.3^{Aa}	11.3 ± 0.2^{Bb}	10.7 ± 0.4^{Bb}	12.8 ± 0.3^{Aa}

The highest g_s was found in mid-morning in all treatments, after which g_s decreased progressively throughout the day. Changes in g_s were closely tracked by changes in A (Table 1), as further highlighting the strong relationship between A and g_s ($r \geq 0.85$; data not shown). Overall, the microclimate alterations caused by the shade around the SM plants in the morning appeared to enable better gas-exchange performance (higher g_s, E and A) in comparison to both SA and FS clones in both afternoon evaluations. This enhanced performance was translated into a higher diurnal carbon gain in the SM leaves. In any case, the F_v/F_m remained unchanged ($\geq$0.76) independent of the treatments (Table 1).

There were no significant differences in LSP among the treatments. In both clones, the LCP did not differ significantly when comparing the FS and SA plants;

Table 3. Photosynthetic variables derived from the net photosynthetic rates (A) and irradiance curves [light compensation point (LCP; μmol photons m^{-2} s^{-1}), light saturation point (LSP; μmol photons m^{-2} s^{-1}), dark respiration (R_d; μmol CO_2 m^{-2} s^{-1}) and light-saturated A (A_{max}) per unit leaf area (μmol CO_2 m^{-2} s^{-1}) or leaf mass (μmol kg^{-1} DW s^{-1})], and from A and internal CO_2 concentration (A/C_i) curves [maximum rate of carboxylation limited by electron transport (J_{max}; μmol e^- m^{-2} s^{-1}) and maximum rate of carboxylation (V_{cmax}; μmol CO_2 m^{-2} s^{-1})] in two clones of robusta coffee subjected to three light treatments, defined as follows: clones shaded in the morning and exposed to full sunlight in the afternoon (SM); clones receiving full sunlight during most of the day (FS); and clones exposed to full sunlight during the morning and shaded in the afternoon (SA). Statistics are defined as in Table 1.

	Clone 03			Clone 120		
Parameters	SM	FS	SA	SM	FS	SA
LCP	10.7 ± 0.3^{Cb}	27.6 ± 0.3^{Aa}	20.7 ± 0.3^{Ba}	18.7 ± 1.8^{Aa}	24.8 ± 1.5^{Aa}	20.7 ± 1.2^{Aa}
LSP	599 ± 52^{Aa}	567 ± 45^{Aa}	660 ± 29^{Aa}	552 ± 47^{Aa}	560 ± 15^{Aa}	582 ± 447^{Aa}
R_d	1.0 ± 0.1^{Ba}	1.4 ± 0.2^{Aa}	1.4 ± 0.2^{Aa}	1.3 ± 0.2^{Aa}	1.6 ± 0.1^{Aa}	1.4 ± 0.1^{Aa}
A_{max} (area)	10.7 ± 0.3^{Aa}	7.2 ± 0.2^{Ca}	8.8 ± 0.1^{Ba}	9.2 ± 0.5^{Aa}	7.0 ± 0.5^{Ba}	7.8 ± 0.1^{Bb}
A_{max} (mass)	138 ± 6^{Aa}	85 ± 3^{Ca}	110 ± 5^{Ba}	103 ± 4^{Ab}	76 ± 6^{Ba}	100 ± 4^{Aa}
J_{max}	73.3 ± 7.4^{Bb}	72.1 ± 8.5^{Ba}	102 ± 6^{Aa}	100 ± 4^{Aa}	76.3 ± 8.9^{Aa}	100 ± 8^{Aa}
V_{cmax}	81.3 ± 11.7^{Ab}	89.6 ± 2.9^{Aa}	91.0 ± 4.8^{Aa}	109 ± 6^{Aa}	79.1 ± 4.4^{Ba}	98.0 ± 3.7^{Aa}

however, the lowest LCP was found in SM individuals, which was a significant difference in the case of clone 03 (Table 3). The R_d tended to be lower in SM plants when compared with plants from the other light treatments, and again, the difference was significant for clone 03. In this clone, the highest values of A_{max} (both on area and mass bases) were found in SM plants and the lowest in FS plants, with intermediate values in SA individuals; in clone 120, area-based A_{max} was higher in SM than in FS and SA plants, which did not differ from one another, whereas mass-based A_{max} was similar in SM and SA plants, but higher than in their FS counterparts (Table 3).

Responses of A to C_i (A/C_i curves) reveal that J_{max} was significantly lower (24–30%) in both the SM and FS plants than in their SA counterparts in clone 03; in clone 120, J_{max} was unresponsive to the PAR treatments (Table 3). The V_{cmax} did not vary among the light regimens in clone 03; in clone 120, it was lower in FS plants than in plants from the other treatments (Table 3).

Photosynthetic pigments, antioxidant enzymes and cellular damages

The SM plants of clone 03 displayed larger concentrations of Chl (63% on average) and Car (25%) and Chl/N ratios (58% on average) than the FS and SA plants, which were similar to one another. The Chl/Car ratio was larger in SM than in FS plants, with intermediate values in SA plants (Table 4). These parameters were unresponsive to the light treatments in clone 120. Clonal differences were noted in SA plants, but only for the Chl and the Chl/N ratio, which were lower in clone 03 than in clone 120. The Chl *a*/*b* ratio remained unchanged irrespective of treatments (Table 4).

Table 4. Concentrations of total chlorophylls (Chl; g kg^{-1} DM) and carotenoids (Car; g kg^{-1} DM)) and the ratios of Chl/N (mmol mol^{-1}), Chl/Car and Chl *a*/*b*, activities of antioxidant enzymes [superoxide dismutase (SOD; kU min^{-1} g^{-1} FM), ascorbate peroxidase (APX; μmol min^{-1} g^{-1} FM), glutathione reductase (GR; μmol min^{-1} g^{-1} FM) and catalase (CAT; μmol min^{-1} g^{-1} FM)], and the concentration of malondialdehyde (MDA; μmol kg^{-1} FM) in leaves of two clones of robusta coffee subjected to three light treatments defined as follows: clones shaded in the morning and exposed to full sunlight in the afternoon (SM); clones receiving full sunlight during most of the day (FS); and clones exposed to full sunlight during the morning and shaded in the afternoon (SA). Statistics are defined as in Table 1.

	Clone 03			Clone 120		
Parameters	SM	FS	SA	SM	FS	SA
Chl	11.1 ± 1.3^{Aa}	6.7 ± 0.3^{Ba}	7.2 ± 0.3^{Bb}	9.9 ± 0.7^{Aa}	8.0 ± 0.7^{Aa}	9.7 ± 0.5^{Aa}
Car	2.0 ± 0.1^{Aa}	$1.5 \pm 0.1A^{Ba}$	1.5 ± 0.2^{Ba}	1.9 ± 0.2^{Aa}	1.7 ± 0.2^{Aa}	1.9 ± 0.2^{Aa}
Chl/N	5.2 ± 0.6^{Aa}	3.2 ± 0.2^{Ba}	3.4 ± 0.1^{Bb}	4.8 ± 0.2^{Aa}	3.9 ± 0.3^{Aa}	4.5 ± 0.3^{Aa}
Chl/Car	5.5 ± 0.3^{Aa}	4.4 ± 0.1^{Ba}	4.9 ± 0.1^{ABa}	5.4 ± 0.4^{Aa}	4.9 ± 0.2^{Aa}	5.4 ± 0.4^{Aa}
Chl *a*/*b*	2.5 ± 0.2^{Aa}	2.9 ± 0.1^{Aa}	2.9 ± 0.1^{Aa}	2.4 ± 0.1^{Aa}	2.8 ± 0.1^{Aa}	2.6 ± 0.2^{Aa}
SOD	1.1 ± 0.1^{Ba}	1.5 ± 0.1^{Aa}	1.2 ± 0.1^{ABa}	1.1 ± 0.1^{ABa}	1.4 ± 0.1^{Aa}	1.0 ± 0.1^{Ba}
APX	22.4 ± 3.0^{Ba}	36.2 ± 4.3^{Ab}	23.8 ± 2.4^{Ba}	30.9 ± 3.4^{Ba}	45.7 ± 2.4^{Aa}	31.4 ± 2.7^{Ba}
GR	1.8 ± 0.1^{Ba}	2.7 ± 0.1^{Aa}	1.8 ± 0.2^{Ba}	1.6 ± 0.2^{Ba}	3.1 ± 0.2^{Aa}	1.9 ± 0.1^{Ba}
CAT	1.2 ± 0.1^{Ba}	2.9 ± 0.1^{Ab}	1.3 ± 0.1^{Ba}	1.1 ± 0.2^{Ba}	4.3 ± 0.1^{Aa}	1.3 ± 0.1^{Ba}
MDA	32.1 ± 1.7^{Aa}	32.7 ± 1.3^{Aa}	31.8 ± 1.5^{Aa}	35.2 ± 1.2^{Aa}	32.7 ± 1.1^{Aa}	33.0 ± 1.0^{Aa}

Table 5. The construction (CC; g glucose g^{-1} DM) and maintenance (MC; mg glucose g^{-1} DM day^{-1}) costs, photosynthetic nitrogen use efficiency (PNUE; µmol CO_2 g^{-1} N s^{-1}), average daily instantaneous water use efficiency (*A*/*E*; mmol mol^{-1}) and carbon isotope discrimination ($\Delta^{13}C$; ‰) in two clones of robusta coffee subjected to three light treatments defined as follows: clones shaded in the morning and exposed to full sunlight in the afternoon (SM); clones receiving full sunlight during most of the day (FS); and clones exposed to full sunlight during the morning and shaded in the afternoon (SA). Statistics are defined as in Table 1.

	Clone 03			Clone 120		
Parameters	SM	FS	SA	SM	FS	SA
CC	1.28 ± 0.02^{Aa}	1.33 ± 0.02^{Aa}	1.29 ± 0.02^{Aa}	1.28 ± 0.0^{Aa}	1.29 ± 0.02^{Aa}	1.28 ± 0.03^{Aa}
MC	16.1 ± 0.4^{Aa}	15.7 ± 0.3^{Aa}	16.3 ± 0.3^{Aa}	16.9 ± 0.6^{Aa}	15.9 ± 0.2^{Aa}	16.6 ± 0.3^{Aa}
PNUE	4.56 ± 0.07^{Aa}	2.92 ± 0.07^{Ba}	3.72 ± 0.06^{Ca}	3.49 ± 0.08^{Ab}	2.59 ± 0.01^{Aa}	3.37 ± 0.03^{Ba}
A/*E*	1.98 ± 0.18^{Aa}	1.80 ± 0.23^{Aa}	2.13 ± 0.22^{Aa}	1.82 ± 0.14^{Aa}	1.67 ± 0.25^{Aa}	1.95 ± 0.23^{Aa}
$\Delta^{13}C$	21.0 ± 0.5^{Ba}	23.0 ± 0.2^{Aa}	19.4 ± 0.1^{Ca}	19.5 ± 0.5^{Bb}	21.8 ± 0.2^{Ab}	18.9 ± 0.1^{Ba}

Little, if any, differences in the activity of key antioxidant enzymes were found in either clone when comparing plants from the SM and SA treatments (Table 4). In contrast, increases in the activities of SOD (though not significant in clone 03), APX, GR and particularly in CAT were found in FS plants in both clones, suggesting an augmented oxidative pressure in these plants. However, the MDA concentration was unresponsive to the PAR treatments regardless of the clone analyzed (Table 4).

Construction and maintenance costs and resource use efficiencies

The CC and MC were unaffected by the treatments (Table 5). This response was accompanied by small, if any, changes in the leaf chemical composition based on the major constituents analyzed (Table S1).

In both clones, the PNUE was significantly lower in FS plants than in SM and SA plants. In clone 03, PNUE was higher in SM than in SA plants, whereas in clone 120 it did not differ between the SM and SA individuals (Table 5).

In both clones, the daily instantaneous WUE (*A*/*E*) did not differ significantly in response to varying PAR conditions. In contrast, a relatively large amplitude (18.9–23.0‰) in $\Delta^{13}C$ (a proxy inversely related to long-term WUE) was noted in response to the treatments. In clone 03, $\Delta^{13}C$ was higher in FS plants and lower in SA plants, with intermediate values in SM individuals (Table 5). In clone 120, $\Delta^{13}C$ was higher in FS plants than in their SM and SA counterparts, which did not differ from one another. In either clone, significant correlations ($r \geq 0.57$) between *A*/*E* and $\Delta^{13}C$ were found (Fig. S1).

Discussion

The experimental design created an environment in which the total, daily integrated PAR was quite similar over the canopies of SM and SA clones, and therefore, we could examine the effects of temporal variations of light availability under similar total daily radiation inputs in the shaded plants (Fig. 2). In contrast to our working hypothesis, we found that SM clones displayed better gas-exchange performance throughout the day (Table 1). We additionally demonstrated that there were varying abilities for coping with PAR alterations between the clones we analyzed. This information suggests that considerable phenotypic plasticity may exist in robusta coffee, which may be explored for selecting promising genotypes to be intercropped with shelter trees.

Gas exchanges

The highest Ψ_w at midday, as found in SM plants despite their higher g_s and *E* (Tables 1 and 2), suggests improved tissue hydration that, together with low leaf temperature and leaf-to-air VPD throughout the morning, should have allowed the SM plants to sustain higher *A* in comparison with the SA and FS plants (Table 1).

The FS plants were subjected to the harshest environmental conditions (high cumulative temperature, leaf-to-air VPD and radiation loads), which could directly affect their *A*. Despite the strong relationship between *A* and g_s, we believe that stomatal limitations should not have played a major role in constraining carbon fixation in FS plants. Compelling evidence for this conclusion comes from the fact that the FS individuals from both clones displayed the lowest A_{max} and, in addition, V_{cmax} was also depressed in FS plants relative to SM and SA individuals, as found in clone 120. Notably, the FS plants displayed the highest discrimination against $^{13}CO_2$ (higher $\Delta^{13}C$) (Table 5). Because increases in $\Delta^{13}C$ (which expresses the magnitude of gas exchange over time instead of a discrete measurement) can arise as of high g_s or low *A* (Farquhar et al. 1989), it is likely that, in the long term, biochemical limitations at the chloroplast level are the primary constraints to carbon fixation in FS plants.

In the case of SA plants, although area-based A_{max} was lower than in SM plants, both J_{max} and V_{cmax} were kept at high values (Table 3), suggesting that the biochemical capacity for CO_2 fixation was preserved. However, the relatively low in situ A (Table 1) suggests that other resources (e.g. water) and environmental conditions were less favorable for carbon gain. In any case, the strong relationship between A and g_s coupled with the absolute lowest $\Delta^{13}C$ values (Table 5) displayed by these plants indicates that stomatal factors played a prominent role in limiting A.

Antioxidative protection

Considering that carbon fixation, the usual main sink for the absorbed PAR in chloroplasts, was depressed, especially in FS and SA plants and particularly in the afternoon, adjustments in light capture, use and dissipation are required to provide photoprotection to the photosynthetic apparatus. Here, we showed that adjustments in the activity of key antioxidant enzymes (Table 4) associated with alternative pathways for electron flow, such as photorespiration (CAT) and the Mehler-peroxidase reaction (APX, GR) (Logan et al. 2006, Wilhelm and Selmar 2011), could play important roles in dissipating the excess reducing power. Regardless of both the clone and temporal changes in A, such adjustments were notably responsive to the total amount of PAR received because of the enzyme activities differed minimally when comparing the SM and SA plants, but were much larger in FS plants. Additionally, increases in non-photochemical quenching, which has been associated with large zeaxanthin pools and higher de-epoxidation state of the xanthophyll cycle with increasing light availability in coffee (Matos et al. 2009), may also provide photoprotection through thermal dissipation (Rodríguez-Calcerrada et al. 2008, Wilhelm and Selmar 2011). Collectively, these adjustments proved to be sufficient for avoiding photoinhibition and photooxidative damage even under the harsh environmental conditions shown here, as judged from the high F_v/F_m ratio (Table 1) and unchanged MDA concentration (Table 4).

Acclimation to varying PAR supply: costs and efficiencies

Both the CC and MC were virtually unchanged regardless of the clone and PAR treatments (Table 5), probably reflecting the minimal changes in the leaf chemical composition among the treatments (Poorter et al. 2006). In any case, the higher diurnal carbon gain per unit leaf area (with unaltered CC) in SM leaves compared to their FS and SA counterparts suggests a lower time span in which the SM leaves must photosynthesize to recover (amortize) the carbon investment used in their construction (payback time) (Poorter et al. 2006).

We showed that clone 03 was better able than clone 120 to acclimate to temporal alterations of PAR supply to enhance light capture. Such acclimation ability should help clone 03 to optimize the carbon gain when the environmental conditions are more conducive for higher rates of gas exchanges, as found in the morning even though the PAR supply is limiting. In this clone, the SM and SA individuals displayed relatively high SLAs (Table 2), which may improve light harvesting per unit of resources invested in construction of photosynthetic tissues (Walters 2005, Lusk et al. 2008); however, the SM plants were better able than the SA plants to acclimate to low PAR via physiological traits, e.g. changes in Chl pools and Chl/N and Chl/Car ratios (Table 4), which indicate an improved ability for light capture. Furthermore, the SM leaves acclimated to the PAR supply by decreasing both the R_d and LCP (Table 3). Overall, these responses suggest an improved light use efficiency when PAR is limiting. Additionally, the SM leaves of clone 03 displayed improved PNUE and higher mass-based A_{max}, which are indicative that, for a given investment (N or biomass), the photosynthetic return is likely to be higher in SM than in SA individuals. Collectively, these acclimations may be interpreted as an evidence of coordinated physiological strategies associated with efficient use of resources in the SM leaves. In clone 120, acclimation to temporal changes of PAR was much less apparent because R_d, LCP, PNUE and mass-based A_{max} were similar between the SM and SA leaves, whereas the SLA was even higher in SA leaves. In any case, both PNUE and mass-based A_{max} were higher in these kinds of leaves than in their FS counterparts, suggesting impaired resource use efficiency under full sunlight conditions under the present experimental conditions.

The slight differences in instantaneous WUE among the treatments were promptly reflected in $\Delta^{13}C$ (which has been used as an inverse proxy for long-term WUE; Farquhar et al. 1989), a pattern (Fig. S1) consistent with other studies (e.g. Erice et al. 2011). Thus, the lowest $\Delta^{13}C$ of FS plants (Table 5) may be assumed as a compelling evidence of lower long-term WUE of these plants, implying that the FS plants were unable to use the extra PAR received by them. Taking this and all the above information together, our results demonstrate that full sunlight conditions may indeed be detrimental for the efficient use of resources by the robusta coffee bushes grown in harsh environments.

Concluding remarks

We demonstrated that shading, particularly in the morning, may improve the physiological performance of coffee bushes growing in a harsh, tropical environment. We also demonstrated that photosynthetic acclimation in response to varying PAR depends on both the clone and nature of the light environment. Importantly, acclimations to varying PAR supplies had no apparent additional cost for constructing and maintaining the leaves regardless of the PAR supply received by them. Overall, both the SM and SA individuals displayed higher return in terms of revenue streams (e.g. higher area- and mass-based A_{max}, PNUE and long-term WUE) than their FS counterparts. Overall, our data lend support for explaining, at least partially, the successful cultivation of coffee bushes intercropped with shelter trees, as has empirically been observed in agroforestry systems implanted recently in warm, marginal regions (DaMatta et al. 2010). Finally, when adopting intercropping systems, it is important to select coffee genotypes with adequate phenotypic plasticity to cope with reduced light supply, as particularly found in clone 03.

Acknowledgements – We gratefully acknowledge the Institute for Research and Rural Assistance of the Espírito Santo State (INCAPER) for providing the working facilities. This research was supported by the Brazilian Consortium for Coffee Research and Development. Scholarships granted by the Ministry of Foreign Relations (N. F. R.-L.), the National Council for Scientific and Technological Development, CNPq (P. C. C., S. C. V. M.) and the Brazilian Federal Agency for Support and Evaluation of Graduate Education (P. E. M. S., L. E. M., E. F. M.), all from the Brazilian Government, are gratefully acknowledged. Research fellowships granted by CNPq and FAPEMIG to F. M. D. are also acknowledged.

References

Araújo WL, Dias PC, Moraes GABK, Celin EF, Cunha RL, Barros RS, DaMatta FM (2008) Limitations to photosynthesis in coffee leaves from different canopy positions. Plant Physiol Biochem 46: 884–890

Boardman NK (1977) Comparative photosynthesis of sun and shade plants. Annu Rev Plant Physiol 28: 355–377

Cavatte PC, Oliveira AAG, Morais LE, Martins SCV, Sanglard LMVP, DaMatta FM (2012a) Could shading reduce the negative impacts of drought on coffee? A morphophysiological analysis. Physiol Plant 144: 111–122

Cavatte PC, Rodríguez-López NF, Martins SCV, Mattos MS, Sanglard LMVP, DaMatta FM (2012b) Functional analysis of the relative growth rate, chemical composition, construction and maintenance costs, and the payback time of *Coffea arabica* L. leaves in response to light and water availability. J Exp Bot 63: 3071–3082

Chaves ARM, Caten AT, Pinheiro HA, Ribeiro A, DaMatta FM (2008) Seasonal changes in photoprotective mechanisms of leaves from shaded and unshaded field-grown coffee (*Coffea arabica* L.) trees. Trees 22: 351–361

DaMatta FM (2004) Ecophysiological constraints on the production of shaded and unshaded coffee: a review. Field Crops Res 86: 99–114

DaMatta FM, Chaves ARM, Pinheiro HA, Ducatti C, Loureiro ME (2003) Drought tolerance of two field-grown clones of *Coffea canephora*. Plant Sci 164: 111–117

DaMatta FM, Ronchi CP, Maestri M, Barros RS (2010) Coffee: environment and crop physiology. In: DaMatta FM (ed) Ecophysiology of Tropical Tree Crops. Nova Science Publishers, New York, pp 181–216

Erice G, Louahlia G, Irigoyen JJ, Sánchez-Díaz M, Alami T, Avice JC (2011) Water use efficiency, transpiration and net CO_2 exchange of four alfalfa genotypes submitted to progressive drought and subsequent recovery. Environ Exp Bot 72: 123–130

Evans JR, Poorter H (2001) Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. Plant Cell Environ 24: 755–767

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. Annu Rev Plant Physiol Plant Mol Biol 40: 503–537

Lichtenthaler HK (1987) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Meth Enzymol 148: 350–382

Lima ALS, DaMatta FM, Pinheiro HA, Totola MR, Loureiro ME (2002) Photochemical responses and oxidative stress in two clones of *Coffea canephora* under water deficit conditions. Environ Exp Bot 47: 239–247

Logan BA, Kornyeyev D, Hardison J, Holaday AS (2006) The role of antioxidant enzymes in photoprotection. Photosynth Res 88: 119–132

Lusk C, Reich PB, Montgomery RA, Ackerly DD, Cavender-Bares J (2008) Why are evergreen leaves so contrary about shade? Trends Ecol Evol 23: 299–303

Matos FS, Wolfgramm R, Gonçalves FV, Cavatte PC, Ventrella MC, DaMatta FM (2009) Phenotypic plasticity in response to light in the coffee tree. Environ Exp Bot 67: 421–427

Merino JA, Field CB, Mooney HA (1984) Construction and maintenance costs of Mediterranean-climate evergreen and deciduous leaves. II. Biochemical pathway analysis. Acta Oecol 5: 211–229

Niinemets U (2007) Photosynthesis and resource distribution through plant canopies. Plant Cell Environ 30: 1052–1071

Ort DR, Baker NR (2002) A photoprotective role for O_2 as an alternative electron sink in photosynthesis? Curr Opin Plant Biol 5: 193–198
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. Academic Press, San Diego, pp 175–208
Penning de Vries FWT, Brunsting AHM, van Laar HH (1974) Products, requirements and efficiency of biosynthesis: a quantitative approach. J Theor Biol 45: 339–377
Pinheiro HA, DaMatta FM, Chaves ARM, Fontes EPB, Loureiro ME (2004) Drought tolerance in relation to protection against oxidative stress in clones of *Coffea canephora* subjected to long-term drought. Plant Sci 167: 1307–1314
Poorter H, Villar R (1997) The fate of acquired carbon in plants: Chemical composition and construction costs. In: Bazzaz FA, Grace J (eds) Resource Allocation in Plants. Academic Press, San Diego, pp 39–72
Poorter H, Pepin S, Rijkers T, DeJong Y, Evans JR, Körner C (2006) Construction costs, chemical composition and payback time of high- and low-irradiance leaves. J Exp Bot 57: 355–371
Prieto JA, Louarn G, Perez-Peña J, Ojeda H, Simonneau T, Lebon E (2012) A leaf gas exchange model that accounts for intra-canopy variability by considering leaf nitrogen content and local acclimation to radiation in grapevine (*Vitis vinifera* L.). Plant Cell Environ 35: 1313–1328
Rodríguez-Calcerrada J, Reich PB, Rosenqvist E, Pardos JA, Cano FJ, Aranda I (2008) Leaf physiological versus morphological acclimation to high-light exposure at different stages of foliar development in oak. Tree Physiol 28: 761–771
SAEG (2007) Sistema de análises estatísticas e genéticas: versão 9.1. Fundação Arthur Bernardes Viçosa
Silva PEM, Cavatte PC, Morais LE, Medina EF, DaMatta FM (2012) The functional divergence of biomass partitioning, carbon gain and water use in *Coffea canephora* in response to the water supply: implications for breeding aimed at improving drought tolerance. Environ Exp Bot. DOI: 10.1016/j.envexpbot.2012.09.005
Sims DA, Pearcy RW (1993) Sunfleck frequency and duration affects growth rate of the understorey plant, *Alocasia macrorrhiza*. Funct Ecol 7: 683–689
Valladares F, Niinemets U (2008) Shade tolerance, a key plant feature of complex nature and consequences. Annu Rev Ecol Evol Syst 39: 237–257
Walters RG (2005) Towards an understanding of photosynthetic acclimation. J Exp Bot 56: 435–447
Wayne P, Bazzaz FA (1993) Birch seedling responses to daily time courses of light in experimental forest gaps and shadehouses. Ecology 74: 1500–1515
Wilhelm C, Selmar D (2011) Energy dissipation is an essential mechanism to sustain the viability of plants: The physiological limits of improved photosynthesis. J Plant Physiol 168: 79–87
Williams K, Percival F, Merino J, Mooney HA (1987) Estimation of tissue construction cost from heat of combustion and organic nitrogen content. Plant Cell Environ 10: 725–734

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. The leaf chemical composition in two clones of robusta coffee subjected to three light treatments.

Fig. S1. The relationship between instantaneous water use efficiency and carbon isotope discrimination in two clones of robusta coffee.

Edited by A. Krieger-Liszkay