ECOPHYSIOLOGICAL ACCLIMATION OF COFFEE (*Coffea arabica* AND *C. canephora*) PLANTS TO COPE WITH TEMPORAL FLUCTUATIONS OF LIGHT SUPPLY

Thesis submitted to Federal University of Viçosa, as part of the requirements for obtaining of *Doctor Scientiae* degree in Plant Physiology.

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Thesis submitted to Federal University of Viçosa, as part of the requirements for obtaining of Doctor Scientiae degree in Plant Physiology.

ATTESTED IN: July 21th, 2012.

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To my mother Elvia and my father Efrain and my brothers Fabiola and Edgar,
for all their love, sacrifice and constant support, always present in my heart

With all my love to my wife Yenis, and my three treasures:
    Esteban, Daniela and Sofia

To my mother in law Juana,
for her help and support throughout this adventure

In Memoriam

My friend and professor, Ernesto Arrieta Maza,
for showing me a way of life ... I still walk and want to improve myself!

My grandfather Manuel Facundo López Mendoza

My friends and colleagues, Nuris and Ulises
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RESUMO


Geralmente, assume-se que a quantidade total de luz disponível é o condutor principal para o crescimento e produtividade das plantas. Porém, como pouco se conhece acerca dos efeitos das variações temporais diurnas na disponibilidade da luz, no acúmulo de biomassa e no desempenho fisiológico, neste estudo se examinaram caracteres morfológicos, fisiológicos e bioquímicos em plantas de cafeeiro em dois experimentos independentes. No primeiro experimento, plantas de café arábica (C. arabica) foram submetidas a vários tratamentos de luz: totalmente sob 100%, 40% ou 10% de luz solar (S100, S40 e S10, respectivamente), sob 40% ou 10% de luz solar durante a manhã e, em seguida, submetidas a 100% de luz solar até o anoitecer (S40/100 e S10/100, respectivamente), e em 100% de luz solar do amanhecer ao meio-dia e depois submetidas a 40% ou 10% de luz (S100/40 e S100/10, respectivamente). As plantas em S100 acumularam maior biomassa, com alterações mínimas nas razões alométricas, quando comparadas com indivíduos de outros tratamentos. Essa maior biomassa foi, aparentemente, independente das taxas fotossintéticas por unidade de área foliar e de alterações na disponibilidade de carboidratos; sendo associado principalmente à rápida formação de área foliar com o aumento da disponibilidade de irradiância. Em contraste, o menor acúmulo de biomassa nas plantas em S10/100 e S10 deve ter sido conseqüência das limitações de carbono e de uma menor área foliar. O acúmulo de biomassa não foi dependente apenas da irradiância total interceptada, mas também das variações temporais do fornecimento de luz, como evidenciado pela comparação da maior biomassa (35%) em indivíduos em S100/10 do que em S10/100. As alterações nas taxas fotossintéticas entre os tratamentos foram aparentemente não relacionadas com o acúmulo de carboidratos ou fotoinibição. Em geral, em nível de folha, apenas pequenas alterações nos caracteres fisiológicos respondentes à luz foram observadas, principalmente, quando comparado os indivíduos em S-10 com aqueles dos outros tratamentos. É pouco provável que o maior acúmulo de biomassa e melhor desempenho fisiológico das plantas em S-100 sejam diretamente associados a ganhos de carbono por
unidade de área foliar, mas sim a processos morfogenéticos induzidos pela luz relacionados a uma rápida formação de área foliar, que, por sua vez, devem ter levado a uma maior produtividade fotosintética em nível de planta inteira. O acúmulo de biomassa em grande parte depende não unicamente da quantidade total de luz interceptada, mas também das escalas temporais de variações diurnas no suprimento de luz, isto é, a quantidade de luz recebida pelas plantas durante a manhã teve um papel importante para incrementar a biomassa. Esta informação tem importância prática para o uso de árvores de sombra em fazendas localizadas em zonas montanhosas, onde o uso do sombreamento deve ser evitado em terrenos com exposição para o oeste se um melhor crescimento (e produção) é o principal objetivo a ser alcançado. No segundo experimento, foi usado um desenho experimental onde dois clones de café robusta (*C. canephora*) foram consorciados com árvores de seringueira de modo que foi permitido a comparação de arbustos de café sombreados na manhã (SM) com aqueles sombreados na tarde (SA), e então comparando ambos com arbustos expostos a pleno sol durante o curso do dia (FS). Os arbustos em SM apresentaram melhor desempenho nas trocas gasosas ao longo do dia em comparação com aqueles em SA e FS, os quais exibiram limitações devido a fatores estomáticos (arbustos em SA) e bioquímicos (arbustos em FS). Os caracteres fisiológicos associados com a captura de luz mostraram uma maior resposta às variações temporais da luz do que à quantidade de luz recebida, embora esse comportamento possa ser uma resposta específica dos clones estudados. As atividades de enzimas do sistema antioxidativo exibiram diferenças mínimas quando comparando os clones em SM e SA, e, em contraste, foram maiores nos clones em FS. Independentemente dos tratamentos de luz, não foram encontrado sinais de fotoinibição ou danos celulares. A aclimatação à variação temporal da luz não teve custos adicionais aparentes para a construção e manutenção das folhas entre os tratamentos. Ambos os indivíduos em SM e SA apresentaram um alto retorno em termos de fluxo de investimento (maiores $A_{\text{max}}$ em base de área e massa, PNUE e WUE no longo prazo, por exemplo) do que sua contraparte FS. Em resumo, o sombreamento na manhã pode melhorar o desempenho fisiológico do cafeeiro em ambientes marginais tropicais, no entanto, é importante selecionar genótipos com adequada plasticidade fenotípicos, como encontrada no clone 03, para lidar com a redução na disponibilidade de luz.
ABSTRACT


It is generally assumed that the daily quantum input is what drives plant growth and productivity. However, as little is known about how temporal diurnal variations of light availability influence biomass accumulation and physiological performance, this study examined the morphological, physiological and biochemical traits of coffee plants in two independent experiments. In the first experiment, Arabica coffee (*C. arabica*) plants were grown in pots and subjected to seven light treatments as follows: plants grown entirely under 100%, 40% or 10% sunlight (S100, S40 and S10, respectively); plants grown at either 40% or 10% sunlight throughout the morning (until midday) and then submitted to full sunlight until sunset (S40/100 and S10/100, respectively); and plants grown under full sunlight from sunrise to midday and then submitted to either 40% or 10% sunlight throughout the afternoon (S100/40 and S100/10, respectively). The S100 plants accumulated more biomass compared to plants grown under other treatments and showed minimal changes in biomass allocation. An increased biomass was associated with faster leaf area formation and increasing irradiance that was independent of the photosynthetic rates per unit leaf area and consequent changes in carbohydrate availability. In contrast, the lower biomass in S10/100 and in S10 individuals was likely a consequence of carbon limitations as well as decreased leaf areas. Changes in the photosynthetic rates between treatments were apparently unrelated to carbohydrate accumulation or photoinhibition. Overall, only minor physiological alterations in traits were observed at the leaf level; significant changes were only apparent in S10 individuals with the other treatments. In summary, the growth and physiological performance of coffee plants depends on the total amount of photosynthetic active radiation (PAR) received by the plant per day and temporal order of diurnal variations in the PAR supply. That is, plants that received high light in the morning grew faster than those receiving high light in the afternoon. In the second experiment, a trial was designed with two Robusta coffee (*Coffea canephora*) clones that were intercropped with shelter trees in a way that allowed us to compare coffee
bushes that were shaded in the morning (SM) with those shaded in the afternoon (SA), and we compared both treatments with bushes that received full sunlight over the course of the day (FS). The SM bushes displayed better gas exchange performance than their SA and FS counterparts, which means that the capacity for CO₂ fixation was mainly constrained by stomatal (SA bushes) and biochemical (FS bushes) factors. The physiological traits associated with light capture were more responsive to temporal light changes rather than the amount of light received, although this behavior could be a clone-specific response. The activity of key antioxidant enzymes differed minimally when compared between the SM and SA clones but was much greater in the FS clones. No signs of photoinhibition or cell damage were observed regardless of the light treatment. Acclimation to varying light supplies had no apparent additional energy cost for constructing and maintaining the leaves regardless of the light supply. The SM and SA individuals displayed higher returns 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 summary, shading may improve the physiological performance of coffee bushes that are grown in harsh, tropical environments; however, it is important to select coffee genotypes with adequate phenotypic plasticity to cope with a reduced light supply, as was noted in clone 03.
Although light is a crucial environmental resource that affects photosynthesis and ultimately influences plant growth, low and high light can limit plant performance. Shortages of key resources, such as light, can compromise survival and growth; however, plants face heat, desiccation and excessive irradiance under high sunlight (Valladares and Niinimets, 2008). The consequences of variable light environments on plant growth and photosynthesis are currently best understood in the case of sunflecks, in which the duration and frequency of light patches affect carbon assimilation and biomass accumulation through responses to an array of physiological and morphological processes (Valladares and Niinimets, 2008). In crop plants, the effects of different light environments have been examined by comparing plants grown entirely at high light with individuals grown at a fixed level of shade (e.g., using netting with varying degrees of light transmittance) or in agroforestry systems with homogeneous ground cover that varies from sparse to deep shade depending on the shelter tree attributes and management (e.g., crown architecture, spacing and pruning). Thus, local photosynthetically active radiation conditions to which individual leaves are exposed can vary tremendously throughout the canopy of a tree (Niinemets, 2007). Furthermore, the effects of variable light environments are influenced by the temporal scale of diurnal changes in light environments, even when the total amount of light is constant (Sims and Pearcy, 1993; Wayne and Bazzaz, 1993). To the best of our knowledge, no efforts have been made to examine the consequences of temporal diurnal light availability changes on the physiological performance of tropical crop species in the field or in greenhouse trials.

Leaf acclimation to sun and shade conditions through 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 that developed in high light are generally thicker and/or heavier with a higher nitrogen 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 and light-saturated photosynthesis and increased photoprotective pigments as well as decreased susceptibility to photosynthesis photoinhibition when compared with their low-light counterparts (Walters, 2005; Niinemets, 2007; Cavatte et al., 2012). Whenever absorbed light energy exceeds the capacity of the leaves to use trapped energy through
photosynthesis or to dissipate the energy as heat, photosystem II damage may occur. Protection against excess energy may be achieved through the down-regulation of photochemical efficiency through the xanthophyll cycle or by maintaining electron flux using alternative pathways, such as photorespiration and the Mehler-peroxidase reaction (Ort and Baker, 2002; Logan et al., 2006).

Among agricultural commodities, coffee has a monetary value that is surpassed only by oil. From some 100 species of the Coffea genus, only C. arabica L. (Arabica coffee) and C. canephora Pierre ex A. Froehner (Robusta coffee) are economically important worldwide, and these two species are responsible for approximately 99% of the worldwide coffee production. Robusta trees are generally more vigorous than Arabica trees, and produce more coffee beans with lower production costs and contain approximately twice as much caffeine per bean. Both species evolved in the forest as understory trees; therefore, they are considered to be a shade-demanding species. Most cultivars were derived from wild Arabica populations, such as the germplasm collections of Ethiopia, and they become severely stressed when grown without overhead shade and provide low yields (Van Der Vossen, 1985). However, according to Van Der Vossen (2005), virtually all current cultivars are descendants of early coffee introductions from Ethiopia to Arabia (Yemen), where they were subjected to a relatively dry ecosystem without shade for a thousand years before being introduced to Asia and Latin America. Most of these cultivars have retained the physiological attributes as shade tolerant plants and can respond to various conditions, such as a mild drought and full sunlight. However, some cultivars (e.g. ‘Typica’) are not suited to the open, showing excessive symptoms of photodamage when grown at full exposure. In any case, modern high-yielding coffee cultivars have been selected in test trials with high external inputs conducted under full sunlight and wide spacing. Hence the performance of Arabica coffee cultivars in full sunlight is likely to have been improved (DaMatta, 2004). Therefore, under intensive crop management, coffee will often produce much higher yields in sunlight than under shade.

Shading has been abandoned as a regular cultural practice in several coffee regions. Even in countries like Colombia, where coffee was predominantly cultivated in the shade until just a few years ago, approximately two-thirds of the crop is grown full sun exposure (DaMattia et al., 2007). In Brazil, shading has almost been completely abandoned since the 1950s in response to low crop yields from shaded plantations, which was most likely the result of excessive water competition from other tree species
as well as excessive shading (DaMattia, 2004). However, shaded plantations have various beneficial features, including less sun scorch damage to the berries, greater natural resource conservation, increased biodiversity and greater stability in coffee production. Thus, these shade-grown coffee characteristics have stimulated renewed interest in the use of shade trees, especially in areas where they had previously been eliminated (Beer et al., 1998; DaMattia et al., 2007).

Whether Arabica coffee trees benefit from an association with shelter trees has been disputed for more than a century (Lock, 1888; Beer et al., 1998; DaMattia, 2004; Vander Vossen, 2005; DaMattia et al., 2007; Bosselmann et al., 2009). For Robusta coffee, this controversy has been virtually ignored, most likely because this species, which constitutes a relatively new commercial crop, has been cultivated in full sunlight conditions since its introduction (1950s-1960s) to such countries as Brazil, where it produces a greater crop yield than Arabica regardless of shade conditions. While a range of studies have been undertaken to examine the effects of shading on the ecophysiology and production of Arabica coffee (for reviews see DaMattia, 2004; DaMattia et al., 2010), virtually nothing is known about this subject in Robusta coffee.

Overall, shading (in agroforestry systems) has been recommended for marginal areas when adverse climatic conditions may limit the successful exploitation of a coffee crop. When the agroforestry system is correctly managed [with the proper choice of shade tree species (often deeply rooted species), judicious evaluation of planting density, appropriate frequency of canopy pruning and tree thinning, soil type, water and thermal regimes], water-use efficiency is expected to rise, making the use of shade trees in agroforestry systems a highly recommended option. This practice should translate into obvious productivity advantages for coffee plantations in dry and hot environments (DaMattia, 2004; DaMattia and Ramalho, 2006) provided that shading is not excessive. Nevertheless, it should be emphasized that shading provides little if any benefit to the crop under optimal or near-optimal edaphoclimatic conditions for coffee cultivation; in many cases, it can even be detrimental (Camargo, 1990; Campanha et al., 2004; Morais et al., 2006).

The protective effects of shading have been associated with a lower radiation input at the coffee canopy level, which may reduce the extent of photooxidative damage, a phenomenon that is frequently observed in coffee grown at full sun exposure in marginal zones, and ultimately increase crop life expectancy (DaMattia, 2004). In addition, the effects from shade trees on coffee physiology are associated with reduced
wind speeds and temperature changes, higher relative humidity and changes in the aerodynamic roughness of the cropped area. Thus, these alterations decrease the leaf-to-air vapor pressure deficit, which in turn allows for longer stomatal opening (favoring CO$_2$ uptake) without a proportional increase in transpiration rates. Hence, water loss because of excessive crop evapotranspiration should decline, an effect that is enhanced by increased ground cover and a decrease in the abundance of weeds (Maestri et al., 2001).

Coffee crops and other species of agricultural interest may be subjected to pronounced temporal diurnal variations in the light supply, as has been empirically noted in agroforestry systems. Even in unshaded plantations, this situation may also be common, such as in plantations in hilly zones. Depending on the relative position of the crop on a hill, the plant may be mostly shaded during the morning or afternoon (with terrain exposure facing west and east, respectively). Empirical observations from the field have shown that when they are shaded in the morning, coffee plant growth (and production) may be depressed relative to plants that receive a more continuous diurnal light supply; an inverse relationship is usually noted in plants under full sunlight in the morning and subjected to some degree of shading in the afternoon. It is believed that these patterns might be associated with increased carbon gains in parallel with higher stomatal aperture in the morning when light is non-limiting (Araújo et al., 2008; DaMatta et al., 2008; Batista et al., 2012). Therefore, our hypothesis is that the physiological performance of a coffee plant is dependent not only on the total amount of light received but also on the temporal scales of diurnal light availability.

In this study, the effects of varying light supply over a range of morphological and physiological traits were investigated in two independent experiments. In the first experiment, Arabica coffee seedlings were grown in pots and observed under the following light treatments: plants grown entirely at three light supplies (100, 40 and 10% sunlight) as well as plants subjected to full sunlight in the morning and shaded (40 and 10% sunlight) in the afternoon, and vice-versa. In the second experiment, a field trial with clones of Robusta coffee intercropped with rubber trees was designed so that the following three light treatments could be established: coffee bushes mostly shaded in the morning and exposed to full sunlight in the afternoon, bushes receiving full sunlight during most of the day and bushes exposed to full sunlight during the morning and shaded in the afternoon.
References


CHAPTER 1

Growth and physiological abilities of *Coffea arabica* L. plants to cope with temporal changes in light availability

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Abstract

The coffee plant has traditionally been considered a shade-demanding species, although it performs well without shade and can even out-yield shaded coffee. We hypothesized that the physiological performance and growth of the coffee plant is dependent on the total amount of light received and on temporal variations in light availability. To test this hypothesis, pot-grown Arabica coffee seedlings were subjected to seven light treatments as follows: plants grown entirely under 100%, 40% or 10% sunlight (S100, S40 and S10, respectively); plants grown at either 40% or 10% sunlight throughout the morning (until midday) and then submitted to full sunlight until sunset (S40/100 and S10/100, respectively); and plants grown under full sunlight from sunrise to midday and then submitted to either 40% or 10% sunlight throughout the afternoon (S100/40 and S100/10, respectively). The S100 plants accumulated more biomass with minimal changes in biomass allocation compared to individual plants from other treatments. The increased biomass was apparently independent of photosynthetic rates per unit leaf area as well as the consequential changes in carbohydrate availability; however, the biomass increase was associated with faster leaf area formation with increasing irradiance. In contrast, the lower biomass in the S10/100 and especially in S10 individuals should have been a consequence of carbon limitations as well as lower leaf areas. Biomass accumulation depended on the total photosynthetic active radiation (PAR) and on the daily course of PAR supply, as determined by the 35% larger biomass in the S100/10 individuals relative to the S10/100 individuals. Changes in photosynthetic rates between the treatments were apparently unrelated to carbohydrate accumulation or photoinhibition. Overall, only minor physiological trait alterations were observed at the leaf level; such changes were
mainly present when comparing the S-10 individuals with those from other treatments. In summary, growth and physiological performance depends on the total amount of PAR received by the plant per day and on the temporal order of diurnal variations in the PAR supply. Thus, plants receiving high amounts of light in the morning grew faster than those receiving high amounts of light in the afternoon.

1. Introduction

Although light is a crucial environmental resource that affects photosynthesis and ultimately influences plant growth, low and high sunlight can limit plant performance. Shortages of key resources, such as light, can compromise growth and survival, whereas plants face heat, desiccation and excessive irradiance in high sunlight (Valladares and Niinemets, 2008). To cope with these stresses, plants have evolved a number of well-known biochemical, physiological and structural changes at the leaf and whole-plant levels that enable them to adjust to a particular set of light conditions (Lusk et al., 2008; Walters, 2005). Many studies of plant light responses have been aimed at unveiling the morphological and physiological mechanisms as well as the ecological implications of tolerance to extremes, such as tolerance to either sun or shade. These responses have often been examined by comparing plants grown entirely at high light with individuals grown at a fixed level of shade (using netting with varying degrees of light transmittance) or plants grown in gaps compared with plants grown in the understory of a forest. Noticeably fewer studies have explored trends in growth and physiological responses to temporal scales of diurnal variations in light availability, particularly in tropical tree crops.

In general, plant growth and biomass allocation patterns at the whole plant level are limited by resource availability, and its evaluation is essential to compare performances under different environmental conditions (Poorter and Sack, 2012). Although the plants may be constrained to different extents within their whole plant allocation pattern, they still tend to show universal plastic responses, such as increasing mass allocation to the leaf plus stem rather than to the roots when growing in the shade and increased allocation to the root relative to the leaf plus stem when growing in soils with a low nutrient or water supply (Poorter et al., 2012). Other factors may also strongly affect biomass allocation patterns, such as the growth form, evolved niche and ontogeny (McConhaguay and Coleman, 1999; Poorter and Nagel, 2000; Niklas and
Enquist, 2002). In any case, the patterns of accumulation and biomass allocation in plants that are subjected to temporal variations in light availability throughout the day have been barely explored.

Coffee is one of the most important commodities of international agricultural trade. It evolved in the understory of the African forest and is considered a shade-demanding species. In early plantations, Arabica coffee bushes were planted under taller shade trees to simulate their natural habitat. However, modern coffee cultivars grow well without shade in many situations and even out-yield shaded coffee; therefore, shading has been abandoned as a regular cultural practice in several regions worldwide (DaMatta, 2004; DaMatta et al., 2010). Even in unshaded plantations, the coffee plant may be subjected to pronounced temporal scales of diurnal variations in light supplies, which are frequently found in hilly zone plantations. Depending on the relative crop position on a hill, the plant may be mostly shaded during either the morning or afternoon (with terrain exposure facing west and east, respectively). Empirical observations from the field have shown that when shaded in the morning, the growth (and production) of coffee plants may be depressed relative to plants that receive a more continuous diurnal light supply; an inverse relationship is usually noted in plants grown under full sunlight in the morning and subjected to some degree of shade in the afternoon. We believe that these patterns might be associated with increased carbon gains in combination with higher stomatal conductance in the morning when light is non-limiting (Araújo et al., 2008; Batista et al., 2012; DaMatta et al., 2008). However, the consequences of this type of temporal variation in light supply on photosynthesis and growth has never been tested in coffee or other tropical tree crop species.

We hypothesized that physiological performance, accumulation and biomass allocation in coffee plants are dependent on the total amount of light received and on the temporal scale of diurnal light availability. To test this hypothesis, biomass accumulation and key photosynthetic traits of coffee plants were examined over the following range of light treatments: plants grown entirely at three light exposures (100, 40 and 10% sunlight) as well as plants that were subjected to full sunlight in the morning and shaded (40 or 10% sunlight) in the afternoon and vice-versa.
2. Materials and Methods

2.1. Plant material, growth conditions and experimental design

The experiments were conducted in Viçosa (20°45’S, 42°54’W; 650 m a.s.l.) in southeastern Brazil. Uniform coffee seedlings (Coffea arabica L. cv ‘Red Catuaí IAC 44’) were grown from seeds and transplanted after growing three leaf pairs (January 7th, 2010) into 12-L pots containing a mixture of soil, sand and composted manure (4:1:1 v/v/v). After transplantation, the seedlings were randomly submitted to seven light treatments as follows: plants grown entirely under 100%, 40% or 10% sunlight (S-100, S-40 and S-10, respectively); plants grown at either 40% or 10% sunlight throughout the morning (until midday) and then submitted to full sunlight until sunset (S-40/100 and S-10/100, respectively) and plants grown under full sunlight from sunrise to midday and then submitted to either 40% or 10% sunlight throughout the afternoon (S-100/40 and S-100/10, respectively). Every day, the shade shelter, which was provided by neutral density black nylon netting, was removed or added at midday according to the treatment. The plants were irrigated and fertilized as required, and no apparent restriction was observed in the root development at the end of the experiment. The pot positions were periodically randomized to minimize any variation within each light environment. The light treatment combinations were applied for 150 days, after which the plants were harvested. The experiment was established in a completely randomized design with eight replicate plants per treatment, and the experimental plot consisted of one individual in each pot. When measurements were made on a single leaf (for photosynthetic parameters), the youngest, most fully expanded leaves from five individuals were used.

Throughout the experiment, the average air temperature was 21.4 ± 0.8°C (the maximum and minimum average temperatures were 27.7°C and 14.7°C, respectively), and the relative humidity was 79.2%, as measured with sensors that were installed at the experimental site. The PAR was measured using three LI-190SA quantum sensors (Li-Cor; Lincoln, NE, USA) that were positioned 1 m above the plant canopies. All sensors were connected to an LI-1400 data logger (Li-Cor) that acquired data from the sensors every minute and stored them as 5 min averages.
2.2. Morphological traits, biomass accumulation and allocation

At the end of the experiment, various morphological traits [height, total leaf number per plant, single leaf area, number of plagiotropic branches (PB), stem diameter and height/stem diameter ratio (H/D)] were recorded. Total leaf areas were estimated by putting the maximum leaf widths and lengths into the equations described by Antunes et al. (2008), which were evaluated in the plants that were subjected to different lighting regimens. In addition, the plants were harvested and separated into orthotropic and plagiotropic branches, leaves and roots. Roots were washed thoroughly with tap water over a 0.5-mm screen sieve. Plant tissues were oven-dried at 70°C for 72 h, after which the dry weights of the leaves, branches and roots were determined. Based on these data, the total biomass (TB), leaf mass fractions (LMF), orthotropic branch mass fractions (OMF), plagiotropic branch mass fractions (PMF), root mass fractions (RMF) and leaf area ratios (LAR) were obtained. In addition, the specific leaf area (SLA) was estimated with 20 leaf discs (14 mm in diameter).

2.3. Photosynthetic measurements

The net rate of carbon assimilation (A), stomatal conductance (gs) and internal-to-ambient CO₂ concentration ratio (Ci/Ca) were measured in an open system under ambient temperature and CO₂ partial pressure using two cross-calibrated infrared gas analyzers (LI-6400, Li-Cor). Measurements were made during two periods of the day: 0800-1000 h and 1400-1600 h (solar time) under artificial PAR, i.e., 1000, 500 and 130-µmol photons m⁻² s⁻¹ at the leaf level (for 100, 40 and 10% sunlight, respectively). These PAR intensities approximately corresponded to the ambient irradiance that was intercepted by sampled leaves (in their natural angles) for each light treatment at 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 CO₂ values. The measurements were repeated three times (two at the end of April and one in mid-May) so that the gas-exchange parameters for each replicate were computed as the average values for the measured days.

The chlorophyll a fluorescence parameters were determined in the same leaves that were used for the gas exchange measurements with a portable pulse amplitude modulation fluorometer (Mini PAM, Walz; Effeltrich, Germany). Following dark adaptation for 30 min, the leaf tissue was illuminated with a weak modulated measuring
beam (0.03 μmol m$^{-2}$ s$^{-1}$) to obtain the initial fluorescence ($F_0$). A saturating white light pulse of 6000 μmol m$^{-2}$ s$^{-1}$ was applied for 0.8 s to ensure maximum fluorescence emission ($F_m$) from which the variable-to-maximum fluorescence ratio was calculated as $F_v/F_m = [(F_m - F_0)/F_m]$. The leaf tissue was exposed to actinic PAR (intensities equal to those used for gas exchange measurements) for 60 s to obtain the steady-state fluorescence yield ($F_s$). A saturating white light pulse (6000 μmol m$^{-2}$ s$^{-1}$; 0.8 s) was subsequently applied to achieve the maximum light-adapted fluorescence ($F_m'$). The light-adapted initial fluorescence ($F_0'$) was estimated according to Oxborough and Baker (1997). Using these parameters along with the photochemical ($q_P$) and non-photochemical (NPQ) quenching coefficients, the actual quantum yield of PSII electron transport ($\Phi_{PSII}$) and apparent electron transport rate (ETR) were calculated as previously described (Chaves et al., 2008).

Photosynthetic light-response curves ($A$/PAR) were produced by increasing the PAR in ten steps from 0 to 1500 μmol m$^{-2}$ s$^{-1}$ at 25°C. The leaf tissues were initially exposed to a 5-Pa CO$_2$ partial pressure for 5 min to allow stomatal opening; the $A$/PAR curves were subsequently obtained at 40 Pa of CO$_2$ partial pressure. The dark respiration rates ($R_d$), light compensation point (LCP), light saturation point (LSP) and light-saturated $A$ ($A_{\text{max}}$) were determined from these curves. Further details are presented 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 began at 35-Pa CO$_2$ partial pressure, and once the steady state was reached, the CO$_2$ partial pressure was gradually lowered to 5 Pa and increased stepwise to 160 Pa. The maximum rate of carboxylation ($V_{cmax}$) and 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 during the early morning in attached leaves.

The fractions of partitioned N in carboxylation ($P_c$), bioenergetics associated with electron transport ($P_b$), thylakoid light-harvesting components ($P_l$) and structural components ($P_s$) were calculated according to the method of Niinemets and Tenhunen (1997) using the given values for $V_{cmax}$, $J_{max}$, SLA and leaf concentrations for chlorophyll and N (see below).
2.4. Biochemical assays

Leaf discs were flash frozen in liquid nitrogen and stored at -80°C until analysis. A 10-mg sample of ground tissue was added to pure methanol, and the mixture was incubated at 70°C for 30 min. After centrifugation (13,000 g, 5 min), the hexoses (glucose and fructose) and sucrose in the supernatant were quantified; the concentration of starch was determined from the methanol-insoluble pellet as previously detailed (Praxedes et al., 2006; Ronchi et al., 2006). Total Chl and carotenoids (Car) were extracted using 80% (v/v) aqueous acetone and quantified according to a procedure reported by Lichtenthaler (1987). Total nitrogen (N) was estimated as described in DaMatta et al. (1999).

2.5. Statistics

Data were statistically analyzed following a completely randomized design with five to eight replicates. Assumptions of normality and homoscedascity were checked. The data were analyzed by one-way ANOVA, and the means were examined using the Newman-Keuls test at \( P \leq 0.05 \). All statistical analyses were performed using SAEG software version 9.1 (SAEG, 2007).

3. Results

3.1. Environmental conditions

The incident PARs above the plant canopies were 26.4, 10.5 and 2.9 mol m\(^{-2}\) d\(^{-1}\) for S-100, S-40 and S-10 individuals, respectively, with values ranging from 13.5 to 19.2 mol m\(^{-2}\) d\(^{-1}\) over the plant canopies plants that were subjected to changing PAR supplies (Table 1). The plants that were shaded only in the morning received the largest proportion of PAR during this period, and the opposite was true for plants shaded only in the afternoon (Table 1).

3.2. Morphological traits

The morphological traits under study showed a significant response to differences in varying light regimes (\( P < 0.05 \), Table 2). The greatest reductions in plant
heights were observed in the S-10 treatment with 18% less height compared to the other treatments. The highest leaf numbers were observed in S-100 individuals and decreased to 23% and 71% in the S-40 and S-10 treatments, respectively. In plants that were subjected to temporal variations in light availability, the leaf number in the S-40/100 and S-100/40 plants were similar, whereas the number in S-100/10 individuals was 20% greater than in the S10/100 individuals. The single leaf area was reduced in S-100 relative to its counterparts in the S-40 and S-10 treatments. The S-40/100 and S-100/40 individuals exhibited similar single leaf areas to the plants in S-40. Furthermore, the S-100/10 individuals displayed higher single leaf area than their S-10/100 relatives. Strong decreases in total leaf areas, up to 21% and 64%, were observed in S-40 and S-10 plants, respectively, in comparison to S-100. Minor changes in the total leaf area in S-40/100 and S-100/40 plants and a strong decrease in S-10/100 plants were observed in contrast to their counterparts in S-100/10 plants. Reductions in the PB were observed, particularly in S-10 individuals and not as great in the S-40 and S-100 plants. The PB was remarkably lower in S-10 individuals and did not differ among plants from the other treatments. The stem diameter decreased in relation to light availability, primarily in S-10 individuals and not as much in the S-40 and S-100 individuals. The stem diameters were similar in S-40/100 and S-100/40 plants; however, this trait had lower values in S-10/100 plants compared to the S-100/10 plants. The height/stem diameter ratio (or Slenderness index) was higher in S-10 plants than in S-40 and S-100 plants. This ratio did not differ significantly among plants from the other treatments.

The SLA increased significantly with decreasing PAR for individuals grown at a fixed PAR; for individuals grown under varying PAR, the SLA from S-40/100 and S-100/40 plants was similar to the S-100 plants (~11 m² kg⁻¹), whereas for the S-10/100 and S-100/10 plants, the SLA was similar to that of the S-40 plants (~13 m² kg⁻¹) (Table 2). Changes in the SLA were mostly responsible for alterations in LAR (Table 2), which ranged from 5.3 m² kg⁻¹ (S-100) to 10.2 m² kg⁻¹ (S-10).

3.3. Biomass accumulation and biomass allocation

The total biomass decreased dramatically in plants grown at a fixed level of solar radiation and a reduction in the PAR supply with numbers ranging from 53.8 g in S-100 plants to 9.4 g in their S-10 counterparts (Fig. 1). However, biomass accumulation responded not only to total PAR but also to temporal scales of PAR supply, which was
observed by the larger (52%) biomass in S-100/10 than in S-10/100 individuals despite the small differences in total PAR supply, whereas the differences (18%) in biomass between S-100/40 and S-40/100 did not reach statistical significance. However, greater (35%) biomass was observed in S-100/40 compared to S-40 plants (Fig. 1). Collectively, these results suggest that the amount of PAR received by plants during the morning rather than the amount of light received during the afternoon positively improved growth with a given total daily PAR supply.

Despite the biomass differences, there were no significant alterations in the patterns of biomass allocation among the treatments with two exceptions: (i) the RMF was larger (approximately 20%) in S-100 plants than in S-40 and S-10 individuals (leading to an increased root-to-shoot ratio; data not shown) but similar in plants from other PAR treatments; (ii) the overall proportion of stem biomass invested into plagiotropic (lateral) branches decreased (at the expense of orthotropic branches) with a decreasing PAR supply (Fig. 1).

### 3.4. Photosynthetic measurements

Gas exchange parameters were assessed under the prevailing PAR availability during their measurements, thus more properly reflecting the differences in actual CO₂ fixation capacity between treatments. In the morning, \( A \) did not significantly differ among individuals receiving 40% or 100% sunlight, whereas the absolute lowest \( A \) values were found in S-10 and S-10/100 individuals (Table 3). Changes in \( A \) were highly correlated with variations in \( g_s \) \((r = 0.87, P = 0.011, n = 35)\). Overall, the largest \( g_s \) values were observed in S-100 individuals; \( g_s \) did not differ significantly between the plants from the other groups. The largest \( C_i/C_a \) (0.69) was observed in S-100 and S-10/100 plants, and the lowest values (0.59) were in S-40 plants with intermediate values in the other groups (Table 3). In the afternoon, the \( A \) and \( g_s \) decreased markedly relative to their values in the morning; these traits, as well as the \( C_i/C_a \), did not differ significantly regardless of the treatment (Table 3).

Irrespective of PAR treatments and time of the day, no signs of photoinhibition were observed because the \( F_v/F_m \) ratio remained at high values (~0.80). Overall, the \( q_P \) and \( \Phi_{PSII} \) tended to decrease and NPQ tended to increase with increasing PAR availability in the morning (Table 4). The ETR was markedly lower in S-10 and S-10/100 plants than in other plants, which suggests photochemical limitations to
photosynthesis in those plants (Table 4). These trends were also observed in the afternoon (Table 4). Notably, the highest absolute values of NPQ in the afternoon were observed in plants that were shaded in the morning (S-40/100 and S-10/100 individuals).

The largest $A_{\text{max}}$ values were observed in S-100/40 and S-100/10 individuals, the lowest were in S-40 and S-10 individuals and intermediate values were observed for plants with the other PAR treatments (Table 5). The largest LSP values were obtained in plants that were subjected to full sunlight in the morning compared to their counterparts when submitted to some degree of shade. The LCP decreased with decreasing PAR supply in plants grown at a fixed level of PAR, although this trait did not vary consistently among plants that were subjected to varying PAR supplies (Table 5). In general, the changes in LCP paralleled those in $R_d$ ($r = 0.89$, $P = 0.007$, $n = 35$). The $V_{\text{cmax}}$ was unresponsive to PAR treatments, whereas the $J_{\text{max}}$ was significantly lower in S-10 plants than in plants from the other PAR treatments, which did not differ from one another (Table 5).

Overall, differences in the fractions of N that were allocated into different components of the photosynthetic apparatus (Table 5) were only evident when comparing S-10 individuals with individuals from other PAR treatments. The S-10 plants showed higher $P_t$ and $P_l$ (and lower $P_s$) values than the other groups ($P_b$), which suggests a greater N content in the photosynthetic apparatus of the S-10 plants.

3.5. Biochemical assays

Starch and sugar (fructose and glucose) concentrations tended to increase throughout the day, whereas the sucrose concentrations only showed minor changes and were associated with different light regimens, particularly in the plants grown at a fixed level of light (Table 6). Overall, there were no large diurnal differences in starch and soluble sugars among the treatments (Table 6).

The concentrations of Chl and Car and the Chl/N ratio were larger in S-10 plants than in the other PAR treatments when assessed before midday, which did not differ from one another (Table 6). The Chl/Car and Chl $a/b$ ratios were unresponsive to the PAR supply (Table 6). No signs of Chl degradation were detected regardless of plant transfer from low-to-high (and vice versa) PAR environments (data not shown), as assessed at the end of the day.
4. Discussion

4.1. Morphological traits, biomass accumulation and allocation

The biomass offers the most direct measure of plant performance as a product of growth (Dawson et al., 2012); therefore, any changes in biomass should offer the clearest indicator of a plant’s ability to respond to and take advantage of varying resource (light) availability. We showed that the ability of the coffee plant to produce biomass was significantly and positively dependent on total PAR, as previously suggested by Cavatte et al. (2012a). It is unlikely that improved biomass accumulation in high PAR was an exclusive result of higher photosynthetic rates per unit leaf area and, consequently, changes in the carbohydrate availability of leaves during the day, particularly when comparing the S-100 plants with plants grown entirely or partially under 40% sunlight. Rather, the improved biomass in high PAR conditions was mostly associated with increased total leaf areas that appear to be largely related to earlier plagiotropic branch development (higher PMF and PB), which implies that there were more nodes for anchoring the larger number of leaves that are produced with increasing PAR. In contrast, lower biomass accumulation in S10/100 and especially in S-10 individuals should have been the consequence of carbon limitations as well as light-induced morphogenetic processes related to leaf formation. In agreement with our working hypothesis, biomass accumulation depended on total PAR and on the temporal scales of PAR supply, as determined in a comparison of S-10/100 with S-100/10 individuals. Both groups of individuals received similar total diurnal PAR supplies; however, the larger biomass of the latter should reflect improved $A$ (and greater carbohydrate availability) in the long term and be associated with higher PAR in the morning when the environmental conditions are more conducive for higher gas exchange rates (Batista et al., 2012). In addition, leaf formation and expansion may be constrained according to the carbohydrate availability in plants that are subjected to deep shade, which would affect the early phases of leaf development (Pantin et al., 2011) at the whole plant level and was observed in plants grown under the S-10 and S-10/100 treatments.

Changes in biomass allocation in response to varying PAR supplies were relatively small and mostly observed when comparing the extreme treatments, such as the S-100 with S-10 individuals. These results contrast with those of Cavatte et al.
(2012a), who reported considerable allometric alterations when comparing older coffee saplings grown under 100% or 15% sunlight. Taken together with our results, biomass partitioning in coffee may be dependent on age-related ontogenetic factors (Poorter and Nagel, 2000; Wright and McConnaughay, 2002). Interestingly, all morphological traits under study were virtually unresponsive to temporal scales of PAR supply when comparing S-40/100 with S-100/40 and S-10/100 with S-100/10 individuals. Therefore, any differences in biomass accumulation between those groups are unlikely to have resulted from morphological adjustments (e.g., SLA and RAF), and physiological adjustments (e.g., net assimilation rate) must have played increased roles to explain the differences in biomass, as previously noted when comparing coffee plants grown under 100% or 15% sunlight (Cavatte et al., 2012a).

4.2. Photosynthetic performance and acclimation to varying PAR

In the morning, the differences in actual $A$ were largely associated with stomatal factors, although photochemical limitations likely played a role in constraining $A$ in S-10 and S-10/100 individuals, as determined by their low ETR and because their PAR supplies were at a level below their LSP. In the afternoon, the strong decreases in $g_s$ imposed large limitations on $A$; however, because $C_i/C_a$ did not decrease relative to their morning values, an additional diffusive (mesophyll) limitation or some impairment to CO$_2$ fixation at the chloroplast level cannot be ruled out (Flexas et al., 2007). Therefore, the decreases in $A$ in the afternoon were apparently unrelated to feedback regulation by carbohydrate accumulation or the photoinhibition of photosynthesis, which is in agreement with previous studies in coffee (Araújo et al., 2008; Batista et al., 2012; DaMatta et al., 2008). The pronounced increases in NPQ, which has been associated with large zeaxanthin pools and a higher de-epoxidation state of the xanthophyll cycle in coffee (Matos et al., 2009; Pompelli et al., 2010) upon transfers from low-to-high PAR coupled with elevated values of $F_v/F_m$, which suggests that the coffee plants possess well-developed photoprotection mechanisms to cope with excessive radiation loads (Chaves et al., 2008; Moraes et al., 2010; Pompelli et al., 2010).

We noted some differences in $A_{max}$ but not in the $V_{cmax}$ between treatments. Together, these results suggest that the capacity of mesophyll cells to engage in carbon fixation should be greater than necessary to cope with the PAR received by coffee
leaves. These data are in agreement with the results of Araújo et al. (2008), which were found in field-grown coffee trees and in line with a suggestion from Laisk et al. (2005), which stated that the power of the photosynthetic machinery is over-dimensioned, likely implying an inefficient use of resources.

Overall, only minor physiological acclimations were observed in response to varying PAR supplies in this study. Such differential acclimations were mainly apparent when comparing the S-10 individuals with plants from other treatments. The S-10 individuals displayed some attributes of shade-acclimated leaves, such as low LCP and \( R_d \), and high SLA, Chl and Car pools, a high Chl/N ratio as well as \( P_t \) and \( P_l \), which may improve light harvesting and use such that a positive carbon balance may be maintained even under low-PAR conditions (Lusk et al., 2008; Walters, 2005). In summary, our results indicate a poor plasticity of traits, which are believed to cope with the temporal scales of varying PAR. Thus, these results are concordant with those reported by Matos et al. (2009), who showed that major phenotypic changes are only observed when comparing deeply shaded with sun-exposed leaves; they also showed that the responses of leaf traits to changing PAR are non-linear and depend on the suite of traits under consideration.

5. Concluding remarks

Despite their characterization as a shade-demanding species, modern coffee cultivars, such as those used in this study, may grow better in full sunlight. This behavior is unlikely to have been directly associated with carbon gains per unit leaf area but rather with light-induced morphogenetic processes related to faster leaf formation, which may lead to increased photosynthetic productivity on a whole-plant level. Even in deep shade (e.g., S-10 individuals) in which carbon gain may obviously limit growth, the biomass accumulation was much more depressed than the actual \( A \) per unit leaf area, which lends further support for the key role of light as a major driver in coffee developmental processes. Thus, we demonstrated that biomass accumulation largely depends on the total amount of PAR and on the temporal scales of diurnal variations in PAR supply, such as the amount of PAR that was received by the plants during the morning played a major role in improving the biomass accumulation. This information has practical importance when selecting shelter trees for farms located in hilly zones.
where the use of shading should be avoided in terrain exposure facing west if better growth (and production) is a major goal.

6. Acknowledgements

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Total biomass (A) and biomass allocation traits (B) [roots mass fraction (RMF), orthotropic branch mass fraction (OMF), plagiotropic branch mass fraction (PMF) and leaf mass fraction (LMF)] in arabica coffee plants subjected to seven light treatments, defined as follows: plants grown entirely under 100%, 40% or 10% full sunlight (S-100, S-40 and S-10, respectively); plants grown at either 40% or 10% full sunlight throughout the morning (until midday) and then submitted to full sunlight until sunset (S-40/100 and S-10/100, respectively); and plants grown under full sunlight from sunrise to midday and then submitted to either 40% or 10% full sunlight throughout the afternoon (S-40/100 and S-10/100, respectively). Means followed by a same letter do not differ significantly from one another (P < 0.05, Newman-Keuls). n = 8 ± SE.
Table 1. The average incident PAR (mol m\(^{-2}\) d\(^{-1}\)) and the proportion of PAR from sunrise to midday (PAR\(_{SR-MD}\)) and from midday to sunset (PAR\(_{MD-SS}\)) over the coffee plants subjected to seven light treatments during 149 days, defined as follows: plants grown entirely under 100%, 40% or 10% full sunlight (S-100, S-40 and S-10, respectively); plants grown at either 40% or 10% full sunlight throughout the morning (until midday) and then submitted to full sunlight until sunset (S-40/100 and S-10/100, respectively); and plants grown under full sunlight from sunrise to midday and then submitted to either 40% or 10% full sunlight throughout the afternoon (S-100/40 and S-100/10, respectively). \(n = 149 \pm SE\).

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Table 2. Morphological traits [height (cm), total leaf number per plant, single leaf area (cm$^2$), total leaf area (m$^2$), plagiotropic branches number (PBN), stem diameter (mm), height/stem diameter ratio (H/D)] and specific leaf area (SLA, m$^2$ kg$^{-1}$) and leaf area ratio (LAR, m$^2$ kg$^{-1}$) in arabica coffee plants subjected to seven light treatments, defined as follows: plants grown entirely under 100%, 40% or 10% full sunlight (S-100, S-40 and S-10, respectively); plants grown at either 40% or 10% full sunlight throughout the morning (until midday) and then submitted to full sunlight until sunset (S-40/100 and S-10/100, respectively); and plants grown under full sunlight from sunrise to midday and then submitted to either 40% or 10% full sunlight throughout the afternoon (S-40/100 and S-10/100, respectively). Means followed by a same letter do not differ significantly from one another ($P < 0.05$, Newman–Keuls). $n = 8 \pm SE.$

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Table 3. Gas exchange parameters [net CO₂ assimilation rate (\(A\); \(\mu\)mol CO₂ m⁻² s⁻¹), stomatal conductance (\(g_s\); mmol H₂O m⁻² s⁻¹), internal-to-ambient CO₂ concentration ratio (\(C_i/C_a\); \(\mu\)mol CO₂ mol⁻¹ air)] and leaf-to-air vapour pressure deficit (VPD; kPa)] as measured in two time points (08:00-10:00 h and 14:00-16:00 h) in arabica coffee plants subjected to seven light treatments, defined as follows: plants grown entirely under 100%, 40% or 10% full sunlight (S-100, S-40 and S-10, respectively); plants grown at either 40% or 10% full sunlight throughout the morning (until midday) and then submitted to full sunlight until sunset (S-40/100 and S-10/100, respectively); and plants grown under full sunlight from sunrise to midday and then submitted to either 40% or 10% full sunlight throughout the afternoon (S-100/40 and S-100/10, respectively). Means followed by a same letter do not differ significantly from one another (\(P < 0.05\), Newman–Keuls test). \(n = 5 \pm SE\).
Table 4. Chlorophyll $a$ fluorescence parameters [variable-to-maximum fluorescence ratio ($F_v/F_m$), photochemical quenching coefficient ($q_p$), non-photochemical quenching coefficient (NPQ), quantum yield of PSII electron transport ($\Phi_{PSII}$) and electron transport rate (ETR)] in arabica coffee plants subjected to seven light treatments. See further details in the legend of Table 2.

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<td>0.79±0.01$^A$</td>
<td>0.80±0.01$^A$</td>
<td>0.79±0.01$^A$</td>
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</tr>
<tr>
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<td>0.80±0.00$^A$</td>
<td>0.80±0.00$^A$</td>
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<td>0.78±0.01$^A$</td>
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<tr>
<td>$q_p$</td>
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<td>0.17±0.01$^D$</td>
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<td>0.23±0.02$^D$</td>
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<td>0.25±0.02$^C$</td>
<td>0.09±0.01$^D$</td>
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<td>ETR</td>
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<td>28±1$^C$</td>
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<td>47±3$^B$</td>
<td>22±2$^C$</td>
<td>63±4$^A$</td>
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<td>8:00-10:00 h</td>
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<td>67±7$^A$</td>
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Table 5. Photosynthetic variables derived from the net photosynthetic rates ($A$) and irradiance curves [light-saturated $A$ ($A_{\text{max}}$; µmol CO$_2$ m$^{-2}$ s$^{-1}$), light compensation point (LCP; µmol photons m$^{-2}$ s$^{-1}$), light saturation point (LSP; µmol photons m$^{-2}$ s$^{-1}$), and dark respiration ($R_d$; µmol CO$_2$ m$^{-2}$ s$^{-1}$)] and from $A$ and internal CO$_2$ concentration ($A/C_i$) curves [maximum rate of carboxylation limited by electron transport ($J_{\text{max}}$; µmol e$^-$ m$^{-2}$ s$^{-1}$) and maximum rate of carboxylation ($V_{\text{cmax}}$; µmol CO$_2$ m$^{-2}$ s$^{-1}$)], and traits associated with nitrogen partitioning into carboxylation (mainly Rubisco, $P_r$), bioenergetics ($P_b$), thylakoid light-harvesting components ($P_l$) and structural components ($P_s$) in arabica coffee plants subjected to seven light treatments. See further details in the legend of Table 2.

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Table 6. Leaf starch (g kg\(^{-1}\) DW) and soluble sugars concentrations (Glucose+Fructose and Sucrose, mmol kg\(^{-1}\) DW) as measured in three time points (5:00, 11:00 and 17:00 h) in arabica coffee plants subjected to seven light treatments. See further details in the legend of Table 2.

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<td>Sucrose</td>
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<td>Sucrose</td>
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Table 7. Leaf concentrations of total chlorophylls (Chl; g kg\(^{-1}\) DM) and carotenoids (Car; g kg\(^{-1}\) DM), the ratios of Chl/Car, Chl \(a/b\) and Chl/N (mmol mol\(^{-1}\)) in arabica coffee plants subjected to seven light treatments. See further details in the legend of Table 2.

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<td>Chl (a/b)</td>
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<td>Chl/N</td>
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</table>
CHAPTER 2

Physiological and biochemical abilities of *Coffea canephora* leaves for acclimation to cope with temporal changes in light availability


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

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Abstract

The effect of varying intensities of light on plants depends 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 shelters trees in such 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 that their SA and FS counterparts, in that the capacity for CO2 fixation was mainly constrained by stomatal (SA bushes) and biochemical (FS bushes) factors. Physiological traits associated with light capture were more responsive to temporal changes of light rather than to the amount of light received, although this behavior could be a clone-specific response. The activity of key antioxidants enzymes differed minimally when comparing the SM and SA clones, but was much larger in FS clones. No signs of photoinibition or cell damage were found regardless of the light treatments. Acclimation 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–sutured 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.

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1. 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 at a fixed level of shade (e.g., using nettings with varying degrees of 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) conditions 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, no efforts have been undertaken in field conditions to examine the effects of the temporal scale of diurnal changes 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 down regulation 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 C. arabica (arabica coffee) and C. 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 growing interest in the cultivation of coffee bushes intercropped with shelters trees, due specially to the benefits to shaded plantations, including the conservation of natural resources, increased biodiversity and 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 full sunlight conditions 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 vapour pressure deficit (VPD) and temperature conditions (Da Matta et al., 2010). Given this fact, we hypothesized that the physiological performance of robusta coffee 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 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 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 chemical composition, construction and maintenance costs of leaves were assessed under real plantation conditions using two clones of robusta coffee with contrasting photosynthetic rates.
2. Material and methods

2.1. 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 (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 bushes (C. canephora; Rubiaceae) with 2.5 x 1.0 m spacing in east-west-oriented hedgerows. Thirty-one rows of coffee bushes, each from a single clone, were randomly distributed along the alley. 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, that is, 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) the tree rows was considered to be the border) in the SM and SA treatments; 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. 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.

2.2. Environmental parameters

The total daily PAR over January 2010 was measured using LI-190SA quantum sensors (LI-COR, Lincoln, NE, USA) 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 that were analysed 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).

2.3. 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, USA). 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 and afternoon, 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 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 ten 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, cut submerged in water to prevent embolism of xylem, and immediately brought to the laboratory with their bases immersed in water.

2.4. Chemical composition, construction and maintenance costs of leaf tissues

Leaf tissues were collected at midday, frozen in liquid nitrogen, freeze-dried, ground 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 analyser (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 one gram 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).

### 2.5. 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 analysed through malondialdehyde (MDA) accumulation, estimated as the content of total 2-thiobarbituric acid-reactive substances, as detailed in Lima et al., (2002).
2.6. Others measurements

The leaf water potential (Ψw) was measured before dawn (04:30-05:30 h) and at midday using a Schollander-type pressure chamber (model 1000, PMS Instruments, Albany, USA). The specific leaf area (SLA; leaf area per unit leaf dry mass) was estimated using 20 leaf discs (each 14 mm in diameter).

2.7. 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).

3. Results

3.1. Environment

As can be seen in Fig. 2, the total daily PAR over the coffee canopies was 54.0 mol m$^{-2}$ d$^{-1}$ in the FS treatment and decreased by 26.9% and 29.4% 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), whilst 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 the full sunlight 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 in the early afternoon. Notably, in these measurements, and independent of the clone, both the leaf temperature and leaf-to-air VPD were significantly higher in FS plants than in either SM or SA individuals (Table 1).
3.2. Leaf water potential and specific leaf area

Independent of the clones studied, the $\Psi_w$ before dawn was greater than -0.20 MPa regardless of the light treatments, whereas the $\Psi_w$ 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 SLA of SM and FS plants was similar and lower than in SA individuals (Table 2).

3.3. Gas exchange

Differences in the magnitude of gas exchange between the clones studied were particularly evident in the morning evaluations, at which time clone 03 displayed higher $g_s$ and $A$ values than clone 120 (Table 1). Regardless of the clone and light treatments, the highest $g_s$ values were found in mid-morning, after which $g_s$ decreased progressively throughout the day. Changes in stomatal aperture 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 $g_s$ and $A$) in comparison to both SA and FS individuals in both afternoon evaluations. This enhanced performance was translated into a higher diurnal carbon gain in the SM leaves. Despite the differences in both the microclimate conditions and $A$, 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; 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 $A_{\text{max}}$ (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 $A_{\text{max}}$ was higher in SM than in FS and SA plants, which did not from differ from one another, whereas mass-based $A_{\text{max}}$ 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 responsive to 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 than in plants from the other treatments (Table 3).

3.4. 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 individuals, with intermediate values in SA plants (Table 4). These pigment parameters were unresponsive to the light treatments in clone 120. Clonal differences were noted in SA plants, but only for the Chl concentration and the Chl/N ratio, which were significantly lower in clone 03 than in clone 120. The Chl a/b ratio remained unchanged (ranging from 2.4 to 2.90) irrespective of treatments (Table 4).

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).

3.5. 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 based in 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 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, relatively large amplitude (18.9 to 23.0‰) in Δ13C (a proxy inversely related to long –term WUE) was noted in response
to 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 one other. In either clone, significant correlations ($r \geq 0.57$) between $A/E$ and $\Delta^{13}C$ were found (Fig. S1).

4. Discussion

The experimental design created an environment in which the total, daily integrated PAR was quite similar over the coffee canopies shaded 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 the 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.

4.1. Gas exchange

The $\Psi_w$ at midday was higher in SM plants than in both FS and SA plants despite the higher $g_s$ (Tables 1 and 2) suggest improved tissue hydration which, 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 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 impact 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 $\Delta^{13}C$). Because increases in $\Delta^{13}C$ (which expresses the magnitude of gas exchange over time
instead of a discrete measurement) can arise because 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 \( A_{\text{max}} \) was lower than in SM plants, both \( J_{\text{max}} \) and \( V_{\text{cmax}} \) were kept at high values (Table 3), suggesting that the biochemical capacity for \( \text{CO}_2 \) fixation was preserved. However, the relatively low \( \text{in situ} \ A \) 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 \( \Delta^{13}\text{C} \) values displayed by these plants indicates that stomatal factors played a prominent role in limiting \( A \).

### 4.2. 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 the enzyme activities differed minimally when comparing the SM and SA plants, but were remarkably larger in FS plants. Additionally, increases in non-photochemical quenching, which has been associated with large zeaxanthin pools and higher pools de-epoxidation state of the xanthophyll cycle with increasing light availability in coffee (Matos et al., 2009), may also provide photoprotection through thermal dissipation (Rodriguez-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).
4.3. Acclimation to varying PAR supply: costs and efficiencies

Both 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 counterpart suggests a lower time span in which the SM 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 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, 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 improved light use efficiency when PAR is limiting. Additionally, the SM leaves of clone 03 displayed improved PNUE and higher mass-based \( A_{\text{mass}} \), 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 and SA leaves, whereas the SLA was even higher in SA leaves. In any case, both PNUE and mass-based \( A_{\text{mass}} \) 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 \( \Delta^{13} \text{C} \) (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 \( \Delta^{13} \text{C} \) 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 resources by the robusta coffee bushes grown in harsh environments.

5. Conclusions

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 not apparent additional costs for constructing and maintaining the leaves regardless of the PAR supply received by them. Overall, both the SM and SA individual displayed higher return in terms of revenue streams (e.g. higher area-and-mass-based $A_{\text{max}}$, 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 shelters trees, as has empirically been observed in agroforestry system 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.

6. Acknowledgments

We gratefully acknowledge the Institute for Research and Rural Assistance of the Espirito 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 CAPES-PEC-PG(NFR-L), the National Council for Scientific and Technological Development, CNPq (PCC, SCVM) and the Brazilian Federal Agency for Support and Evaluation of Graduate Education (PEMS, LEM, EFM), all from the Brazilian Government, are gratefully acknowledged. Research fellowships granted by CNPq to FMD are also acknowledged.
7. References


8. Tables and figures

Table 1. The air ($T_A; ^\circ C$) and leaf ($T_l; ^\circ C$) temperatures, leaf-to-air vapour pressure deficit (VPD; kPa), net CO$_2$ assimilation rate ($A; \mu$mol CO$_2$ m$^{-2}$ s$^{-1}$), stomatal conductance ($g_s; \text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$), internal-to-ambient CO$_2$ concentration ratio ($C/C_a; \mu$mol 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, 11:00-1300 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). $n = 6 \pm SE$

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Table 2. The leaf water potential at predawn ($\Psi_{pd}$; MPa) and midday ($\Psi_{md}$; 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

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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$^{-1}$), light-saturated $A$ ($A_{\text{max}}$; μmol CO$_2$ m$^{-2}$ s$^{-1}$) and dark respiration ($R_d$; μmol CO$_2$ m$^{-2}$ s$^{-1}$)] and from $A$ and internal CO$_2$ concentration ($A/C_i$) curves [maximum rate of carboxylation limited by electron transport ($J_{\text{max}}$; μmol e$^{-}$ m$^{-2}$ s$^{-1}$) and maximum rate of carboxylation ($V_{\text{cmax}}$; μmol CO$_2$ m$^{-2}$ s$^{-1}$)] and carbon isotope discrimination ($\Delta^{13}$C; %o) 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.

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<tr>
<td>$A_{\text{max}}$ (mass)</td>
<td>138±6$^{Aa}$</td>
<td>85±3$^{Ca}$</td>
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<tr>
<td>$J_{\text{max}}$</td>
<td>73.3±7.4$^{Bb}$</td>
<td>72.1±8.5$^{Ba}$</td>
</tr>
<tr>
<td>$V_{\text{cmax}}$</td>
<td>81.3±11.7$^{Ab}$</td>
<td>89.6±2.9$^{Aa}$</td>
</tr>
</tbody>
</table>
Table 4. Leaf 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 \(a/b\) 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.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Clone 03</th>
<th>Clone 120</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>SM</td>
<td>FS</td>
</tr>
<tr>
<td>Chl</td>
<td>11.1±1.3(^{Aa})</td>
<td>6.7±0.3(^{Ba})</td>
</tr>
<tr>
<td>Car</td>
<td>2.0±0.1(^{Aa})</td>
<td>1.5±0.1(^{Aa})</td>
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<tr>
<td>Chl/ N</td>
<td>5.2±0.6(^{Aa})</td>
<td>3.2±0.2(^{Ba})</td>
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<tr>
<td>Chl/Car</td>
<td>5.5±0.3(^{Aa})</td>
<td>4.4±0.1(^{Aa})</td>
</tr>
<tr>
<td>Chl (a/b)</td>
<td>2.5±0.2(^{Aa})</td>
<td>2.9±0.1(^{Aa})</td>
</tr>
<tr>
<td>SOD</td>
<td>1.1±0.1(^{Bb})</td>
<td>1.5±0.1(^{Aa})</td>
</tr>
<tr>
<td>APX</td>
<td>22.4±3.0(^{Ba})</td>
<td>36.2±4.3(^{Ab})</td>
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<td>GR</td>
<td>1.8±0.1(^{Bba})</td>
<td>2.7±0.1(^{Aa})</td>
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<tr>
<td>CAT</td>
<td>1.2±0.1(^{Ba})</td>
<td>2.9±0.1(^{Ab})</td>
</tr>
<tr>
<td>MDA</td>
<td>32.1±1.7(^{Aa})</td>
<td>32.7±1.3(^{Aa})</td>
</tr>
</tbody>
</table>
Table 5. The construction (CC; g glucose g\textsuperscript{-1} DM) and maintenance (MC; mg glucose g\textsuperscript{-1} DM day\textsuperscript{-1}) costs, photosynthetic nitrogen use efficiency (PNUE; μmol CO\textsubscript{2} g\textsuperscript{-1} N s\textsuperscript{-1}), average daily instantaneous water use efficiency (A/E; mmol mol\textsuperscript{-1}) and carbon isotope discrimination (Δ\textsuperscript{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.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Clone 03</th>
<th></th>
<th>Clone 120</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>SM</td>
<td>FS</td>
<td>SA</td>
<td>SM</td>
</tr>
<tr>
<td>CC</td>
<td>1.28±0.02\textsuperscript{Aa}</td>
<td>1.33±0.02\textsuperscript{Aa}</td>
<td>1.29±0.02\textsuperscript{Aa}</td>
<td>1.28±0.00\textsuperscript{Aa}</td>
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<td>MC</td>
<td>16.1±0.4\textsuperscript{Aa}</td>
<td>15.7±0.3\textsuperscript{Aa}</td>
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<td>PNUE</td>
<td>4.56±0.07\textsuperscript{Aa}</td>
<td>2.92±0.07\textsuperscript{Ba}</td>
<td>3.72±0.06\textsuperscript{Ca}</td>
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<tr>
<td>A/E</td>
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<td>1.80±0.23\textsuperscript{Aa}</td>
<td>2.13±0.22\textsuperscript{Aa}</td>
<td>1.82±0.14\textsuperscript{Aa}</td>
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<tr>
<td>Δ\textsuperscript{13}C</td>
<td>21.0±0.5\textsuperscript{Ba}</td>
<td>23.0±0.2\textsuperscript{Aa}</td>
<td>19.4±0.1\textsuperscript{Ca}</td>
<td>19.5±0.5\textsuperscript{Bb}</td>
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</table>
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).
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 facing the west rows, which were exposed to full sunlight during the morning and shaded in the afternoon (SA).

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 facing the west rows, which were exposed to full sunlight during the morning and shaded in the afternoon (SA).
**Table S1.** The chemical composition (expressed in g kg\(^{-1}\) DM) of leaf tissues [starch, total soluble sugars (TSS), structural carbohydrates plus lignin (SCL), proteins, lipids, organic acids (OA), amino acids (AA), minerals, phenols and alkaloids] and the construction (CC; g glucose g\(^{-1}\) DM) and maintenance (MC; mg glucose g\(^{-1}\) DM day\(^{-1}\)) costs 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.

<table>
<thead>
<tr>
<th>Parameters</th>
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<tr>
<td>Starch</td>
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<td>146±1(^{\text{Aa}})</td>
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<td>TSS</td>
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<td>77±4(^{\text{Ab}})</td>
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<td>SCL</td>
<td>290±30(^{\text{Aa}})</td>
<td>239±7(^{\text{Aa}})</td>
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<td>Proteins</td>
<td>84±10(^{\text{Ab}})</td>
<td>79±5(^{\text{Ba}})</td>
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<td>Lipids</td>
<td>107±3(^{\text{Bb}})</td>
<td>107±3(^{\text{Ba}})</td>
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<tr>
<td>AO</td>
<td>81±5(^{\text{Ab}})</td>
<td>89±6(^{\text{Aa}})</td>
</tr>
<tr>
<td>AA</td>
<td>7.4±0.3(^{\text{Aa}})</td>
<td>7.8±0.2(^{\text{Aa}})</td>
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<td>Minerals</td>
<td>52±6(^{\text{Aa}})</td>
<td>41±3(^{\text{Aa}})</td>
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<tr>
<td>Phenols</td>
<td>140±14(^{\text{Ab}})</td>
<td>140±13(^{\text{Ab}})</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>21±1(^{\text{Ab}})</td>
<td>26±1(^{\text{Ab}})</td>
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</table>
Fig. S1. The relationship between instantaneous water use efficiency and carbon isotope discrimination in two clones of robusta coffee.
Our predictions that the physiological performance and biomass accumulation of plants depend on the total amount of light received and on the temporal scales of diurnal light availability were confirmed in the experiments that were conducted with C. arabica seedlings. The higher biomass accumulation increased in association with morphogenetic processes that were induced by higher light availability during the morning (faster leaf area formation) and ultimately resulted in better photosynthetic productivity on the whole-plant level. Exposing plants to light during the morning promoted an increased $A$ and higher carbohydrate availability in the long term, which led to improved growth with no major alterations in biomass allocation patterns; this could be a consequence of ontogenetic drift in coffee plants. Photosynthetic rate changes during the day were associated with stomatal limitations and were apparently unrelated to carbohydrate accumulation or photoinhibition within the varying light regimes. In summary, growth and physiological performance depends not only on the total amount of PAR received by the plant per day but also on the temporal order of diurnal variations in PAR supply.

In the second experiment, in contrast to our working hypothesis, it was shown that C. canephora clones grown under harsh environmental conditions and that were shaded during the morning displayed better gas exchange performance throughout the day, and there were varying abilities to cope with PAR alterations between the clones under evaluation. It was also confirmed that the acclimation of Robusta coffee to temporal changes in PAR were more associated with morphological and physiological traits, particularly those related to light capture. In addition, C. canephora clones were shown to have a robust antioxidative system that allows adequate photoprotection in response to the total amount of PAR received, which was observed by the absence of cellular damage and photoinhibition. Furthermore, we showed that morning shade may increase the resource use efficiencies with minimal changes in CC, MC and leaf chemical composition under temporal variations of light availability. Overall, the present data indicates that this species has considerable phenotypic plasticity, which may be used to select promising genotypes for intercropping with shelter trees to improve light use efficiency when PAR is limiting and could lead to an increased carbon gain, as found in clone 03.
Finally, the results obtained in both experiments are of great practical importance and should be considered for the management of light availability as an alternative to the current cultural management of coffee plantations in response to climate change scenarios, particularly in mountainous areas or in marginal environments where better growth (and production) is a major goal.