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Why is it better to produce coffee seedlings in full sunlight than in the shade? A morphophysiological approach

G.A.B.K. MORAES, A.R.M. CHAVES, S.C.V. MARTINS, R.S. BARROS, and F.M. DaMATTA+

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

Abstract

The coffee plant is native to shaded environments and its seedlings are often produced in shaded nurseries. However, some nursery managers, in an effort to improve the acclimation of seedlings to field conditions after transplantation, produce seedlings in full sun exposure. In this study, the morphological and physiological parameters of arabica coffee (*Coffea arabica*) seedlings produced in full sun (T1) and in shade (T2) were examined. The biomass accumulation and relative growth rate of T1 and T2 seedlings were similar. The T1 seedlings had less biomass allocation to shoots, a lower leaf mass ratio and a lower leaf area ratio; however, they had a greater net assimilation rate (rate of increase in plant mass per unit leaf area), which was associated with a greater net photosynthetic rate. There were no alterations in the concentrations of total chlorophylls or in the chlorophyll *a/b* ratio when comparing T1 and T2 seedlings. No indications of photoinhibition or photooxidative damage were observed in the T1 plants, which were shown to have a more robust antioxidant system than the T2 plants. Seedlings transferred from shade to full sun (T3) were not capable of utilising the incident extra light to fix CO_2. These seedlings showed a remarkable nocturnal retention of zeaxanthin and a significantly increased deepoxidation state of the xanthophyll cycle, even at predawn, but the activity of antioxidant enzymes was lower than in the T1 and T2 plants. Despite the acclimation capacity of T3 seedlings to the new light environment, they exhibited chronic photoinhibition and considerable photooxidative damage throughout the seven days following the transfer to full sun exposure. We further discuss the practical implications of producing coffee seedlings in full sunlight and under shade.

Additional key words: biomass allocation; *Coffea*; growth; oxidative stress; photoinhibition; photosynthesis; xanthophylls.

Introduction

When plants are exposed to high irradiance, the intercepted light energy can surpass the requirements of the photosynthetic machinery, resulting in photoinhibition of photosynthesis. This process, characterised by a decrease in the maximum photochemical efficiency of the photosystem (PS) II (F_v/F_m ratio), can be dynamic or chronic (Osmond 1994). Dynamic photoinhibition is reversible and is associated with a thermal dissipation of excess absorbed energy, indicating that the reduction in photochemical efficiency is a partial result of photoprotection mechanisms rather than oxidative damage to the photosynthetic machinery (Demming-Adams *et al*. 1996, Thiele *et al.* 1998). Chronic photoinhibition, on the other hand, occurs when excess absorbed light generates a series of reactive oxygen species (ROS), which can cause damage to the photosynthetic apparatus (Mittler

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+Corresponding author; fax: +55 31 3899 2580, e-mail: fdamatta@ufv.br
Abbreviations: A – antheraxanthin; APX – ascorbate peroxidase; Car – carotenoid; CAT – catalase; Chl – chlorophyll; DAT – days after transference; DEPS – de-epoxidation state of the xanthophyll cycle; F_v/F_m – variable-to-maximum Chl fluorescence ratio; GR – glutathione reductase; g_s – stomatal conductance; LAR – leaf area ratio; LMR – leaf mass ratio; MDA – malondialdehyde; NAR – net assimilation rate; NPQ – non-photochemical quenching; P_N – net carbon assimilation rate; PS – photosystem; q_P –photochemical quenching coefficient; RGR – relative growth rate; RMR – root mass ratio; ROS – reactive oxygen species; SLA – specific leaf area; SMR – stem mass ratio; SOD – superoxide dismutase; S/R – shoot-to-root ratio; T1 – seedlings grown in full sun; T2 – seedlings grown in shade; T3 – seedlings grown in shade and transferred to full sun conditions; V – violaxanthin; Z – zeaxanthin; Φ_{PSII} – quantum yield of PSII electron transport.
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2002). Plants can protect themselves from excessive absorbed light through the down-regulation of photochemical efficiency by way of the xanthophyll cycle (Demmig-Adams *et al*. 1996) or by maintenance of electron flux involving alternative pathways such as photorespiration and the Mehler-peroxidase reaction (Asada 1999, Ort and Baker 2002). Failure of the antioxidant defence system may result in damage when metabolites and components of the cellular machinery react with ROS, resulting in lipid peroxidation and the oxidation of proteins and nucleic acids.

Among the 103 species of the *Coffea* genus (Davies *et al*. 2006), *C. arabica* (arabica coffee) dominates the world coffee trade, representing about 65% of global coffee production. Coffee is one of the most important commodities in international agricultural trade, generating over US$ 90 billion each year and involving about 500 million people in its management, from cultivation to preparation of the final product for consumption. It is currently grown in some 80 countries on four continents. Brazil is the world's largest coffee producer, followed by Colombia and Vietnam. Many African countries, including Uganda, Burundi, Rwanda and Ethiopia, have coffee as their main source of foreign exchange (DaMatta and Ramalho 2006).

Coffee evolved in African forest understories and has therefore traditionally been considered a shade-requiring species. In many situations, however, coffee grows well without shade, even out-yielding shaded coffee (DaMatta 2004). It could be assumed, therefore, that adult coffee trees should have sufficient phenotypic plasticity to acclimate themselves to contrasting light environments (Matos *et al.* 2009). In fact, shading has been abandoned as a regular cultural practice in several regions, such as in southeastern Brazil, where it has been almost completely abandoned since the 1950s (DaMatta 2004). However, it is generally believed that coffee trees grow better in shade than in full sun in the seedling phase (Maestri and Barros 1977); therefore, the production of seedlings is normally conducted in shaded nurseries. Nevertheless, shaded seedlings usually suffer considerable photooxidative damage when transplanted to the field. Aiming to reduce the costs of seedling production and to improve acclimation to the harsh conditions of the field after transplantation, some nursery managers have started to produce coffee seedlings in full sun exposure (Paiva *et al*. 2003). Seedlings produced in this way are less susceptible to both tipping and photooxidative damage, which reduces the necessity of replacing deceased or badly developed seedlings, thus ultimately resulting in a reduction of costs when implementing a coffee plantation (Silva *et al*. 2000).

Most comparative results obtained with coffee seedlings grown in full sun or in the shade have been based on simple morphological evaluations such as variations in leaf area and biomass accumulation, without an emphasis on the physiological mechanisms that would explain the acclimation of seedlings to light availability. The objective of the present study was to investigate the morphological and physiological performance of coffee seedlings grown in full sun and in shade, in such a way as to obtain a better understanding of the physiological mechanisms involved in acclimation to light. Additionally, seedlings grown in the shade were transferred to full sun to explore the short-term response of the photosynthetic machinery to a sharp increase in light availability.

Materials and methods

Experiments were conducted from October 2006 to March 2007 in Viçosa (20º45'S, 42º54'W, 650-m altitude), southeastern Brazil. Coffee (*Coffea arabica* L. cv. 'Catuaí Vermelho IAC 44') seeds were conditioned in humidified *germistest* paper and placed in a germinator at 30ºC. After the germination process, the seedlings ("soldier" stage; about 2 cm high) were transferred (20 October 2006) to polyethylene bags (one for each bag) with the usual dimensions for coffee (11 × 22 cm). Of a total of 300 seedlings, half were cultivated under full exposure, and the other half under 50% shade (nursery conditions), using nylon screens of neutral density. The average irradiance (08:00 h – 16:00 h) incident on the coffee seedlings was 1,283 and 617 μmol(photon) m^{-2} s^{-1}, respectively for plants under full sunlight and shaded conditions. The plants were grown under naturally fluctuating conditions of temperature and air relative humi-dity, and were irrigated as necessary. To avoid edge effects, the plants in each light environment taken for analysis were protected by a double border. Two independent experiments were completed and analysed separately.

Experiment I: The growth traits of seedlings in both full sun (T1) and shade (T2) were evaluated at 135 and 160 days after transplanting (DAT) the seedlings into bags. An additional sampling was made at 100 DAT for estimating the relative growth rate (RGR) and the net assimilation rate (NAR). Ten seedlings per sampling per treatment were used, with a total of 60 seedlings. Each experimental plot was composed of one seedling. After sampling, the plants were separated into stem, leaf, and root sections. The roots were completely washed with faucet water in a 0.5-mm sieve. For the determination of total leaf area, the leaves were digitalised with a table scanner, and the images were analysed by *Image-Pro® Plus* (version *4.1, Media Cybernetics, Inc.*, Silver Spring, USA). The plant tissues were oven-dried at 70ºC for 72 h, after which the dried leaf, stem and root material was obtained. Based on the above data, the leaf mass ratio (LMR), stem mass ratio (SMR), root mass ratio (RMR), specific leaf area (SLA), leaf area ratio (LAR), shoot-to-root ratio (S/R), RGR, and NAR were calculated, as described in Dias *et al*. (2007).

Experiment II: This experiment was conducted about five months after the seedlings were transplanted into bags, with a total of 105 seedlings. The experimental plot was composed of three seedlings to allow for leaf material harvests in sufficient amounts for biochemical evaluations (*see* below). The evaluations were conducted with recently expanded leaves (third and fourth pairs from the base of the seedlings, when four or five pairs of expanded leaves were presented). The seedlings were about 15 cm tall, so minimal self-shading took place. This experiment consisted of three treatments: T1 – seedlings grown in full sun; T2 – seedlings grown in shade; and T3 – seedlings grown in shade and transferred to full sun conditions. Samplings and measurements were conducted during the acclimation phase to high irradiance of T3 plants [1, 3, and 7 days after transference (DAT) of seedlings]: for the gas-exchange and F_v/F_m data (Fig. 1), plants from all treatments were concomitantly evaluated at 1, 3, and 7 DAT; for fluorescence parameters under photosynthetic steady-state conditions (Table 2), measurements in all treatments were only made at 3 DAT; for pigments and enzymes (Table 3, Fig. 3), evaluations were performed at 1 DAT for the T1, T2, and T3 plants and additionally at 3 and 7 DAT for the T3 seedlings.

Gas exchange and chlorophyll (Chl) fluorescence parameters: The net carbon assimilation rate (P_N) and the stomatal conductance of water vapour (g_s) were measured using a portable open-flow infrared gas analyser (*LI-6400, LI-COR Inc.*, Lincoln, USA). Measurements were made under ambient conditions from 08:00 to 09:00 h, when photosynthetic rates were maximal. During the measurements, the air temperature varied from 23.7 to 27.5ºC, the atmospheric CO_2 partial pressure was about 38.5 Pa, and the irradiance was about 1,000 and 600 μmol(photon) m^{-2} s^{-1} at the leaf level [respectively, for plants under full sunlight (T1 and T3) and shaded (T2) conditions]. These irradiances were high enough to saturate the photosynthetic apparatus of the coffee plants.

The Chl *a* fluorescence parameters were determined using a portable pulse amplitude modulation fluorometer (*FMS2, Hansatech*, Norfolk, UK). Previously dark-adapted leaf tissues (30 min) were initially exposed to a weak pulse of far-red light (1–2 μmol m^{-2} s^{-1}) for a determination of the initial fluorescence (F_0) and to a saturated light pulse [6,000 μmol(photon) m^{-2} s^{-1}; 1-s duration] to estimate the maximum fluorescence (F_m) in order to calculate the maximum PSII photochemical efficiency [$F_v/F_m = (F_m - F_0)/F_m$]. The leaf samples were then illuminated for 300 s using an artificial irradiance of 600 and 1,000 μmol m^{-2} s^{-1} at the leaf level, to obtain a steady-state fluorescence yield (F_s). These irradiances corresponded approximately to the ambient irradiance intercepted by the leaves (08:00 – 09:00 h) of plants of the T2 and T1 treatments, respectively. Subsequently, a saturating white-light pulse [6,000 μmol(photon m^{-2} s^{-1}] was applied to achieve the maximum fluorescence (F_m'). The actinic light was then turned off and a far-red illumination (735 nm) was turned on to measure the light-adapted F_0 (F_0'). The photochemical quenching coefficient (q_P), the actual quantum yield of PSII electron transport (Φ_{PSII}) and the non-photochemical quenching (NPQ) were then determined. Further details have been described previously (DaMatta *et al.* 2002, Lima *et al.* 2002). Measurements were conducted at predawn (F_v/F_m only) and immediately after gas-exchange measurements.

Biochemical analyses: Leaf discs (1.4-cm diameter) were collected *in situ* at pre-dawn (only for photosynthetic pigments) and at midday, flash frozen in liquid nitrogen and then stored at –80ºC until they were analysed. Total Chl (*a*+*b*) and total carotenoids (Car) were assayed according to Lichtenthaler (1987). Xanthophylls (neoxanthin, violaxanthin, antheraxanthin, lutein, and zeaxanthin) and carotenes (α-carotene and β-carotene) were assayed by HPLC as reported in detail by Matos *et al*. (2009). The de-epoxidation state of the xanthophyll cycle (DEPS) was calculated as (Z+ 0.5A)/(V+A+Z), where Z is zeaxanthin, A is antheraxanthin and V is violaxanthin.

Key enzymes of the antioxidant system, including superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), ascorbate peroxidase (APX, EC 1.11.1.11) and glutathione reductase (GR, EC 1.6.4.2), were assayed as described by Pinheiro *et al.* (2004). Cellular damage, which indicates the occurrence of oxidative stress, was evaluated by lipid peroxidation by the accumulation of malondialdehyde (MDA), as described by Lima *et al*. (2002).

Statistics: Data were statistically analysed following a completely randomised design, with ten (Experiment I) or seven (Experiment II) replicates. Significant treatment differences were examined using the Newman-Keuls test at $P \leq 0.05$. Assumptions of normality and homoscedascity were determined and, when necessary, data were transformed to attain a normal distribution using the Lilliefors test. All of the statistical analyses were performed using the software *SAEG version 9.1* (*Funarbe*, Viçosa, Brazil) and *Statistica 7.0* (*StatSoft, Inc., Tulsa*, Oklahoma, USA).

Results

Experiment I: At 135 DAT, biomass accumulation and the relative growth rate (RGR) were similar between plants grown in full sun (T1) and in the shade (T2); however, NAR was greater in the former (Table 1). The T1 plants had better root development, which is associated with a larger RMR, whereas the T2 plants presented greater shoot growth, based on the larger total leaf area, larger LMR and larger LAR. As a whole, these

allometric alterations resulted in a larger shoot-to-root (S/R) ratio in the T2 plants (Table 1). In general, the differences found at 135 DAT were also demonstrated at 160 DAT, although they were less pronounced than those observed at 135 DAT (Table 1).

Table 1. The growth traits of arabica coffee seedlings grown in full sun (T1) and in shade (T2), evaluated at 135 and 160 days after transplantation (DAT) into bags. The different letters denote significant differences between the means within each time. The asterisks represent a significant difference for a given parameter between sampling times (*ANOVA*, $P \leq 0.05$). $n = 10 \pm$ SE.

Growth traits	135 DAT		160 DAT	
	T1	T2	T1	T2
Biomass [g]	$1.36 \pm 0.08^{a*}$	$1.33 \pm 0.05^{a*}$	2.58 ± 0.17^{a}	2.305 ± 0.18^{a}
Total leaf area [dm^2]	$1.23 \pm 0.06^{b*}$	$1.54 \pm 0.06^{a*}$	2.31 ± 0.00^{a}	2.50 ± 0.00^{a}
Specific leaf area [$m^2\ kg^{-1}$]	$20.7 \pm 0.2^{b*}$	$23.6 \pm 0.6^{a*}$	18.3 ± 0.5^{b}	20.4 ± 0.3^{a}
Leaf area ratio [$m^2\ kg^{-1}$]	9.1 ± 0.1^{b}	$11.6 \pm 0.3^{a*}$	9.10 ± 0.29^{b}	11.0 ± 0.3^{a}
Leaf mass ratio [$g\ g^{-1}$]	$0.44 \pm 0.01^{b*}$	$0.49 \pm 0.01^{a*}$	0.50 ± 0.01^{b}	0.54 ± 0.01^{a}
Root mass ratio [$g\ g^{-1}$]	$0.38 \pm 0.01^{a*}$	$0.34 \pm 0.01^{b*}$	0.33 ± 0.01^{a}	0.28 ± 0.01^{b}
Stem mass ratio [$g\ g^{-1}$]	0.19 ± 0.00^{a}	$0.17 \pm 0.00^{b*}$	0.18 ± 0.00^{a}	0.18 ± 0.00^{a}
Shoot-to-root ratio [$g\ g^{-1}$]	$1.60 \pm 0.02^{a*}$	1.96 ± 0.03^{b}	2.07 ± 0.01^{a}	2.56 ± 0.02^{b}
Relative growth rate [$mg\ g^{-1}\ d^{-1}$]	27.0 ± 1.7^{a}	24.4 ± 1.1^{a}	22.3 ± 2.2^{a}	20.0 ± 1.7^{a}
Net assimilation rate [$g\ m^{-2}\ d^{-1}$]	2.92 ± 0.20^{a}	2.03 ± 0.10^{b}	2.49 ± 0.28^{a}	1.79 ± 0.17^{b}

Experiment II: The gas exchange (as well as the F_v/F_m ratio) was analysed for the plants of all treatments during the acclimation phase to high irradiance of the T3 plants (Fig. 1). With the exception of 3 DAT, the T1 plants presented a greater P_N than the T2 plants. The T2 and T3 plants displayed a similar P_N at 1 and 7 DAT, whereas at 3 DAT, P_N was significantly greater in the T2 plants than in the T3 plants. Overall, there were no large changes in g_s among treatments.

The F_v/F_m ratio was always greater than 0.80 in the T1 and T2 plants, but it was smaller than this value in the T3 plants (Fig. 1). The decreased F_v/F_m ratio in these plants was also observed at predawn (< 0.73, as depicted in Fig. 2).

The values for q_P, Φ_{PSII} and NPQ, which were obtained only at 3 DAT, are presented in Table 2. At 600 μmol(photon) $m^{-2}\ s^{-1}$, q_p was similar in the T1 and T2 plants and significantly smaller in the T3 plants. The Φ_{PSII} values were greater in the T2 plants and smaller in the T3 plants, with intermediate values in the T1 plants; NPQ was smaller in the T2 plants and similar between the T1 and T3 plants. In contrast, at 1,000 μmol(photon) $m^{-2}\ s^{-1}$, q_p was similar when comparing the T2 and T3 plants; however, q_p was greater in the T1 plants. There were no differences in Φ_{PSII} among the treatments at 1,000 μmol(photon) $m^{-2}\ s^{-1}$; NPQ was greater in the T1 plants and smaller in the T2 plants, with intermediate values in the T3 plants.

The Chl (*a*+*b*) and Car concentrations and the Chl *a*/*b* and Chl/Car ratios did not vary between the T1 and T2 plants (Table 3). The T3 plants presented a lower Chl concentration in relation to the T1 and T2 plants, except at 3 DAT. The Car concentration did not change consistently in the T3 plants during the period of acclimation to the high-light conditions. In the T3 plants, the Chl/Car ratio was significantly smaller than in the T1 and T2 plants, while the Chl *a*/*b* ratio was not altered in these plants (Table 3).

The concentrations of violaxanthin, antheraxanthin, lutein, zeaxanthin, β-carotene, the VAZ (violaxanthin + antheraxanthin + zeaxanthin) pool, and DEPS were all similar at predawn when comparing the T1 and T2 plants; only neoxanthin and α-carotene concentrations were lower in the T1 plants than in the T2 plants (Table 3). The differences observed at predawn persisted at midday; however, there was a substantial increase in the zeaxanthin pool along with a reduction of the violaxanthin concentration, resulting in an increased DEPS, particularly in the T1 plants. Comparing the T3 plants to those from the other treatments, the principal alterations in carotenoid composition were found in the strong rise of the zeaxanthin pool along with a reduction in the violaxanthin pool, producing great increases in DEPS even at predawn, especially at 1 and 3 DAT. In addition, α-carotene and β-carotene concentrations decreased substantially in the T3 plants, particularly at midday. It should be noted, however, that the VAZ pool did not differ between treatments at predawn, but was smaller in the T3 plants than in the T1 and T2 plants at midday. The VAZ/Car ratio was similar between T2 and T3 and lower in relation to the T1 seedlings, regardless of the time of evaluation (Table 3).

The correlations between the F_v/F_m ratio and VAZ, as well as between F_v/F_m and DEPS, were not significant for the T1 or T2 plants (data not shown), most likely due to the very small changes in F_v/F_m. In the T3 plants, however, there was a significant correlation between the F_v/F_m ratio and DEPS obtained at predawn (Fig. 2), suggesting a possible cause/effect relationship between the reduction of F_v/F_m and the deepoxidation state of the

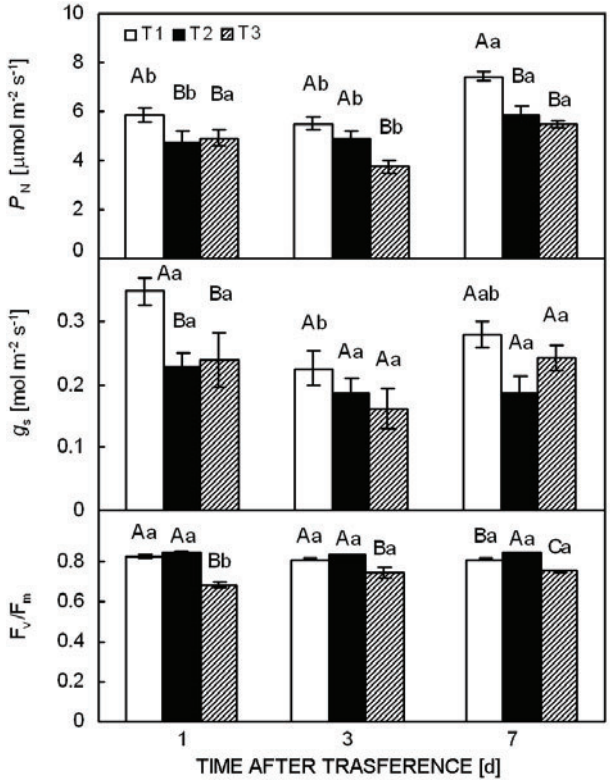

Fig. 1. The rate of net carbon assimilation (P_N), stomatal conductance (g_s) and maximum photosystem II photochemical efficiency (F_v/F_m ratio) in arabica coffee seedlings grown in full sun (T1), in shade (T2) or in shade and transferred to full sun exposure (T3). The P_N and g_s measurements were made under ambient irradiance [*ca.* 600 or 1,000 µmol(photon) m^{-2} s^{-1} at the leaf level, respectively for the plants under shade or full sunlight conditions. For all treatments, evaluations were made at 1, 3, and 7 days after transference of T3 seedlings to full sun. The different capital letters denote significant differences among the means within the same day; the different lowercase letters express a statistical difference between the days within each treatment (Newman-Keuls, $P \leq 0.05$). $n = 7 \pm$ SE.

xanthophyll cycle.

The activities of APX, CAT and GR were greater in the T1 plants than in the T2 ones, whereas the SOD activity was similar between both treatments (Fig. 3). The T3 plants presented the lowest enzyme activity for CAT, GR, and SOD. In the T3 plants, there was an increase in APX activity, but not in the activity of the other enzymes throughout the exposure time to full solar radiation (Fig. 3). The MDA concentration was substantially greater in the T3 plants and similar between the T1 and T2 plants. In any case, the MDA concentration decreased in the T3 plants with the time of exposure to full irradiance (Fig. 3).

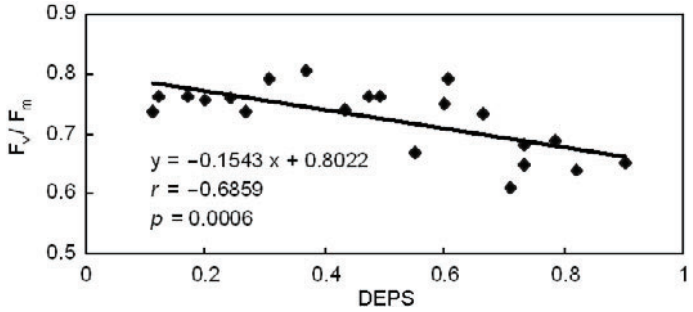

Fig. 2. The relationship between maximum photosystem II photochemical efficiency (F_v/F_m) and the deepoxidation state of the xanthophyll cycle (DEPS) at predawn in coffee seedlings grown in the shade and transferred to full sunlight. Data were collected at 1, 3, and 7 days after transference of the T3 seedlings to full sun exposure.

Table 2. The photochemical quenching coefficient (q_P), quantum yield of photosystem II electron transport (Φ_{PSII}) and non-photochemical quenching (NPQ) in arabica coffee seedlings grown in full sun (T1), in shade (T2) or in shade and transferred to full sun exposure (T3). The measurements were made under artificial irradiance of 600 or 1,000 µmol(photon) m^{-2} s^{-1} at the leaf level. The different letters denote significant differences among the means within each artificial light treatment. The asterisks represent a significant difference for a given parameter between artificial light treatments (Newman–Keuls, $P \leq 0.05$). $n = 7 \pm$ SE.

Irradiance	600 µmol(photon) m^{-2} s^{-1}			1,000 µmol(photon) m^{-2} s^{-1}		
Parameters	T1	T2	T3	T1	T2	T3
q_P	$0.66 \pm 0.01^{a*}$	$0.65 \pm 0.01^{a*}$	$0.59 \pm 0.02^{b*}$	0.33 ± 0.01^{a}	0.26 ± 0.01^{b}	0.28 ± 0.01^{b}
Φ_{PSII}	$0.306 \pm 0.01^{b*}$	$0.33 \pm 0.01^{a*}$	$0.26 \pm 0.01^{c*}$	0.10 ± 0.01^{a}	0.11 ± 0.01^{a}	0.09 ± 0.01^{a}
NPQ	$2.73 \pm 0.06^{a*}$	$2.57 \pm 0.08^{b*}$	$2.83 \pm 0.09^{a*}$	4.15 ± 0.11^{a}	3.41 ± 0.07^{c}	3.94 ± 0.17^{b}

Discussion

Seedlings grown in full sunlight and in the shade: In this study, biomass accumulation and RGR were both similar when comparing the T1 and T2 plants, even though the T1 plants had decreased biomass allocation to shoots and decreased LAR, as found at 135 DAT. As a whole, such behaviour should be associated with a greater NAR of the T1 plants, which should be associated with greater P_N of those plants over time (Lambers *et al.*

2008). Furthermore, the pattern of biomass allocation of the T1 plants should enable them to gain greater access to water and nutrients (larger RMR) in addition to having a smaller total transpiration rate (smaller leaf area), which would allow for a better acclimation after transplantation into the field. The results presented here do not support data reported by Paiva *et al*. (2003), who observed that seedlings of arabica coffee grow better in shade than in full sun. In their study, Paiva *et al*. (2003) observed S/R ratio values of around 6.0 for plants under 50% shading as well as those under full sun. In contrast, in this study, the S/R ratio varied between 1.6 and 2.5. An elevated S/R ratio can indicate a spatial restriction of root growth; in this case, the balance between the roots and the shoot is probably lost, so the roots would not succeed in supplying the shoot properly, especially with water (Poorter and Nagel 2000, Ronchi *et al.* 2006). Therefore, the plants in full sun would not grow well, possibly due to a decreased supply of water in the shoot when the S/R ratio is high.

Leaves developed in shaded environments usually display a high Chl concentration per unit mass and a smaller Chl *a*/*b* ratio in order to increase the capacity for light capture (Lambers *et al*. 2008). However, these classic responses to light availability were not observed here, which agrees with previous studies in coffee (Fahl *et al*. 1994, Araújo *et al.* 2008, Chaves *et al*. 2008). Therefore, adjustments in light-harvesting complexes associated with PSII are not expected when comparing T1 and T2 seedlings (Murchie and Horton 1997, Walters 2005). In this context, greater light absorption should be expected in T1 plants than in T2 plants, which could lead to excessive excitation energy. Nonetheless, no evidence of chronic photoinhibition or photooxidative damages was observed, as the T1 plants exhibited a discreet decrease in the F_v/F_m ratio with recovery at predawn, in addition to showing an MDA concentration similar to that of the T2 plants. Concurrently, these data suggest that the larger excitation pressure suffered by the T1 plants was effectively dissipated, possibly a function of the greater

Table 3. The composition of carotenoids (at predawn and midday), VAZ (violaxanthin + antheraxanthin + zeaxanthin), deepoxidation state of the xanthophyll cycle (DEPS) and VAZ-to-total carotenoid ratio (VAZ/Car) in arabica coffee seedlings grown in full sun (T1), in shade (T2) or in shade and transferred to full-sun exposure (T3). The concentrations of total chlorophylls (Chl) (*a* + *b*) and total carotenoids (Car) and the ratios of Chl *a*/*b* and Chl/Car at midday are also shown. For the T3 treatment, the evaluations were made at 1, 3, and 7 days after transference of the T3 seedlings to full sun. Concentrations are expressed as mg kg^{-1}(FM). The different letters denote significant differences among the means within each time. The asterisks represent a significant difference for a given parameter between sampling times within each treatment. (Newman-Keuls, $P \leq 0.05$). $n = 7 \pm$ SE.

Parameters	T1	T2	T3		
			Days after transferrence		
			1	3	7
Predawn					
Neoxanthin	59.9 ± 4.0^{b}	85.7 ± 5.7^{a}	74.4 ± 7.5^{ab}	86.3 ± 4.4^{a}	62.4 ± 6.1^{b}
Violaxanthin	$94.2 \pm 4.4^{a*}$	$87.8 \pm 6.4^{a*}$	$25.5 \pm 4.5^{c*}$	$55.4 \pm 3.9^{b*}$	$69.5 \pm 5.0^{ab*}$
Antheraxanthin	17.2 ± 3.2^{abc}	10.9 ± 1.3^{c}	20.0 ± 3.1^{abc}	21.6 ± 4.7^{a}	12.5 ± 1.6^{bc}
Lutein	135 ± 6^{c}	144 ± 10^{bc}	178 ± 13^{ab}	207 ± 15^{a}	182 ± 16^{ab}
Zeaxanthin	4.6 ± 0.4^{b}	3.7 ± 0.2^{b}	56.0 ± 8.1^{a}	40.2 ± 8.0^{a}	7.6 ± 3.8^{b}
α-Carotene	28.7 ± 2.7^{b}	56.9 ± 5.3^{a}	30.0 ± 4.3^{b}	42.3 ± 4.6^{ab}	10.1 ± 2.7^{c}
β-Carotene	93.2 ± 4.4^{a}	105 ± 6^{a}	71.2 ± 5.3^{b}	89.9 ± 8.0^{ab}	88.2 ± 13.0^{ab}
VAZ	116 ± 4.8^{a}	1035 ± 8^{a}	102 ± 6.1^{a}	117 ± 120^{a}	89.5 ± 5.4^{a}
DEPS	0.19 ± 0.03^{c}	0.14 ± 0.00^{c}	0.75 ± 0.04^{a}	0.50 ± 0.05^{b}	0.22 ± 0.04^{c}
VAZ/Car	0.27 ± 0.01^{a}	0.21 ± 0.01^{b}	0.23 ± 0.01^{b}	0.21 ± 0.01^{b}	0.21 ± 0.01^{b}
Midday					
Chl (*a* + *b*)	1651 ± 72^{a}	1683 ± 121^{a}	1224 ± 80^{b}	1541 ± 56^{a}	1295 ± 94^{b}
Car	559 ± 26^{ab}	615 ± 36^{a}	438 ± 31^{c}	561 ± 45^{ab}	507 ± 40^{bc}
Chl *a*/*b*	2.2 ± 0.1^{a}	2.4 ± 0.2^{a}	2.3 ± 0.1^{a}	2.3 ± 0.1^{a}	2.4 ± 0.1^{a}
Chl/Car	3.8 ± 0.2^{a}	4.18 ± 0.2^{a}	3.0 ± 0.1^{b}	3.0 ± 0.1^{b}	2.7 ± 0.1^{b}
Neoxanthin	$80.1 \pm 3.4^{ab*}$	102 ± 50^{a}	66.6 ± 5.5^{b}	89.2 ± 8.6^{a}	66.8 ± 5.5^{b}
Violaxanthin	35.1 ± 11.0^{b}	61.1 ± 10.2^{a}	9.1 ± 2.4^{c}	15.1 ± 2.5^{c}	16.7 ± 3.4^{c}
Antheraxanthin	$31.1 \pm 4.9^{b*}$	$28.2 \pm 3.1^{b*}$	13.2 ± 1.6^{c}	21.1 ± 3.8^{bc}	$46.7 \pm 6.2^{a*}$
Lutein	$171 \pm 10^{b*}$	172 ± 11^{b}	169 ± 12^{b}	214 ± 17^{a}	202 ± 170^{a}
Zeaxanthin	$74.1 \pm 13.7^{ab*}$	$32.1 \pm 6.7^{c*}$	$85.7 \pm 10.4^{ab*}$	$96.5 \pm 6.1^{a*}$	$66.2 \pm 9.6^{b*}$
α-Carotene	$43.3 \pm 3.3^{b*}$	$89.5 \pm 8.5^{a*}$	27.3 ± 4.4^{c}	37.0 ± 5.0^{bc}	11.6 ± 2.6^{d}
β-Carotene	$124 \pm 6^{a*}$	$131 \pm 8.3^{a*}$	67.6 ± 3.8^{c}	88.5 ± 7.8^{b}	97.5 ± 9.5^{b}
VAZ	$185 \pm 15^{a*}$	$162 \pm 8.8^{a*}$	108 ± 10^{b}	133 ± 9.1^{b}	$130 \pm 9^{b*}$
DEPS	$0.75 \pm 0.07^{b*}$	$0.50 \pm 0.06^{c*}$	$0.91 \pm 0.03^{a*}$	$0.89 \pm 0.01^{ab*}$	$0.87 \pm 0.03^{ab*}$
VAZ/Car	$0.33 \pm 0.02^{a*}$	$0.27 \pm 0.01^{b*}$	0.25 ± 0.01^{b}	0.24 ± 0.01^{b}	$0.26 \pm 0.01^{b*}$

NPQ, which is associated with greater zeaxanthin concentrations, a high DEPS and a greater VAZ/Car ratio, as found at midday (Morosinotto *et al*. 2003, Horton *et al*. 2008), as well as a greater activity of the enzymes of the antioxidant system, particularly APX, GR, and CAT.

In addition to the changes expected in the violaxanthin and zeaxanthin pools when comparing the T1 and T2 seedlings, we showed a lower concentration of neoxanthin in T1 than in T2 plants, which is, at a first glance, an unexpected result since a higher neoxanthin content may play an important role in preserving PSII from photoinactivation under high-light conditions and protecting membrane lipids from photooxidation by ROS, especially superoxide anions (Dall'Osto *et al.* 2007). However, similar results were found by Ramalho *et al.* (2000) when comparing high-light-acclimated coffee plants with their shaded counterparts. We were unable to find significant alterations in the concentrations of other important carotenoids associated with photoprotection, such as lutein, which is also in accordance with Ramalho *et al.* (2000). In any case, the lower α-carotene/β-carotene ratio in T1 seedlings reflects changes towards characteristics of sun-acclimated plants (Ramalho *et al.* 2000).

Seedlings grown in the shade and transferred to full-sun exposure: The pronounced increases in the zeaxanthin pool and in DEPS, along with the persistent decreases in the F_v/F_m ratio at predawn associated with the outstanding nocturnal retention of zeaxanthin –which probably led to the negative correlation between the F_v/F_m ratio and DEPS at predawn– suggest an increased capacity for photoprotection linked to a higher thermal dissipation rate (Ramalho *et al.* 2003). However, it should also be emphasised that the sustained decreases in the F_v/F_m ratio at predawn may also result from an accumulation of nonfunctional PSII reaction centres and partial photoinactivation of PSII (Niyogi 1999). This may lead to a reorganisation of the light-harvesting complexes of PSII into aggregates of polypeptides containing chlorophylls and xanthophylls in order to maximise thermal energy dissipation (Adams *et al.* 2004). Nevertheless, we believe that the increased capacity for thermal dissipation was not sufficient to avoid protein (*e.g.*, D1) degradation (Ramalho *et al*. 2003, Martinez-Ferri *et al*. 2004) and photodamage. The probable photobleaching of β-carotene in T3 plants, which may trigger the degradation of the D1 protein (Trebst and Depka 1997), is consistent with this suggestion. In fact, the probable rise in ROS production under high light was not accompanied by increased antioxidant capacity in the T3 plants, resulting in photooxidative damage as evidenced by the considerable increase in MDA

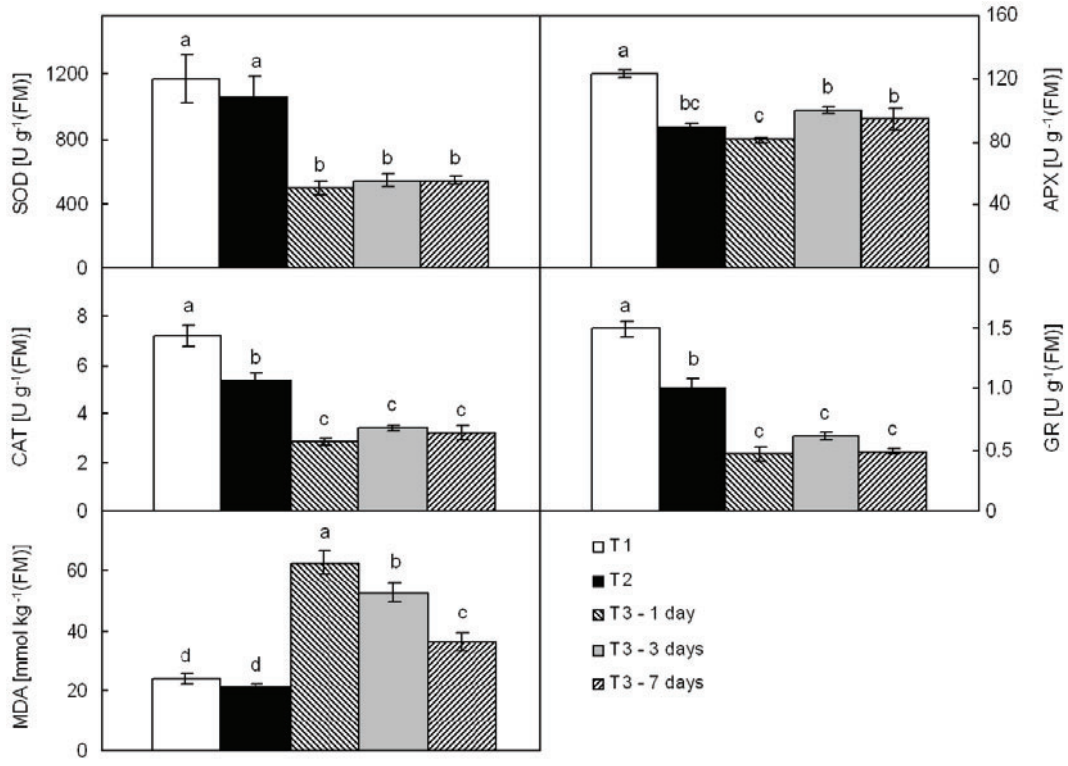

Fig. 3. The activity of ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR), and superoxide dismutase (SOD) and the concentration of malondialdehyde (MDA) in coffee seedlings grown in full sun (T1), in shade (T2) and in shade and transferred to full sun (T3). For the T3 treatment, evaluations were made at 1, 3, and 7 days after transference of T3 seedlings to full sun. The different letters denote significant differences among the means. (Newman-Keuls, $P \leq 0.05$). $n = 7 \pm$ SE.

concentration. Some enzymes, catalase in particular, are very sensitive to elevated ROS concentrations (Smirnoff 1995). Thus, it is suggested that the decreased activity of antioxidant enzymes is a reflection of an excessive increase in ROS production after an abrupt change from a shaded environment to full sun, which ultimately contributed to a lower capacity of the plant to properly adjust to the greater excitation pressure in the new light environment.

It should be noted that, by 7 DAT to full sunlight, the T3 plants displayed a decrease in Chl/Car and α-carotene/β-carotene ratios, which suggests ongoing changes towards acclimation to excess light energy availability (Ramalho *et al*. 2000, 2003). Furthermore, by the same time, nocturnal retention of zeaxanthin and DEPS was strongly reduced along with decreases in MDA concentration, suggesting that the negative impact of excess energy became less important. These results fully agree with those reported by Ramalho and co-workers (1998, 1999, 2000), who showed that coffee plants (cv. 'Catuaí') with adequate nitrogen nutrition were able to recover from the photoinhibitory impact only after the 7th day after transferring plants from shade to high-light conditions. Such a recovery, which involved improved photosynthetic performance and strengthening of antioxidant mechanisms (*e.g.*, zeaxanthin, APX and GR activities), was almost completed after 14 days in high light. Nevertheless, from a practical viewpoint, the mortality of coffee seedlings during the acclimation process under field conditions may be considerable, especially when high light is superimposed with other stresses such as low water availability.

Conclusions: The fact that seedlings present larger leaves (and a larger leaf area) and are greener (usually indicating a larger Chl concentration) when grown in the shade as compared to full sun can transmit a false perception of greater vigour of the plant. Nonetheless, coffee seedlings grown in the open could exhibit better or similar growth relative to plants grown in the shade. In addition, the physiological performance of seedlings grown in full sun was superior to that of seedlings grown in the shade and transferred to full sunlight, as deduced from greater photosynthetic rates (and larger NAR) accompanied by a more robust antioxidative system, which, collectively, was reflected by an adequate capacity for photoprotection, even under high irradiance.

Although seedlings grown in the shade and transferred to the open can partially acclimate to the new light environment, transient photooxidative damage after transfer is inevitable and probably results in high metabolic costs of repair to the cellular structures. Furthermore, this damage can eventually result in a relatively high mortality rate, which therefore increases the costs of formation of a coffee plantation. In summary, the cultivation of coffee seedlings in full sun was shown to be a viable option that should be considered by coffee growers due to the superior performance of these seedlings as compared to seedlings grown in the shade.

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