UNIVERSIDADE FEDERAL DE MINAS GERAIS

INSTITUTO DE CIÊNCIAS BIOLÓGICAS

Departamento de Botânica

Programa de Pós-Graduação em Biologia Vegetal

UFMG

Tiago Vilas Boas da Silva

AVALIAÇÕES MORFOFISIOLÓGICAS EM TRÊS CULTIVARES DE CAFÉ CRESCENDO EM DUAS CONDIÇÕES DE LUMINOSIDADE

Belo Horizonte

2019

Tiago Vilas Boas da Silva

AVALIAÇÕES MORFOFISIOLÓGICAS EM TRÊS VARIEDADES DE CAFÉ CRESCENDO SOB DIFERENTES CONDIÇÕES DE LUMINOSIDADE

Versão Final

Dissertação apresentada ao Programa de Pós-Graduação em Biologia Vegetal do Departamento de Botânica do Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais, como requisito parcial à obtenção do título de Mestre em Biologia Vegetal.

Área de Concentração: Fisiologia Vegetal

Orientador: Prof. Dr. José Pires de Lemos Filho

Belo Horizonte

2019

Dissertação defendida pelo mestrando Tiago Vilas Boas da Silva, aprovada em 29 de julho de 2019, pela banca examinadora constituída pelos professores:

Dr. José Pires de Lemos Filho (orientador)

Dr. Cleber Juliano Neves Chaves

Dra. Andréa Rodrigues Marques Guimarães

AGRADECIMENTOS

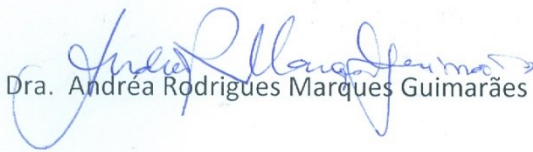Esse texto é apenas uma pequena forma de agradecer a todos que participaram da minha jornada de três anos no laboratório de Fisiologia Vegetal da UFMG, sendo os últimos dois anos como aluno do PPGBV-UFMG. Inicialmente dedico esse trabalho aos meus familiares que sempre contribuíram incansavelmente para meu bem-estar ao longo desses anos de trabalho. A minha mãe, Maria de Lurdes, agradeço pela luta, perseverança, apoio e confiança depositadas em mim durante todas as etapas da minha vida. A minha avó, Maria da Conceição, agradeço por estar presente no meu dia a dia sempre zelando por mim da melhor forma possível. Ao meu pai, Cristiano, minhas tias, Rosana e Tati, meus irmãos, Lucas e Miguel, e primos, Kaio e Bianca, também deixo meu agradecimento por todos os bons momentos durante todos esses anos de vida.

A todos meus amigos, deixo um abraço especial e sincero por todos os momentos vividos durante esse tempo, agradeço pela vida além do Lattes que vocês me proporcionaram. Deixo um agradecimento especial para meu amigo Rodrigo, que é sempre empenhado nos debates acerca da botânica, futebol, política e qualquer outro assunto, sempre me apresentando e ensinando sobre a diversidade de espécies existentes, especialmente das Rubiaceae, e com diversão garantida independente do momento. Aos meus companheiros do laboratório e de outros grupos da botânica, Fernanda, Leilane, Humberto, Tulio, Ailton, Dani Boanares, Jana, Dani Pianetti, Juliana, Natália, Leandro, Val, Elaine e outros agradeço pela convivência e momentos mais que especiais em qualquer lugar que estivéssemos, seja tomando um cafezinho ou para um dia de trabalho no campo. Aos ICs que passaram pelo laboratório, que foram muito importantes durante o período de experimentos, mas que se tornaram amigos, Fernanda e Silvia, fica aqui meu agradecimento especial a vocês. A Roberta, por todo companheirismo nesse tempo que estamos juntos, por todas os momentos que compartilhamos juntos e as mais diversas demonstrações de carinho e amor que recebi por você.

Agradeço imensamente os professores da Botânica e outros departamentos por todo ensinamento, discussões e momentos de risada nesse tempo, além de toda contribuição para meu crescimento pessoal e profissional. Ao meu orientador José Pires de Lemos Filho, pela orientação, diálogos, paciência e ideias sempre bem-vindas para a execução do trabalho. Também agradeço aos funcionários não botânicos, Isabela, Denise, Val por sempre buscarem ajudar os alunos do departamento. Ao CNPq e FAPEMIG pela concessão da bolsa de estudos

durante o período de trabalho. Agradeço a Deus por estar sempre presente na minha vida, atuando de forma inexplicável, mas que ajuda a me tornar uma pessoa melhor a cada dia.

Ao final, dedico esse trabalho especialmente a duas pessoas que contribuíram diretamente ao longo desses dois anos com a execução do trabalho mas que se tornaram meus amigos, Alexandre e Felipe, que sempre confiaram em mim, me motivaram em todos os momentos desde minha chegada ao laboratório e que me ensinaram muito sobre a fisiologia vegetal e sobre a vida. São pessoas muito especiais que, assim como todos os outros, se tornaram meus amigos e vou levar por toda minha vida, sem vocês nada disso seria possível e cada um com sua forma de ser, me ajudou a crescer em todos os contextos possíveis.

Obrigado!

Abstract

Coffee is one of the main agricultural products traded in the world and species *Coffea arabica* L. and *Coffea canephora* Pierre ex A. Froehner are the most cultivated. They are plants that can be grown in environments of different shading conditions, but with productivity and morphophysiological characteristics that can be altered. The objective of the present study was to evaluate the effect of shading on growth, gas exchange and heat tolerance of photosystem II (PSII) in three coffee cultivars, Mundo Novo and Catuaí Amarelo (*C. arabica*) and Conilon (*C. canephora*). We hope that: I - the varieties are capable of acclimatization to the different shading conditions, with important morphophysiological alterations for photosynthesis; II - plants in sun-exposed environments showed higher tolerance of photosynthetic apparatus to heat; III - Conilon variety will have the most heat tolerant photosynthetic apparatus, as *C. canephora* originates from warm habitat. To test these hypotheses, plants of the three varieties were grown in containers with 3 liters of soil under two light conditions (Sun and Shade), with climate monitoring, constant hydration and nutrient replacement. Plant growth parameters (leaf number, plant growth and stem diameter), gas exchange (from light response curves), stomatal traits, pigment content and PSII heat tolerance were evaluated. Temperature and humidity varied significantly between growing conditions. In general, coffee trees growing in sun-exposed environmental conditions presented higher leaf number, higher density and stomatal index, higher gas exchange rates and other parameters evaluated by light response curves. In Shade condition the plant size, stomata size and chlorophyll content were higher. About the thermotolerance of PSII, Conilon was the most heat tolerant variety, and plants in Shade condition plants were more thermotolerant. Growing in a brighter environment may be beneficial to plant growth and photosynthetic rates of Mundo Novo and Catuaí Amarelo, varieties of C. arabica. However, for Conilon (*C. canephrora*), the microclimate variation in Sun condition can be detrimental to PSII growth and thermotolerance in this variety. According to the morphophysiological responses found, coffee varieties generally exhibit similar adaptations for each growing condition, but each variety has distinct responses even if they belong to the same species. Thus, the cultivation of varieties must respect their adaptive limitations to ensure a good physiological status of the plants and consequently good productivity.

Key-words: *Coffea arabica. Coffea canephora.* Temperature. Gas exchange. Shading. Microclim. Thermotolerance.

Resumo

O café é um dos principais produtos agrícolas comercializados no mundo sendo as espécies *Coffea arabica* L. e *Coffea canephora* Pierre ex A. Froehner as mais cultivadas. São plantas que podem ser cultivadas em ambientes com distintas condições de sombreamento, porém com produtividade e características morfofisiológicas podendo ser alteradas em cada condição de luminosidade. O objetivo do presente estudo foi avaliar o efeito do sombreamento no crescimento, trocas gasosas e tolerância do fotossistema II (PSII) ao calor em três cultivares de café, Mundo Novo e Catuaí Amarelo (*C. arabica*) e Conilon (*C. canephora*). Esperamos que: I - as variedades são capazes de aclimatação às distintas condições de sombreamento, com alterações morfofisiológicas importantes para fotossíntese; II - plantas em ambientes mais ensolarados apresentaram maior tolerância do aparato fotossintético ao calor; III – a variedade Conilon terá o aparato fotossintético mais tolerante ao calor, pois *C. canephora* é originaria de habitat quente. Para testar essas hipóteses, plantas das três variedades foram crescidas em recipientes com 3 litros de terra sob duas condições de luminosidade (Sol e Sombra), havendo monitoramento climático, constante hidratação e reposição de nutrientes. Foram avaliados parâmetros de crescimento das plantas (número de folhas, crescimento e diâmetro do caule), trocas gasosas (a partir de curvas de resposta a luz), características estomáticas, teor de pigmentos e termotolerância do PSII. Temperatura e umidade variaram significativamente entre as condições de crescimento. No geral, cafeeiros crescendo em condição ambiental mais ensolarada apresentaram, maior numero de folhas, maior densidade e índice estomático, maiores taxas de trocas gasosas e demais parâmetros avaliados pelas curvas de resposta a luz. Em condição sombreada o tamanho da planta, tamanho dos estômatos e o teor de clorofila foram maiores. Acerca da termotolerância do PSII, Conilon foi a variedade mais tolerante ao calor, sendo que nessa variedade plantas de ambiente mais sombreados foram mais termotolerantes. Crescer em ambiente mais luminoso pode ser benéfico ao crescimento das plantas e taxas fotossintéticas de Mundo Novo e Catuaí Amarelo, variedades de *C. arabica*. Porem para Conilon (*C. canephrora*), a variação microclimática existente no ambiente mais ensolarado pode ser prejudicial ao crescimento e termotolerância do PSII nessa variedade. De acordo com as respostas morfofisiológicas encontradas, as variedades de café exibem no geral adaptações semelhantes para cada condição de crescimento, porem cada variedade tem respostas distintas mesmo pertencendo a mesma espécie. Sendo assim, o cultivo das variedades deve respeitar suas

limitações adaptativas para garantir um bom status fisiológico as plantas e consequentemente boa produtividade.

List of Figures

Figure 1. Photosynthetically photon flux density (PPFD) in January 28 (**a**) and February 04 (**b**), the days with more and less PPFD, respectively. The continuous line represents the Sun and the dotted line the Shade growth condition.

Figure 2. Temperature, air relative humidity and precipitation during January to March in Sun, Shade and local weather station. Minimal, mean and maximum temperature registered, in Sun (**a**), Shade (**b**) and for weather station (**c**). Air relative humidity for Sun (**d**), Shade (**e**) and for weather station.

Figure 3. Example of stomatal structure and epidermis cells in abaxial surface of the coffee varieties growth in the sun (left column) and shade (right column) conditions. Catuaí Amarelo is represented by (**a**) and (**b**); Mundo Novo is represented by (**c**) and (**d**); Conilon is represented by (**e**) and (**f**). Letters inside the structures mean: ST, stomatal; EP, epidermis cells. Scale bars = *200 μm*.

Figure 4. Result of SPAD index and DMSO pigments quantification are shown in the following graphs: SPAD readings (**a**), Total Chlorophylls content (**b**), Chlorophyll a/b ratio (**c**), Chlorophyll/Carotenoid ratio (**d**) and Carotenoids content (**e**) in coffee varieties in each shading condition (sun and shade). Vertical bars are mean ± SE. Different letters in columns indicate statistically significant differences in that variety (n = 10, $p < 0.05$, Two-way ANOVA and Tukey test).

Figure 5. Relationship between stomatal traits and gas exchange. Significant relationships are shown for P_{Nmax} and SD (**A**), and P_{Nmax} and SI (**B**), *E* and SD (**C**) and *gs* and SD (**D**). Filled symbols represent the varieties in the shade and open symbols the varieties in the sun. Each symbol represents a variety, square (Conilon), triangle (Mundo Novo) and circle (Catuaí Amarelo).

Figure 6. PSII thermotolerance of coffee varieties under different shading conditions, using two methodologies, ramping assay with results of T_{50} (**a**) and in static assay with results of H_{50} (**b**). Means ± SE followed by the same uppercase letter do not differ significantly from one another for sun and shade treatments within varieties. (n = 5, $p < 0.05$, Two-way ANOVA and Tukey test).

Summary

1. INTRODUCTION

Plants being sessile organisms require adjustments in their morphology of structures that participate in the light capture process, and physiology of metabolic processes dependent on light (Valladares, 2003), to develop properly in the habitat. At the foliar level, the entire morphological and physiological structure of this organ is subject to significant adjustments in relation to environments with different light conditions, being that this differentiation results in called Sun or Shade leaves (Naidu and DeLucia, 1998; Rozendaal *et al.*, 2006). The characteristics presented by Sun leaves compared to Shade leaves are summarized by a higher thickness of tissues and cells, high stomatal density, lower concentration of photosynthetic pigments, higher concentration of photoprotective pigments and the use of greater amounts of light to reach the maximum yield of photosynthetic processes (Valladares and Niinemets, 2008; Nobel, 2009). These structural changes are to optimize the capture and use of light, which would ensure better photosynthetic efficiency and therefore plants would survive in that habitat. (Naidu and DeLucia, 1998; Niinemets and Valladares, 2004; Terashima *et al.*, 2006).

Shaded environments frequently present a more stable microclimate in relation to sun-exposed environments (Barradas and Fanjul, 1986; Morais *et al.*, 2006). In contrast, sun-exposed environments are subject to high temperatures, low available water in soil and low relative humidity in daytime (Mittler, 2006). The functioning of photosynthetic apparatus is related to climatic variation that occurs naturally in one day, and under stressful conditions the photosynthetic activity may be compromised (Bita and Gerats, 2013; Yamori *et al.*, 2014). Above the upper limit of optimal temperature for a species, the photosynthetic activity tends to fall until the moment of total interruption of this process, limiting plant growth (Berry and Bjorkman, 1980; Damatta and Ramalho, 2006; Wahid *et al.*, 2007). The temperature increase up to 10-15°C above local average temperature, already causes damage to the photosynthetic apparatus in most species, which is characterized as shock or thermal stress (Wahid *et al.*, 2007; Allakhverdiev *et al.*, 2008). Thus, the intensification of stressful events, such as high temperatures, tend to produce greater impacts in sun-exposed regions that can severely damage the cellular structure of plants (Davis *et al.*, 2012; Suzuki *et al.*, 2014; Müller *et al.*, 2017). As a consequence, the ongoing climate changes may contribute to significantly reducing or losses in the productivity of the crops as well as a reduction of areas suitable for agriculture (Mittler, 2006; DaMatta *et al.*, 2010; Bita and Gerats, 2013; IPCC, 2014).

Coffee is classified as second most traded natural product in the world, behind oil, being among the most consumed beverages today and a source of income for many farmers around the planet (Vega *et al.*, 2003; Damatta *et al.*, 2018). *Coffea arabica* L. and *Coffea. canephora* Pierre ex A. Froehner are the two most cultivated coffee species, representing approximately 99% of the world coffee production (Damatta *et al.*, 2018). The greatest coffee productions currently occur in intertropical region of the world, and generate one of the most valuable natural commodities (Chaves *et al.*, 2008; Damatta *et al.*, 2018). The cultivation of *C. arabica*, corresponds to 60-75% of current coffee production, with the remainder being *C. canephora*, which can account for up to 40% of coffee production (DaMatta *et al.*, 2007; Mussatto *et al.*, 2011; Covre *et al.*, 2016). Compared to other coffee species, *C. canephora* varieties present higher resistance to diseases, higher productivity, concentration up to three times higher caffeine in the grain and may require lower expenditures with agricultural inputs in the production, but the quality of the drink coming from *C. arabica* beans is superior and then this makes this species more cultivated (DaMatta *et al.*, 2007; Mussatto *et al.*, 2011).

The species *C. canephora* is naturally spread over an extensive area, from east to west in the tropical and subtropical region of the African continent, occupying humid and gallery forests, seasonally dry forests, and forests present in the savanna (Davis *et al.*, 2006). The annual rainfall in this region can exceed 2000 mm, the average temperature can vary from 22-26°C and the populations of the species often occupy lower regions, about altitudes of 875 meters (Noirot *et al.*, 2016). Meanwhile, *C. arabica* occupies regions about altitudes of 1500 meters, and natural distribution is restricted to the northeastern region of African continent, mainly in the south of Ethiopia, where there are forests of humid and perennial altitude (Davis *et al.*, 2006; Noirot *et al.*, 2016). It is cooler habitats than those occupied by *C. canephora*, with average temperature between 18-23°C and annual precipitation of 1600-2000 mm (De Camargo, 2010). Currently, *C. arabica* species is on the IUCN red list (International Union for Conservation of Nature) in Endangered category (EN) due to small distribution, degradation of the natural environment and impacts of climate change in southern Ethiopia (Moat *et al.*, 2018).

Although the species *C. arabica* and *C. canefora* are naturally found in shaded environment, the commercial coffee crop of these species are cultivated under sun-exposed conditions in Brazil, which represent approximately 90% of the production areas (DaMatta, 2004). Due to the most potentially stressful conditions of the sun-exposed cultivation, climate changes may reduce the suitable areas for coffee production (Assad *et al.*, 2004; Bunn *et al.*, 2015). Therefore, an alternative way of maintaining the traditional areas of coffee cultivation

may be the cultivation under shaded conditions, however, few studies have simultaneously tested the effect of distinct sun exposures on distinct varieties of coffee of the *C. arabica* and *C. canephora* (e.g. Carvalho et al. 2001; Batista-Santos et al. 2011; Hurxley 1967, Ramalho et al. 2013; Martins et al. 2016; Rodrigues et al. 2016 and Rodrigues et al. 2018). Understanding how the quality and quantity of shading can affect the development of coffee species is essential for cultivation practices that make plants less susceptible to long and intense periods of stress. Morphophysiological analyzes may be essential in monitoring the health plants that growing under different shading conditions.

Coffee trees are recognized for their low net carbon assimilation rate in comparison to other tree species, on average between 4 and 12 μmol (CO_2) m^{-2} s^{-1}, with stomatal conductance reaching rates up to 150 mmol H_2O m^{-2} s^{-1} (DaMatta *et al.*, 2007). Some studies have shown that maximum carbon assimilation in coffee trees are similar under partial shade conditions throughout the day (Morais *et al.*, 2012) or distinct sun-exposed conditions (Baliza et al., 2012; Chaves *et al.*, 2008; Rodríguez-López *et al.*, 2014). The species *C. arabica* seems to have lower rates of assimilation and stomatal conductance than *C. canephora* despite their similar photosynthetic saturation levels, between 300 e 700 μmol photons m^{-2} s^{-1}, with the lowest values being observed in the shade leaves (DaMatta, 2004; DaMatta *et al.*, 2007).

In sun-exposed coffee production is common the temperature to reach 40°C or higher, which extrapolates optimum temperature range for growth of both *Coffea* species (DaMatta *et al.*, 2007; Chaves *et al.*, 2008; Rodríguez-López *et al.*, 2014). Some of the consequences of high temperatures one coffee are flower abortion and tumor growth harming fruit formation and resulting in coffee drinks with inferior qualities (DaMatta *et al.*, 2007, 2010; Wahid *et al.*, 2007). In addition, temperatures close to 40°C may affect the concentration and activity of photosynthetic enzymes, causing decreases in photochemical efficiency and carbon assimilation in coffee plants (Rodrigues *et al.*, 2016; Marias *et al.*, 2017a,b). These results show that the tolerance of the photosynthetic apparatus and upper limit of the optimum temperature range for the photosynthetic activity in the coffee is around 40°C.

The general aim of this study was to evaluate the growth, photosynthetic behavior and PSII tolerance of varieties of *Coffea arabica* (cv. Mundo Novo and Catuaí Amarelo) and *Coffea canephora* (cv. Conilon) under two shading conditions. To do this, we compared morphological traits and tested the photosynthetic behavior of distinct coffee varieties under high and low sun exposures. We expected that are that plants of both species growing under shade conditions besides altering changes in gas exchange parameters, would present lower thermal tolerance as

compared to the sun adapted ones. We also intent to verify if the Conilon variety of *C. canephora* has greater thermal tolerances, due to the hotter climate of its original areas, compared to *C. Arabica* (Davis et al., 2006; Noirot et al., 2016).

.

2. MATERIAL AND METHODS

2.1 Plant material and experimental design

In September 2016, seeds of *C. arabica* var. Mundo Novo and var. Catuaí Amarelo and *C. canephora* var. Conilon were germinated in a germination chamber (FANEM, model 347 CDG, São Paulo, Brazil) with a constant temperature of 25°C and photoperiod 12/12. After the radicle emission the newly germinated seeds were transferred to black plastic bags with capacity for 3 liters containing soil composed of 70% commercial vegetable substrate (Plantmax®) and 30% sand. Plants were grown at Universidade Federal de Minas Gerais, Brazil (19°52'09.2"S 43°57'59.9"W). Coffee varieties grew under distinct conditions of temperature and humidity and incident light determined by two shading levels: ~ 75% (Sun) and ~ 15% (Shade) of luminosity in relation to external environment. For the sun treatment it was covered by a metallic structure with a white polyethylene mesh with 0.72 x 1.07 mm (Optinet®). For shade treatment was used high density polyethylene mesh (Sombrite®) black color. In total 12 plants of each variety were cultivated in the respective shading conditions, with a total of 72 plants.

Seedlings of each variety were selected and randomly distributed in each shading treatment. Plants were irrigated every two days near the field capacity. The pot positions were periodically randomized to minimize any variation within each light environment. Once a month were added in each pot 50 ml of Hoagland and Arnon (1938) nutrient solution, with modifications, containing ½ of the ionic strength with following final concentration: $Ca(NO_3)_2$ 0.25 mmol 1^{-1}, $(NH_4)_2SO_4$ 0.25 mmol 1^{-1}, KH_2PO_4 0.5 mmol 1^{-1}, $MgSO_4$ 0.5 mmol 1^{-1}, $CaCl_2$ 0.5 mmol 1^{-1}, Fe-EDTA 1 mmol 1^{-1} e Micronutrients 1 mmol 1^{-1}.

2.2 Microclimatic characterization

The density of the photosynthetic active photon flux (PPFD) was determined using quantum sensors coupled datalogger (Li-Cor model LI-1400, Lincoln, EUA), where the incident light was measured every 5 minutes. Relative humidity and air temperature were obtained using EL-USB-2 EasyLog sensors (Lascar electronics, Erie, PA, USA) which were positioned at height of 0.9 m in relation to the seedlings. These data were collected in January to March/2018, period of physiological tests. Meteorological data of the same period for the

Pampulha meteorological station were obtained on the website of Instituto Nacional de Meteorologia (INMET).

2.3 Growth measurements and stomatal traits

After eighteen months of cultivation, in February, we account for total number of leaves, number of plagiotropic branches, height and stem diameter (3 cm above ground) of 10 individuals of each variety in their respective growth condition, totalizing 60 measurements. We used measurement-tape and digimatic caliper (Mitutoyo, Japan) to measure individual heights and diameters of plants, respectively.

For determination of the stomatal density (SD), stomatal index (SI), stomatal size (SS) we selected two completely expanded leaves of the five plants used in gas exchange analysis. From each leaf were excised two fragments about 1cm² in middle third and submitted to dissociation of epidermal layer in Jeffrey solution (10% nitric acid:10% chromic acid). The leaf tissue was stained with 0.5% safranin solution in 95% ethanol and semi-permanent slides were then made using glycerinated gelatin (Johansen, 1940). Importantly, stomatal traits will be defined by analysis of the abaxial epidermis, because coffee leaves are hypostomatous (Fanjul *et al.*, 1985).

Using light microscope (Leica DM2500 LED, Wetzlar, Germany) with coupled camera (Leica ICC50 HD, Wetzlar, Germany) 3 distinct fields were photographed in each leaf fragments, totalizing 60 photographed fields in each variety growing in Sun and Shade. Measurements on stomata were made from photographs using the free software ImageJ version 1.52*a* (National Institutes of Health, USA). We calculated SD from 40 fragments as the number of stomata per leaf area unit (mm²). To calculated SI, in turn, we selected 10 random fragments and performing the following equation: *SI = [(total stomata)/(total cells + total stomata)] ×100*. For SS, 100 randomly selected stomata and their areas were calculated as $SS = \pi ab$ (μm^{-2}), where *a* and *b* are 1/2 length and 1/2 width, respectively, thus assuming that the stomatal shape is an ellipse (Ramalho *et al.*, 2013).

2.4 Quantification of photosynthetic pigment

We used two methods to evaluate the chlorophyll content by way of comparison of these two forms of pigment analysis. We measured the chlorophyll content of ten individuals per variety under each treatment using a portable chlorophyll meter (SPAD-502, Minolta, Japan). The chlorophyll content of each individual represents the average of three distinct measurements made on each of the first three fully expanded leaves, totalizing in each plant nine measurement and ninety per variety in each shading condition.

Photosynthetic pigments contents were also determined in two circular samples of 0.78 cm^2 cut of the middle third of a leaf of each individual used previously and placed in an amber bottle containing 4 mL dimethyl-sulfoxide (DMSO). The amber bottle was incubated in laboratory conditions in the dark during the minimum of 48 hours for total extraction of pigments. An aliquot containing 2 ml was then analyzed spectrophotometrically at 480, 649 and 665 nm (Spectronic® 20 Genesys™, Spectronic instruments, NY, USA). The chlorophyll a (Chl *a*), chlorophyll b (Chl *b*), carotenoid (Car) concentrations were determined according to the equation proposed by Wellburn (1994). The ratio between chlorophyll a and b, total chlorophyll content, ratio between carotenoid and total chlorophylls were determined from the values obtained in pigment extractions. The relationship between the two measurements of chlorophyll content in plants was analyzed.

2.5 Gas Exchange Measurements

The photosynthetic light-response curves (P_N/I curve) were conducted with a portable infrared gas analyzer (IRGA) Model Li-Cor LI 6400 XT (Li-Cor Inc., Lincoln, NE, USA) coupled with LED camera as irradiation source. Measurements were made during the morning (8:00 -11:00 h), from January 16th to 25th, 2018. In this period at the end of each day the pots were watered to ensure the maximum hydration of the plants. The P_N/I curve was performed using the auto program function. In this case, we chose the sequence of desired light settings of 0, 40, 80, 120, 160, 200, 400, 600, 800, 1000 e 1200 μmol (photon) m^{-2} s^{-1}, and a minimum wait time of 180s to maximum wait time of 300s was to reach a steady photosynthetic state with the lowest coefficient of variation possible before measuring, and the sensor was matched for each plant curve. The measurements were obtained with a block temperature of 30 ± 0.5°C, and 50 to 60% relative humidity, and ~385 μmol (CO_2) mol(air)$^{-1}$ of CO_2 concentration inside

the chamber. The first fully expanded leaf was selected from the apex of 8 individuals of each shading treatment applied in the varieties. The net rate of carbon assimilation (P_N), stomatal conductance (g_s), internal CO_2 concentration (C_i) and transpiration rate (E) were measured in each point of light curve.

We used nonlinear mathematical models, which describe the photosynthetic activity in relation to light, compiled by Lobo et al. (2013), to estimate the photosynthetic efficiency in coffee varieties. From worksheets developed in *Microsoft Excel* and using the Solver function, it was possible for us to choose the model that presented the smallest sum of the error squares (SSE) (Lobo *et al.*, 2013). The curves submitted to the non-rectangular hyperbolic equation described by Prioul e Chartier (1977) were the best for all varieties, basing the choice on SSE. From this equation, parameters were estimated as: The dark respiration rate (R_D), light compensation point (LCP), light saturation point (LSP) and the maximum apparent photosynthetic quantum yield at $I = 0$ ($\phi_{(Io)}$). The LSP in light curve was attributed the luminosity necessary to reach 90% of the maximum assimilation, when no significant changes in assimilation has been observed (Rascher *et al.*, 2000; Lobo *et al.*, 2013). The assimilation values obtained in LSP can be close to real maximum assimilation capacity in these plants, then maximum assimilation (P_{Nmax}), g_s, E and C_i were estimated in LSP for each variety in these shading conditions. The determination of values of water use efficiency (WUE), intrinsic water use efficiency (iWUE) and instantaneous carboxylation efficiency (Φc) were made by the following formulas: WUE = P_{Nmax}/E; iWUE = P_{Nmax}/g_s; Φc = P_{Nmax}/C_i.

2.6 PSII heat tolerance

For the evaluation of PSII heat tolerance two methodologies were used, the ramping assay and constant temperature assay. In the ramping assay, leaf fragments of 1,5cm² from five individuals, of each coffee variety and treatment were removed from the first fully expanded leaves and placed between two metal sheets lined with filter paper, with a thermocouple attached to a digital thermometer (TD-880, ICEL, Manaus, Brazil) for temperature monitoring. The metal sheets were then placed in a hermetic polyethylene bag and submerged in ultratherostatic water bath (Model 116-3, FANEM, São Paulo, Brazil) and then subjected to ta gradual increase in temperature. Initially we measure the potential quantum yield initial (F_v/F_m) at room temperature (e.g. ±27°C), after dark adaptation for 30 minutes, using a portable pulse

amplitude modulation fluorometer (Mini-PAM, Walz, Effeltrich, Germany). Excision and adaptation of fragment in the dark were pretested and showed no change in F_v/F_m.

This first measurement of F_v/F_m at room temperature was considered the starting point for PSII heat tolerance evaluation. Already in the ultratermostatic bath, initial temperature set for the evaluations was 34°C, considered the upper limit of the optimum range for most plants (Berry and Bjorkman, 1980). During the experiment the temperature rise occurred every 2°C after the determination of F_v/F_m and finished at 60°C. From the values obtained, decay temperatures of 15% (T15) and 50% (T50) was determined, from the sigmoidal equation adjustment (Gimeno *et al.*, 2009; Godoy *et al.*, 2011; Chaves *et al.*, 2015).

The procedure of collecting, conditioning in the ultrathermostatic bath and evaluation of photosystem II, and the number of leaf replicates used in the constant temperature assay (static assay) was analogous to the ramping assay. The chosen constant temperature was 49.5°C the mean of the T15 values obtained in the ramping assay. The potential quantum yield initial (F_v/F_m), was measured, following dark adaptation for 30 min, using a portable pulse amplitude modulation fluorometer (Mini-PAM, Walz, Effeltrich, Germany), being considered the zero evaluation time. Thereafter, the F_v/F_m was determined at the times of 15, 35, 60, 85 and 120 minutes, at the pre-established temperature. From the exponential regression (*One phase association*) we obtained the decay time required for a 50% of F_v/F_m (H50) in the constant temperature of 49.5°C.

2.7 Statistical analysis

Data were submitted to Kolmogorov-Smirnov normality test and Brown-Forsythe test for homogeneity of variances with 5% significance. Data that did not present normality or homogeneity underwent logarithmic transformation and were again submitted to the previous tests. In this case only the total number of leaves, stomatal density (SD) and stomatal size (SD) required transformation. The only case where even after the transformation was not verified the homogeneity of variances was in the stomatal size (SS) parameter, being thus analyzed through the Kruskal-Wallis and multiple comparison was made by Dunn's test with 5% significance.

All remaining data was submitted to analysis of variance *Two-way* ANOVA and F test was used to determine whether there was interaction or simple difference in some source of variation. The multiple comparison of the means was made by post-hoc test Tukey HSD at *P* <

0.05, to evaluate differences between varieties and shading condition. All data were analyzed using software Graph Pad Prism 6.0 (San Diego, CA, USA). Linear and Nonlinear regressions were analyzed and plotted in the same software.

3. RESULTS

3.1 Microclimatic data

In the analyzed period, the day with the highest recorded daily light incidence occurred on January 28, 2018 (Fig. 1a). Specifically, in this day at Sun condition had 28.38±3.7 mol photons m^{-2} d^{-1} and Shade 6.25±0.9 mol photons m^{-2} d^{-1} (*mean±SE*). The average of daily light incidence was calculated from 13 days that were evaluated between January and March, and average of daily luminosity was (*mean±SE*) 16.49±1.8 mol photons m^{-2} d^{-1} in the Sun and 3.5±0.4 mol photons m^{-2} d^{-1} in Shade condition. However, the maximal instantaneous values were observed on February 6, 2018, being of 2007.4 µmol m^{-2} s^{-1} in the Sun and on February 13, 2018 in the Shade, 551.4 µmol m^{-2} $s^{-}1$. The less bright day was February 4, 2018 in both conditions (Fig. 1b) being the daily light incidence of (*mean±SE*) 1.2±0.1 mol photons m^{-2} d^{-1} in the Shade and 6.6±0.7 mol photons m^{-2} d^{-1} in the Sun.

The temperature was also a very contrasting variable between the two growth conditions. Maximum temperatures were recorded at January 28, 2018, with the maximum of 53.5°C in the Sun and 39 °C in the Shade. In this date it was also registered the highest average temperature in these environments, 30.8°C and 28.3°C, respectively. The minimum temperatures recorded were 14.5°C in the Sun and 15°C in the Shade. In the monitored period (January / March) the temperature averages were 26.6°C in Sun and 24.8 °C in shade, and data obtained from INMET the mean temperature was 23.5°C (Fig 2a,b,c). Days in which the average daily temperature in each growing condition was higher than that recorded by INMET were 45 and 62 days for shade and sun, respectively. In the sun 1/3 of the monitored days, 24 days had a daily average above 28°C whereas in the shade only 2 days the averages exceeded this value. By INMET, no day averaged above 27°C and in only 13 days averages were recorded between 25°C and 27°C. The minimum mean air temperature during the monitored period was ~19°C in both conditions, and the maximum averages were 43.4°C in the Sun and 34.2°C in the Shade. For the meteorological station of INMET the minimum and maximum averages were 29.6°C and 29.2°C, respectively (Fig 2a, b, c). The mean temperature amplitudes throughout the day were approximately 23,6°C in the Sun and 14.5°C in the Shade during that period.

On the sunny day, January 28, was recorded the lowest relative air humidity, 18.5% in the Sun and 31.5% in the Shade. The daily average air relative humidity was 73.2% in the Sun and 76.2% in the Shade treatments. Minimum and maximum average of air relative humidity were 37.5% and 94.9% for the Sun, 51.6% and 91.9% for the shade (Fig. 2d,e). By INMET, the

average of relative humidity of the air was 70% in that period, and the minimum and maximum means for the region were 46.5% and 86%, respectively (Fig 2f). The Sun growth condition showed a greater variation of daily relative humidity, approximately 57% while in the Shade this variation was on average 40%. The precipitation registered in the INMET meteorological station was 678.3 mm in the period from January 16 to March 29, 2018.

3.2 Growth measurements and stomatal traits

Plagiotropic branches were observed only in two varieties, Catuaí Amarelo and Mundo Novo, being that in Catuaí Amarelo only one plant emitted lateral branch. In Mundo Novo variety all plants emitted lateral branch and the average pairs of branches issued was (*mean±SE*) 3.6±0.5 in shade and 4.6±0.6 in sun condition, with no significant difference between then. Mundo Novo was the variety that emitted, on average, more amount of leaves per plant, followed by Catuaí Amarelo and Conilon. In each coffee variety the total number of leaves was higher in plants growing in the Sun condition (Table 1). In relation to plant height, it is observed that plants growing in Shade condition were taller. Statistically only Conilon presented difference in height between Sun and Shade, with Shade plants being twice as tall as Sun plants (Table 1). The stem diameter was larger in Mundo Novo plants growing in Sun condition and Conilon plants growing in Sun condition showed the smallest stem diameter (Table 1).

Coffee plants have stomata only on the abaxial leaf surface and a consistent trend for lower stomatal density was observed in shade plants regarding sun plants (Table 2). Sun Conilon showed the highest stomatal quantity per area, 297.6 (stmm^{-2}), and lowest value in shade condition, 103.6 (stmm^{-2}), thus difference between sun and shade was 65%. In *C. arabica* species difference between sun and shade conditions was 35% for Catuaí Amarelo and 40% for Mundo Novo (Table 1).

Stomatal index was highest in sun leaves, above 17%, and in Mundo Novo and Conilon varieties the highest index was obtained, about 20% of stomates in relation to the rest of epidermal cells by unit of leaf area (Table 2). In shade leaves this fell to 15% in *C. arabica* species and was only 11% in Conilon (Table 1). Meanwhile, the stomatal size (SS) had different patterns. In *C. arabica* species the SS was above 300 μm^{-2} under both growth conditions, while for Conilon variety SS values was below 300 μm^{-2}, with SS in sun leaves lower than 200 μm^{-2} (Table 1). Sun leaves of Catuaí Amarelo showed the highest SS, 378 μm^{-2}, followed by shade

Mundo Novo, 356 μm^{-2} (Table 2). The discrepancy between the stomates found in the sun and shade at Conilon was approximately 38% and in the varieties of *C. arabica* this difference did not reach 15%.

3.3 Photosynthetic pigments

The highest values found in the SPAD-502 were also the highest observed in the concentration of chlorophyll extracted with DMSO with a positive and significantly relationship between the SPAD-502 readings and chlorophyll concentrations. The r² values between SPAD and pigments were, 0.90 (chlorophyll a), 0.87 (chlorophyll b) and 0.88 (total chlorophyll). The leaves of shade presented greater SPAD index in relation to leaves of sun in all the varieties, only in Mundo Novo, which presented the highest values of SPAD, no difference was observed between sun and shade leaves (Fig. 4a).

This same pattern was found for total chlorophyll extracted through DMSO, but in this extraction Sun leaves of Mundo Novo showed lower values than in the shade leaves (Fig. 4b). In both methodologies of pigment quantification, the Mundo Novo variety presented the highest values of chlorophyll in both growth conditions.

There was no difference in concentration of carotenoids, in each variety growing under different shading conditions (Fig. 4e). Mundo Novo also showed the highest values for carotenoids. Shade leaves had higher chlorophyll *a/b* ratio, and Catuaí Amarelo showed the lowest ratio (Fig. 4c). The carotenoids/total chlorophyll ratio difference was observed between shading conditions in all varieties being that ratio was higher in sun leaves (Fig. 4d).

3.4 Gas Exchange

The amount of light needed to reach the light compensation point (LCP) and light saturation points (LSP) of photosynthetic activity were higher in plants growing in Sun condition. Conilon presented the largest values for these variables, in Sun and Shade conditions. Shade plants of Conilon showed the LSP equal to the values of the *C. arabica* Sun plants and almost double in relation to the Shade plants of the varieties of this species. There was also difference between varieties under same shading conditions. The LSP in *C. arabica* had similar responses in each shade, being in the Sun condition the highest values were observed (Table 2).

There was also a relationship between LCP and respiration (R_D), with the decrease of LCP resulting in lower R_D values ($r^2 = 0.96$, $p = 0.0006$). The parameters about assimilation and respiration differ between shading condition. Higher R_D values have been observed in sun leaves of all varieties. Conilon had the highest respiration rate, and in the Sun leaves there was an increase of ~67% of the RD in relation to Shade leaves. In Mundo Novo the respiration rate increased ~139% in the leaves of Sun in relation to those of Shade, whereas in Catuaí Amarelo the increase was ~54% (Table 2).

The maximum carbon assimilation rate in LSP (P_{Nmax}) presented same pathway that R_D in *C. arabica* species, in general sun leaves showed the highest assimilation rate. Sun leaves of Mundo Novo and Conilon had assimilation rate in P_{Nmax} close to 5.5 µmol (CO_2) m^{-2} s^{-1} and the value in shade plants was ~3.6 µmol (CO_2) m^{-2} s^{-1} (Table 2). In Catuaí Amarelo the highest assimilation rates for P_{Nmax} was in Shade leaves, but statistically no significant difference was observed between shading condition (Table 2). Mundo Novo and Conilon presented an increase of approximately 50% more assimilation in Sun leaves, while Catuaí Amarelo had only 8% more in Shade leaves.

The apparent quantum yield ($\phi_{(Io)}$) of leaves was higher in shade condition, being that in *C. arabica* species we detected 0.072 µmol (CO_2) µmol $(photon)^{-1}$ for $\phi_{(Io)}$ and Conilon had the highest value for $\phi_{(Io)}$, 0.097 µmol (CO_2) µmol $(photon)^{-1}$. Leaves growing in sun condition presented $\phi_{(Io)}$ below 0.070, and in Catuaí Amarelo the value of quantum yield was lowest observed, 0.050 µmol (CO_2) µmol $(photons)^{-1}$ (Table 2).

The stomatal conductance (gs) and transpiration (E) were higher in the leaves of the Sun, independent of the variety and the internal carbon concentration (C_i) was superior in *C. arabica*, mainly in Catuaí Amarelo compared to Conilon, *C. canephora* (Table 2). For Ci there was no difference among light conditions in each variety. The water use efficiency was difference only between Sun Catuaí Amarelo, the lowest value, to Shade Conilon, the highest value (Table 2). The intrinsic water use efficiency (iWUE) and instantaneous carboxylation (Φc) were not significantly different (Table 2).

The relationship between the stomatal variables (SD, SI, SS) and the assimilation variables (P_{Nmax}, *gs*, *E*, C_i, WUE, iWUE and Φc) were expressed through linear regressions. The significant relationship observed between stomatal traits and gas exchange were observed for P_{Nmax} and SD ($p = 0.04$), *E* and SD ($p = 0.01$), P_{Nmax} and SI ($p = 0.03$) and *gs* and SI ($p = 0.04$) and all had r^2 above 0.6 (Fig 5a,b,c,d). The relation between *E* and SI was not significant,

but had r^2 similar to previous ones ($p = 0.057$ and $r^2 = 0.64$). In the latter case, gs and SD had no significant relation ($p = 0.1$) but presented an r^2 of 0.5, and the other relations tested had r^2 always below 0.4.

3.5 PSII heat tolerance

The T50 values obtained in the ramping assay for all studied varieties were higher than 55°C, but only the variety factor evaluated presented significant diference ($p = 0.0008$), no differences were observed between leaves from Sun and Shade growth conditions. The difference observed for T50 was among the varieties, being Conilon the most thermotolerant and Catuaí Amarelo the lowest thermotolerant (Fig. 6a).The heat resistance of the PSII on leaf disks of Conilon Shade leaves was at least 1°C above that of all other treatments, and about ~ 2°C above in relation to Catuaí Amarelo. In the static assay there was interaction between the variety and shading factors ($p = 0.0022$). Conilon shade leaves showed the highest H50 value, ~38.8 minutes, and Catuaí Amarelo sun leaves the lowest H50, ~14.6 minutes (Fig. 6b). The H50 values between plants from Sun and Shade for both *C. arabica* varieties were similar, and that observed for Conilon plants was that shade leaves presented higher H50 values then sun leaves (Fig 6b).

4. DISCUSSION

The light incident on plants was experimentally controlled, with a difference about 60% between treatments, but with natural variation of luminosity throughout the day. During the study period it was observed that the temperature and air relative humidity also were different suggesting that in Sun condition the microclimate was drier and hotter. From the assumptions of climate change, warmer and drier periods are expected to become more frequent throughout the year, including in areas for agricultural production (IPCC, 2014). The average air humidity was similar between shading condition, but the minimum recorded in Sun was about 15% lower in relation to the shade, and days where the humidity reached values below 30% were frequent. The mean air temperature was higher in Sun treatment with daily maximum temperatures frequently above 40°C and in some occasions exceeded 50°C. This situation can be characterized as events of extreme temperatures or heat waves, due high temperature recorded regarding to normal temperature and the duration of that condition over a few days, for example (IPCC, 2014; Suzuki *et al.*, 2014; Hatfield and Prueger, 2015; Teskey *et al.*, 2015). Temperature increase associated with other abiotic stresses, such as drought, is a threat to agricultural crops, as affects the reproductive and vegetative stages at the molecular, cellular and physiological levels, resulting in severe plant damages and productivity reduction (Wahid *et al.*, 2007; Lichtenthaler, 2012; Zandalinas *et al.*, 2018).

According ours results, the microclimate of Sun condition may be one of the determining factors for growth impairment mainly of Conilon plants, which had the lowest aerial part growth observed among varieties. Although *C. canephora* is a species adapted to grow in warmer environments, it also requires high humidity conditions or short dry period (DaMatta *et al.*, 2007; Ramalho *et al.*, 2014), characteristics not observed in our Sun experimental conditions. Our Shade conditions propitiate less stressful microclimatic conditions for this variety, with attenuation of high temperatures and promoting higher air relative humidity, which allow the existence of an ideal microclimate for the development of plants (Bote and Struik, 2015; Alves *et al.*, 2016). Morais et al. (2012) evidence for *C. canephora* clones that shading at some time of the day may be beneficial for good physiological performance of plants in relation to plants grown in full sunlight. For this species, a little shading may be interesting for the cultivation, mainly by the microclimate created in these conditions, in order to maintain a better humidity of the air and the soil besides for good development of plants. For *C. arabica* varieties, the Sun microclimate did not seem to affect in

higher extent the growth of plants, since most of analyzed growth variables showed equal or superior values of Shade grown plants. An additional factor that may have been impaired plant growth was small volume of soil which may restrict the development of roots. Combined with drier and hotter conditions of the Sun conditions, the impact of the available volume of soil may have been decisive for this behavior, especially for Conilon variety. Ronchi et al. (2006) showed that the development of *C. arabica* was influenced by the restriction of soil space for root growth, resulting in a reduction in growth under conditions of greater soil volume restriction.

Regarding the possible high light, drier and heat stress on gas exchange variables, our results suggests that plants of all varieties were already acclimated to these conditions since the values of photosynthetic parameters as the assimilation rate at the light saturation point (P_{Nmax}), as well stomata conductance (g_s) and transpiration rate (E) were higher in the most stressful microclimatic condition (Sun) than in Shade. For example, Marias et al. (2017*b*) observed that coffee trees under different duration of heat stress have a reduction in the photosynthetic rate, non-structural carbohydrate content in the leaves, decrease in iWUE and may not produce flowers in the reproductive period. However, the plants were grown all the time under controlled conditions of temperature, humidity and luminosity and were not subjected to any previous heat stress event. Their results are important to characterize the effects of the only an abiotic stress in plants, in this case high temperature, at the first moment of contact with plant with this stress, which is different from growing in an environment with natural variation of abiotic factors as in the present study. So, any type of response to the abiotic stresses has to be attributed to the set of variables and conditions that the plants were subjected to in our work, which in fact is what occurs in a natural environment.

From the gas exchange data there was also no difference between shading condition for Φc, WUE e iWUE in each variety. When the parameters WUE or iWUE are high this indicates that the plants are more adapted to withstand drier environments (Machado *et al.*, 2005) which did not happen in the study and coffee plants used the same quantify of water in the sweating process for the assimilation of CO_2. In a study comparing the effect of soil volume on photosynthesis Ronchi et al. (2006) showed that the CO_2 assimilation in coffee plants growing in 3L pots ranged between 5.5 e 3.9 µmol m^{-2} s^{-1}. These values were closed to those obtained in our study. Conilon and Mundo Novo varieties in Sun presented maximum assimilation close to 5.5 µmol m^{-2} s^{-1}. In Shade conditions, this value decreased to 3.6 and 3.7 µmol (CO_2) m^{-2} s^{-1} respectively. As all plants in our work were subjected to the same amount of soil volume, shading seems to have determined the decrease in assimilation rate. Our results strongly

suggested that the observed differences in the assimilation rate also were associated with morphological differences observed in each shading condition.

The shading becomes restrictive mainly by the reduction in the amount and quality of light incident on the plants (Farquhar and Sharkey, 1982; DaMatta, 2004; Nobel, 2009). In coffee, growing in environments with much light restriction throughout the day, significantly reduces the rate of carbon assimilation (DaMatta, 2004; Franck and Vaast, 2009; Matos *et al.*, 2009; Baliza *et al.*, 2012; Rodríguez-López *et al.*, 2014). In fact, shading, as well as excessive light, are limiting factors of the photosynthetic process, and both conditions require adaptations to increase the efficiency of light capture or photoprotection, respectively (Beer, 1987; Valladares and Niinemets, 2008; Bote and Struik, 2015). Plants that are conditioned to shade have morphophysiological adaptations, in relation to individuals in more luminous environments, to enable the photosynthetic process to be efficient in low light conditions and the development of the plants is not compromised (Niinemets and Valladares, 2004; Valladares and Niinemets, 2008). So, the increase in total chlorophyll would increase the light capture by coffee plants growing in shade, as the lower chlorophyll content in Sun grown plants can be strategy to prevent excessive amounts of light from being absorbed and not being used or dissipated, causing damage to the photosystem II (PSII) (Nobel, 2009). The coffee trees, besides being plants originally found in shade environments, are organisms with high phenotypic plasticity to the contrasts of existing luminosity, and are able to maintain a positive balance of carbon in low luminosities (Matos *et al.*, 2009; Morais *et al.*, 2012). The decrease in respiration rate, high photosynthetic quantum yield, increased concentration of photosynthetic pigments, lower LCP and lower density/stomatal index observed in the work may represent also some of these strategies for shade trees to increase the efficiency of the use of luminosity and CO_2 assimilation (Valladares and Niinemets, 2008; Matos *et al.*, 2009). These characteristics seem to have been fundamental for Catuaí Amarelo presented similar assimilation rates between the environments, and consequently grow in the same proportion in the two shading conditions.

Even shade plants presented characteristics aimed at optimizing photosynthesis, assimilation rates were less than or equal to those observed in Sun plants, showing that another factor was limiting the gas exchange in the plant. Only Catuaí Amarelo appears to benefit from the characteristics presented by the plants in greater shading condition. Then it is possible that the lower values of gas exchange in Shade plants were associated to the decrease in the stomata density (SD) and stomata index (SI) in shade conditions. In this context, for CO_2 assimilation, the structures with a central role in this process are the stomata, because they are directly

associated with the capacity of gas exchanges between the environment and the plant, and can control about 95% of this process (Matthews *et al.*, 2018). The stomatal characteristics, such as SD and SS, have different leaf patterns according light conditions, and are associated with the first limitation barrier of photosynthesis (Martins *et al.*, 2014*b*). In our study, having greater stomatal density or stomatal index represented greater assimilation, stomatal conductance and transpiration, a fact also observed by Matos et al. (2009). The regressions between SD or SI and the parameters of gas exchange reinforce the idea that stomatal characteristics and area occupation are determinant in the process of assimilation.

Thus, the gas exchange process can then be limited at some point by the stomatal conductance, and it directly reflects on the transpiration, as well as the assimilation, parameters directly influenced by the gas diffusion capacity by this structure (Jones, 1998). Shade coffee plants had lower stomatal conductance rates compared to Sun plants, being one of signs of stomatal limitation in the photosynthetic process. The lowest stomatal conductance rate is one of the characteristics expected for shade leaves, and in studies of the photosynthetic activity of coffee under low incident light, close to 90% shading, this behavior is observed (Baliza *et al.*, 2012; Martins *et al.*, 2014*b*; Rodríguez-López *et al.*, 2014). Partial shading between 25% and 75% in contrast may represent higher rates of stomatal conductance in relation to full sun or very shaded environments (Franck and Vaast, 2009). The development of the plants in the respective microclimates allowed the varieties to present different strategies for survival in each condition, and the types of stress throughout growth are crucial for acclimatization and resistance to future stressful events.

For tolerance of PSII to heat, we expected that growing in a more luminous condition, with all microclimatic characteristics presented, could increase the heat tolerance of PSII, as recorded in other species (Gimeno *et al.*, 2009; Godoy *et al.*, 2011; Chaves *et al.*, 2015). In *C. arabica* varieties differences were not observed between Sun and Shade, and the lowest values of T_{50} and H_{50} were for this species. However, *C. canephora* showed to be more heat tolerant, in two methodologies employed (ramping and static assay), in Shade leaves than Sun leaves, and still. This aspect of heat tolerance in *C. canephora* species, shade leaves more resistant to heat, make it more evident that the effect of ambient water conditions influences the development of this species, and in this case, heat tolerance. It may be that the effects of high temperatures, high light irradiance and low air humidity, compared to the Shade condition microclimate, have a more deleterious impact on the photochemical phase, and consequently

PSII, than on the biochemical reactions of the Calvin-Benson cycle and the process of gas exchange of this species.

Under excessive light and stressful temperatures is the increase of reactive oxygen species (ROS) that degrade proteins and cellular structures, such as PSII, photosynthetic pigments and membranes (Wahid *et al.*, 2007; Martins *et al.*, 2014*a*). Intracellular generation of ROS can be occur in many cellular organelles, as chloroplast, mitochondria and peroxisomes, and this sites one of main sites of formation of reactive species are mitochondria, site of the cell respiration (Mittler, 2002; Apel and Hirt, 2004; Mathur *et al.*, 2014) and increased oxidative stress may be associated with higher respiration rates (Tiwari *et al.*, 2002). In coffee trees it has been demonstrated that ROS can be more produced in periods where unfavorable conditions for their growth are observed (Chaves *et al.*, 2008; Martins *et al.*, 2016). The production of antioxidant structures in coffee trees to combat reactive oxygen species may be lower in plants growing under high temperature conditions. Then it is possible that higher respiration rates observed in Sun leaves also lead to higher ROS, and this may have affected the structure of PSII and its consequently the tolerance to heat.

Although Shade Conilon plants presented lower values of transpiration and assimilation of CO_2, their highest values of T_{50} and H_{50} suggests that protective mechanisms, as antioxidant system, has more efficient action, permitting longest time of PSII heat resistance in this condition and do not degrade other structures important for protection. Heat shock proteins (HSPs), for example, are commonly synthesized, accumulated and easily detected at temperatures exceeding the optimum growth limit, with their production increasing in relation to temperature (Kaiser, 1987; Suzuki et al., 2014, Sun et al., 2002; Vierling, 2003; Wahid et al., 2007), which happened under both conditions of growth, but at higher intensities in the Sun. These proteins are essential for many cellular organelles, being associated with the structures to confer greater thermal tolerance, thus preventing the denaturation of molecules and rupture of membranes (Allakhverdiev et al., 2008). Martins et al. (2016) observed that in *C. arabica* the content of HSP70 was similar after plants were submitted to temperature conditions higher than 30°C, in a short term period. However, in *C. canephora* there was a decrease in the content of these proteins at growing temperatures that exceeded 40°C. Another important fact is that some antioxidant enzymes had changes in chloroplastic maximal activities in both species Martins et al. (2016). of HSP in *C. canephora* and this has affected the thermotolerance of PSII. Even our plant growth conditions are different from those of Martins et al. (2016), it may be that some moments of high temperatures in a day also affect the expression of HSP in plants.

In addition to the microclimatic condition of a brighter environment may have impacted the antioxidant system of these plants, which caused significant changes in the heat resistance and another process or structures in this coffee plants, especially Conilon variety.

5. CONCLUSION

In general, the guiding hypotheses of work on gas exchange and heat tolerance of PSII were partially confirmed, while the different results found are important for the greater knowledge about photosynthesis in different coffee trees. All varieties exhibited adaptive morphophysiological characteristics to survive to both sun-exposed and shade conditions, such as changes in pigment content, use of light in photosynthetic processes and stomatal characteristics. However, the coffee varieties respond differently to the amount of light incident to the environment, and within the same species (Mundo Novo and Catuaí Amarelo by *C. arabica*) there may be different patterns of response and these characteristics were not sufficient for CO_2 assimilation rates to be similar between sun and shade condition, except for Catuaí Amarelo. A very intense shading may reduce photosynthetic capacity, but the lesser climatic variation may be more interesting for better development and even greater stress tolerance. Mundo Novo variety is best suited for sun-exposed cultivation and Conilon would perform better in shaded environments, while Catuaí Amarelo would adapt well to the different shading conditions. Then, the most suitable shading condition for coffee trees studied would be intermediate shading, among those imposed in our work, where the incident light restriction is not large and the climate may be less variable with less chance of stressful events reach the plants. In this context, agroforestry environments would be ideal for coffee cultivation, since they consist of some kind of shading and keep the microclimate less variable.

REFERENCES

Allakhverdiev SI, Kreslavski VD, Klimov V V., Los DA, Carpentier R, Mohanty P. 2008. Heat stress: an overview of molecular responses in photosynthesis. Photosynthesis Research **98**, 541–550.

Alves V, Goulart FF, Jacobson TKB, Filho RJ de M, Ribas CEDC. 2016. Shade's Benefit: Coffee Production under Shade and Full Sun. Journal of Agricultural Science **8**, 11.

Apel K, Hirt H. 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annual review of plant biology **55**, 373–99.

Assad ED, Pinto HS, Zullo Junior J, Ávila AMH. 2004. Climatic changes impact in agroclimatic zonning of coffee in Brazil. Pesquisa Agropecuária Brasileira **39**, 1057–1064.

Baliza DP, Cunha RL, Guimarães RJ, Barbosa JPRAD, Ávila FW, Passos AMA. 2012. Physiological characteristics and development of coffee plants under different shading levels. Revista Brasileira de Ciencias Agrarias **7**, 37–43.

Barradas VL, Fanjul L. 1986. Microclimatic chacterization of shaded and open-grown coffee (Coffea arabica L .) plantations in Mexico. Agricultural and Forest Meteorology **38**, 101–112.

Beer J. 1987. Advantages, disadvantages and desirable characteristics of shade trees for coffee, cacao and tea. Agroforestry Systems **5**, 3–13.

Berry J, Bjorkman O. 1980. Photosynthetic Response and Adaptation to Temperature in Higher Plants. Annual Review of Plant Physiology **31**, 491–543.

Bita CE, Gerats T. 2013. Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. Frontiers in Plant Science **4**, 1–18.

Bote AD, Struik PC. 2015. A study of the effects of shade on growth , production and quality of coffee (COFFEA ARABICA) in Ethiopia. International Journal of Agricultural Sciences **5**, 748–752.

Bunn C, Läderach P, Ovalle Rivera O, Kirschke D. 2015. A bitter cup: climate change profile of global production of Arabica and Robusta coffee. Climatic Change **129**, 89–101.

De Camargo MBP. 2010. The impact of climatic variability and climate change on arabic coffee crop in Brazil. Bragantia **69**, 239–247.

Chaves CJN, Leal BSS, de Lemos-Filho JP. 2015. Temperature modulation of thermal tolerance of a CAM-tank bromeliad and the relationship with acid accumulation in different leaf regions. Physiologia Plantarum **154**, 500–510.

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

Covre AM, Partelli FL, Bonomo R, Braun H, Ronchi CP. 2016. Vegetative growth of Conilon coffee plants under two water conditions in the Atlantic region of Bahia State, Brazil. Acta Scientiarum. Agronomy **38**, 535.

DaMatta FM. 2004. Ecophysiological constraints on the production of shaded and unshaded coffee: A review. Field Crops Research **86**, 99–114.

Damatta FM, Avila RT, Cardoso AA, Martins SCV, Ramalho JC. 2018. Physiological and Agronomic Performance of the Coffee Crop in the Context of Climate Change and Global Warming: A Review. Journal of Agricultural and Food Chemistry **66**, 5264–5274.

DaMatta FM, Grandis A, Arenque BC, Buckeridge MS. 2010. Impacts of climate changes on crop physiology and food quality. Food Research International **43**, 1814–1823.

Damatta FM, Ramalho JDC. 2006. Impacts of drought and temperature stress on coffee. Brazilian Journal of Plant Physiology **18**, 55–81.

DaMatta FM, Ronchi CP, Maestri M, Barros RS. 2007. Ecophysiology of coffee growth and production. Brazilian Journal of Plant Physiology **19**, 485–510.

Davis AP, Gole TW, Baena S, Moat J. 2012. The Impact of Climate Change on Indigenous Arabica Coffee (Coffea arabica): Predicting Future Trends and Identifying Priorities. PLoS ONE **7**, 10–14.

Davis AP, Govaerts R, Bridson DM, Stoffelen P. 2006. An annotated taxonomic conspectus of the genus Coffea (Rubiaceae). Botanical Journal of the Linnean Society **152**, 465–512.

Fanjul L, Arreola-Rodriguez R, Mendez-Castrejon MP. 1985. Stomatal responses to environmental variables in shade and sun grown coffee plants in mexico. Experimental

Agriculture **21**, 249–258.

Farquhar GD, Sharkey TD. 1982. Stomatal Conductance and Photosynthesis. Annual Review of Plant Physiology **33**, 317–345.

Franck N, Vaast P. 2009. Limitation of coffee leaf photosynthesis by stomatal conductance and light availability under different shade levels. Trees - Structure and Function **23**, 761–769.

Gimeno TE, Pas B, Lemos-Filho JP, Valladares F. 2009. Plasticity and stress tolerance override local adaptation in the responses of Mediterranean holm oak seedlings to drought and cold. Tree Physiology **29**, 87–98.

Godoy O, de Lemos-Filho JP, Valladares F. 2011. Invasive species can handle higher leaf temperature under water stress than Mediterranean natives. Environmental and Experimental Botany **71**, 207–214.

Hatfield JL, Prueger JH. 2015. Temperature extremes: Effect on plant growth and development. Weather and Climate Extremes **10**, 4–10.

IPCC. 2014. *Climate change 2014.*

Johansen DA. 1940. *Plant Microtechnique*. New York: McGraw-Hill Book Company.

Jones HG. 1998. Stomatal control of photosynthesis and transpiration. Journal of Experimental Botany **49**, 387–398.

Lichtenthaler HK. 2012. Vegetation Stress: an Introduction to the Stress Concept in Plants. Journal of Plant Physiology **148**, 4–14.

Lobo F de A, de Barros MP, Dalmagro HJ, Dalmolin ÂC, Pereira WE, de Souza ÉC, Vourlitis GL, Rodríguez Ortíz CE. 2013. Fitting net photosynthetic light-response curves with Microsoft Excel — a critical look at the models. Photosynthetica **51**, 445–456.

Machado EC, Schmidt PT, Medina CL, Ribeiro R V. 2005. Photosynthetic responses of three citrus species to environmental factors. Pesquisa Agropecuaria Brasileira **40**, 1161–1170.

Marias DE, Meinzer FC, Still C. 2017*a*. Leaf age and methodology impact assessments of thermotolerance of Coffea arabica. Trees - Structure and Function **31**, 1091–1099.

Marias DE, Meinzer FC, Still C. 2017*b*. Impacts of leaf age and heat stress duration on

photosynthetic gas exchange and foliar nonstructural carbohydrates in Coffea arabica. Ecology and Evolution **7**, 1297–1310.

Martins SCV, Araújo WL, Tohge T, Fernie AR, DaMatta FM. 2014*a*. In high-light-acclimated coffee plants the metabolic machinery is adjusted to avoid oxidative stress rather than to benefit from extra light enhancement in photosynthetic yield. PLoS ONE **9**, 1–11.

Martins SCV, Galmés J, Cavatte PC, Pereira LF, Ventrella MC, DaMatta FM. 2014*b*. Understanding the low photosynthetic rates of sun and shade coffee leaves: Bridging the gap on the relative roles of hydraulic, diffusive and biochemical constraints to photosynthesis. PLoS ONE **9**, 1–10.

Martins MQ, Rodrigues WP, Fortunato AS, *et al.* 2016. Protective Response Mechanisms to Heat Stress in Interaction with High [CO2] Conditions in Coffea spp. Frontiers in Plant Science **7**.

Mathur S, Agrawal D, Jajoo A. 2014. Photosynthesis: Response to high temperature stress. Journal of Photochemistry and Photobiology B: Biology **137**, 116–126.

Matos FS, Wolfgramm R, Gonçalves F V., Cavatte PC, Ventrella MC, DaMatta FM. 2009. Phenotypic plasticity in response to light in the coffee tree. Environmental and Experimental Botany **67**, 421–427.

Matthews JSA, Vialet-Chabrand S, Lawson T. 2018. Acclimation to Fluctuating Light Impacts the Rapidity of Response and Diurnal Rhythm of Stomatal Conductance. Plant Physiology **176**, 1939–1951.

Mittler R. 2002. Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science **7**, 405–410.

Mittler R. 2006. Abiotic stress, the field environment and stress combination. Trends in Plant Science **11**, 15–19.

Moat J, O'Sullivan RJ, Gole TW, Davis AP. 2018. The IUCN Red List of Threatened Species: Coffea arabica. The IUCN Red List of Threatened Species **8235**.

Morais H, Caramori PH, De Arruda Ribeiro AM, Gomes JC, Koguishi MS. 2006. Microclimatic characterization and productivity of coffee plants grown under shade of pigeon pea in Southern Brazil. Pesquisa Agropecuaria Brasileira **41**, 763–770.

Morais LE, Rodríguez-López NF, Medina EF, Cavatte PC, DaMatta FM, Martins SC V., Silva PEM. 2012. Physiological and biochemical abilities of robusta coffee leaves for acclimation to cope with temporal changes in light availability. Physiologia Plantarum **149**, 45–55.

Müller L-LB, Albach DC, Zotz G. 2017. 'Are 3 °C too much?': thermal niche breadth in Bromeliaceae and global warming (G Matlack, Ed.). Journal of Ecology **105**, 507–516.

Mussatto SI, Machado EMS, Martins S, Teixeira JA. 2011. Production, Composition, and Application of Coffee and Its Industrial Residues. Food and Bioprocess Technology **4**, 661–672.

Naidu SL, DeLucia EH. 1998. Physiological and morphological acclimation of shade-grown tree seedlings to late-season canopy gap formation. Plant Ecology **138**, 27–40.

Niinemets Ü, Valladares F. 2004. Photosynthetic acclimation to simultaneous and interacting environmental stresses along natural light gradients: Optimality and constraints. Plant Biology **6**, 254–268.

Nobel PS. 2009. *Physicochemical and Environmental Plant Physiology*. Elsevier.

Noirot M, Charrier A, Stoffelen P, Anthony F. 2016. Reproductive isolation, gene flow and speciation in the former Coffea subgenus: a review. Trees - Structure and Function **30**, 597–608.

Prioul JL, Chartier P. 1977. Partitioning of Transfer and Carboxylation Components of Intracellular Resistance to Photosynthetic CO_2 Fixation: A Critical Analysis of the Methods Used. Annals of Botany **41**, 789–800.

Ramalho JC, DaMatta FM, Rodrigues AP, Scotti-Campos P, Pais I, Batista-Santos P, Partelli FL, Ribeiro A, Lidon FC, Leitão AE. 2014. Cold impact and acclimation response of Coffea spp. plants. Theoretical and Experimental Plant Physiology **26**, 5–18.

Ramalho JC, Rodrigues AP, Semedo JN, *et al.* 2013. Sustained photosynthetic performance of Coffea spp. under long-term enhanced [CO2]. PLoS ONE **8**, 1–19.

Rascher U, Liebig M, Luttge U. 2000. Evaluation of instant light-response curves of chlorophyll fluorescence parameters obtained with a portable chlorophyll fluorometer on site in the field. Plant, Cell and Environment **23**, 1397–1405.

Rodrigues WP, Martins MQ, Fortunato AS, *et al.* 2016. Long-term elevated air [CO2] strengthens photosynthetic functioning and mitigates the impact of supra-optimal temperatures in tropical Coffea arabica and C. canephora species. Global Change Biology **22**, 415–431.

Rodríguez-López NF, Martins SC V, Cavatte PC, *et al.* 2014. Morphological and physiological acclimations of coffee seedlings to growth over a range of fixed or changing light supplies. Environmental and Experimental Botany **102**, 1–10.

Ronchi CP, DaMatta FM, Batista KD, Moraes GABK, Loureiro ME, Ducatti C. 2006. Growth and photosynthetic down-regulation in Coffea arabica in response to restricted root volume. Functional Plant Biology **33**, 1013–1023.

Rozendaal DMA, Hurtado VH, Poorter L. 2006. Plasticity in leaf traits of 38 tropical tree species in response to light; relationships with light demand and adult stature. Functional Ecology **20**, 207–216.

Suzuki N, Rivero RM, Shulaev V, Blumwald E, Mittler R. 2014. Abiotic and biotic stress combinations. New Phytologist **203**, 32–43.

Terashima I, Hanba YT, Tazoe Y, Vyas P, Yano S. 2006. Irradiance and phenotype: Comparative eco-development of sun and shade leaves in relation to photosynthetic CO2diffusion. Journal of Experimental Botany **57**, 343–354.

Teskey R, Wertin T, Bauweraerts I, Ameye M, McGuire MA, Steppe K. 2015. Responses of tree species to heat waves and extreme heat events. Plant Cell and Environment **38**, 1699–1712.

Tiwari BS, Belenghi B, Levine A. 2002. Oxidative Stress Increased Respiration and Generation of Reactive Oxygen Species, Resulting in ATP Depletion, Opening of Mitochondrial Permeability Transition, and Programmed Cell Death. Plant Physiology **128**, 1271–1281.

Valladares F. 2003. Light Heterogeneity and Plants: from Ecophysiology to Species Coexistence and Biodiversity. In: Esser K,, In: Lüttge U,, In: Beyschlag W,, In: Hellwig F, eds. Progress in Botany. Progress in Botany. Berlin, Heidelberg: Springer Berlin Heidelberg, 439–461.

Valladares F, Niinemets Ü. 2008. Shade Tolerance, a Key Plant Feature of Complex Nature

and Consequences. Annual Review of Ecology, Evolution, and Systematics **39**, 237–257.

Vega FE, Rosenquist E, Collins W. 2003. Global project needed to tackle coffee crisis. Nature **425**, 343–343.

Wahid A, Gelani S, Ashraf M, Foolad MR. 2007. Heat tolerance in plants: An overview. Environmental and Experimental Botany **61**, 199–223.

Wellburn AR. 1994. The Spectral Determination of Chlorophylls a and b, as well as Total Carotenoids, Using Various Solvents with Spectrophotometers of Different Resolution. Journal of Plant Physiology **144**, 307–313.

Yamori W, Hikosaka K, Way DA. 2014. Temperature response of photosynthesis in C3, C4, and CAM plants: Temperature acclimation and temperature adaptation. Photosynthesis Research **119**, 101–117.

Zandalinas SI, Mittler R, Balfagón D, Arbona V, Gómez-Cadenas A. 2018. Plant adaptations to the combination of drought and high temperatures. Physiologia Plantarum **162**, 2–12.

TABLES AND FIGURES

Table 1. Growing of aerial part and stomatal traits in coffee plants that were subjected to two growing conditions. Means ± SE followed by the same letter do not differ significantly from one another in each treatment. For the results about plant growth, the N = 10; for stomatal characteristics see N in material and methods (P < 0.05, ANOVA and Tukey test for all parameters, and Kruskal-Wallis and Dunn's test for stomatal size).

Parameters	Shading condition	Coffee Varieties		
		C. arabica **Catuaí Amarelo**	*C. arabica* **Mundo Novo**	*C. canephora* **Conilon**
Total number of leaves	Sun	30.0 ± 0.42**C**	55.0 ± 3.70**A**	21.8 ± 1.31**E**
	Shade	28.2 ± 0.62**D**	39.3 ± 2.03**B**	20.3 ± 1.79**F**
Height (cm)	Sun	20.1 ± 1.01**C**	46.8 ± 2.16**A**	13.7 ± 0.79**D**
	Shade	24.8 ± 1.11**BC**	49.0 ± 1.51**A**	27.7 ± 1.28**B**
Stem diameter (mm)	Sun	4.78 ± 0.23**C**	8.54 ± 0.36**A**	3.11 ± 0.25 **D**
	Shade	4.78 ± 0.23**C**	7.43 ± 0.14**B**	5.65 ± 0.18 **C**
Stomatal density (st mm^{-2})	Sun	180.1 ± 5.0 **C**	213.9 ± 8.4 **B**	297.6 ± 10.9 **A**
	Shade	126.5 ± 3.5 **D**	127.9 ± 3.8 **D**	103.6 ± 4.1 **E**
Stomatal index (%)	Sun	17.7 ± 0.6 **AB**	20.9 ± 1.1 **A**	20.2 ± 1.2 **A**
	Shade	15.5 ± 0.6 **B**	15.3 ± 0.9 **B**	11.0 ± 1.1 **C**
Stomatal size (μm^{-2})	Sun	378.0 ± 13.9 **A**	307.0 ± 7.6 **BC**	182.2 ± 5.3 **D**
	Shade	336.1 ± 7.8 **AB**	356.6 ± 7.2 **A**	291.9 ± 8.3 **C**

Table 2. Photosynthetic parameters derived from the photosynthetic light-response curves (P_N/I curve): light compensation point (**LCP**; μmol photons m^{-2} s^{-1}), light saturation point (**LSP**; μmol photons m^{-2} s^{-1}), dark respiration (**R$_D$**; μmol CO_2 m^{-2} s^{-1}), photosynthetic assimilation rate in LSP (**P_{Nmax}**; μmol (CO_2) m^{-2} s^{-1}), maximum apparent photosynthetic quantum yield at $I = 0$ (**ϕ$_{(Io)}$**; μmol (CO_2) μmol (photon)$^{-1}$), stomatal conductance in LSP (**g_s** mol H_2O m^{-2} s^{-1}), transpiration in LSP (**E**; mmol H_2O m^{-2} s^{-1}) and internal CO2 concentration in LSP (**C$_i$**; mmol CO_2 mol^{-1} air), Water use efficiency in LSP (**WUE**), intrinsic Water Use efficiency in LSP (**iWUE**) and instantaneous carboxylation efficiency (**Φc**) in coffee plants that were subjected to shading conditions.

Parameters	Shading condition	Coffee Varieties		
		C. arabica **Catuaí Amarelo**	*C. arabica* **Mundo Novo**	*C. canephora* **Conilon**
LCP	Sun	8.7 ± 1.1**C**	14.2 ± 0.9**B**	20.9 ± 1.5**A**
	Shade	4.1 ± 0.3**D**	5.1 ± 0.4**CD**	8.3 ± 0.6**C**
LSP	Sun	428 ± 29**B**	388 ± 40**B**	710 ± 31**A**
	Shade	192 ± 17**C**	237 ± 15**C**	396 ± 32**B**
R$_D$	Sun	0.57 ± 0.10**Ba**	0.79 ± 0.07**Ba**	0.97 ± 0.08**Aa**
	Shade	0.37 ± 0.04**Bb**	0.33 ± 0.04**Bb**	0.58 ± 0.06**Ab**
P_{Nmax}	Sun	4.2 ± 0.4**AB**	5.5 ± 0.3**A**	5.5 ± 0.4**A**
	Shade	4.6 ± 0.4**AB**	3.6 ± 0.3**B**	3.7 ± 0.5**B**
ϕ$_{(Io)}$	Sun	0.050 ± 0.006**D**	0.067 ± 0.002**CD**	0.063 ± 0.007**C**
	Shade	0.070 ± 0.002**B**	0.072 ± 0.003**AB**	0.089 ± 0.006**Aa**
g_s	Sun	0.067 ± 0.01**A**	0.054 ± 0.004**A**	0.062 ± 0.009**A**
	Shade	0.048 ± 0.01**B**	0.047 ± 0.003**B**	0.031 ± 0.005**B**
E	Sun	1.50 ± 0.09**A**	1.27 ± 0.14**A**	1.66 ± 0.23**A**
	Shade	0.99 ± 0.12**B**	0.81 ± 0.10**B**	0.80 ± 0.13**B**
C$_i$	Sun	267 ± 18**A**	215 ± 7.3**AB**	233 ± 12**B**
	Shade	228 ± 15**A**	232 ± 5.0**AB**	195 ± 14**B**
WUE	Sun	3.2 ± 0.4**B**	4.9 ± 0.2**AB**	3.7 ± 0.4**AB**
	Shade	4.9 ± 0.5**A**	4.4 ± 0.1**AB**	5.0 ± 0.4**A**
iWUE	Sun	73.7 ± 12.6	99.2 ± 3.78	93.9 ± 12.3
	Shade	105.4 ± 15.2	82.5 ± 13.2	126.7 ± 12
Φc	Sun	0.018 ± 0.002	0.025 ± 0.001	0.025 ± 0.002
	Shade	0.024 ± 0.003	0.017 ± 0.001	0.020 ± 0.003

Means ± SE followed by the same uppercase letter do not differ significantly from one another for sun and shade treatments within varieties. Uppercase letters indicate difference between treatments. (n = 8, $p < 0.05$, Two-way ANOVA and Tukey test).
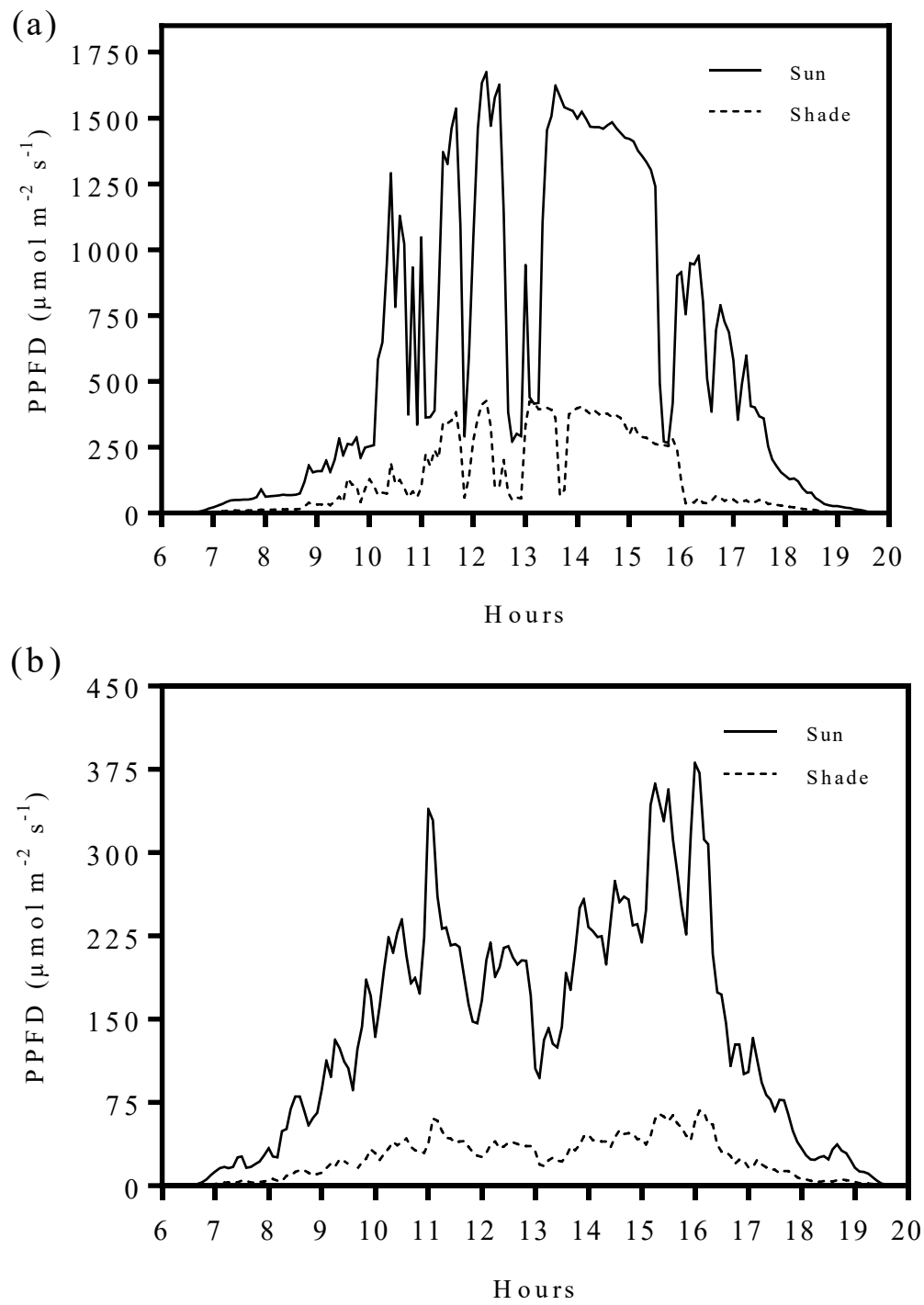
Figure 1. Photosynthetically photon flux density (PPFD) in January 28 (**a**) and February 04 (**b**), the days with more and less PPFD, respectively. The continuous line represents the Sun and the dotted line the Shade growth condition.
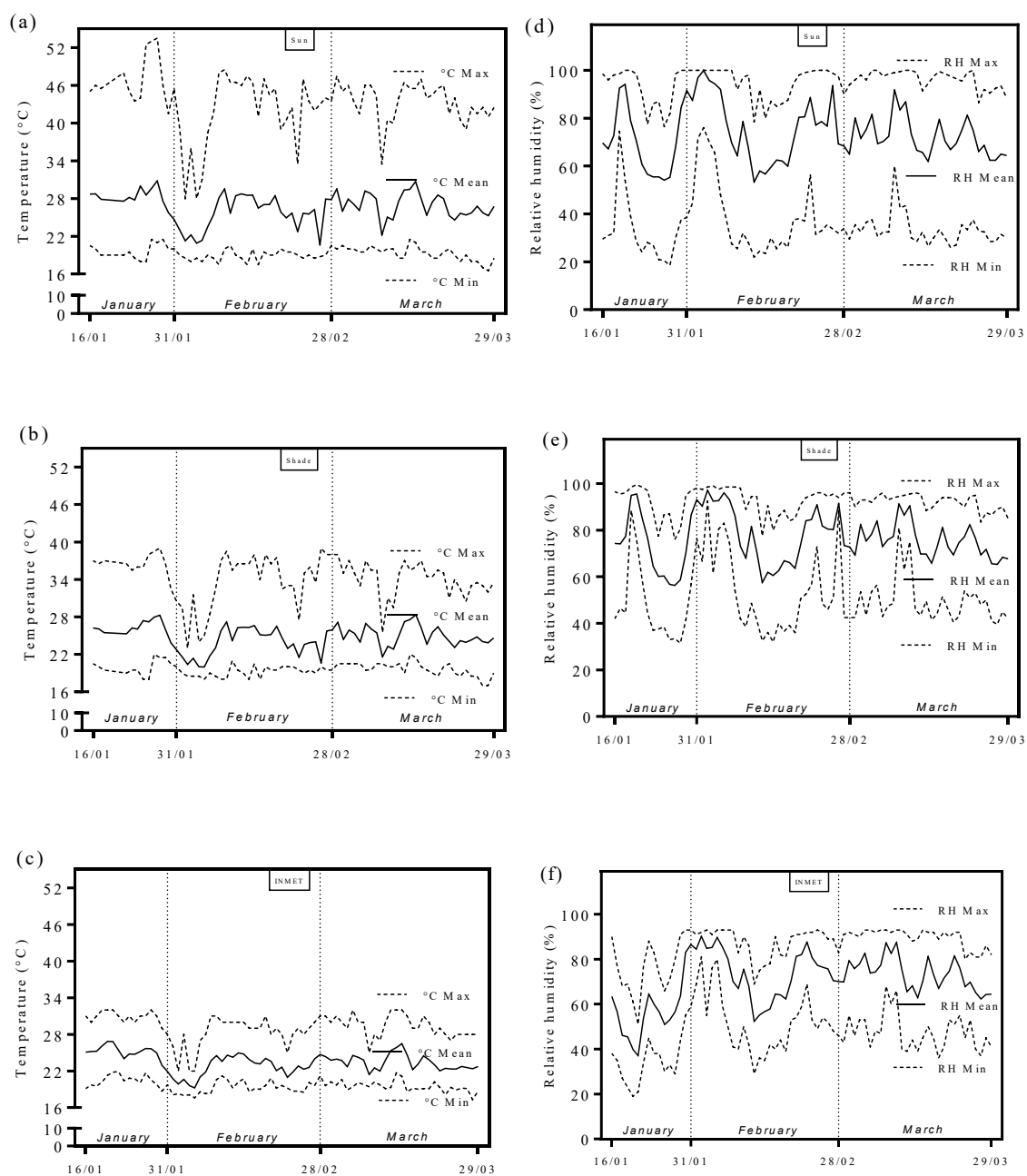
Figure 2. Temperature, air relative humidity and precipitation during January to March in Sun, Shade and local weather station. Minimal, mean and maximum temperature registered, in Sun **(a),** Shade **(b)** and for weather station **(c)**. Air relative humidity for Sun **(d)**, Shade **(e)** and for weather station.
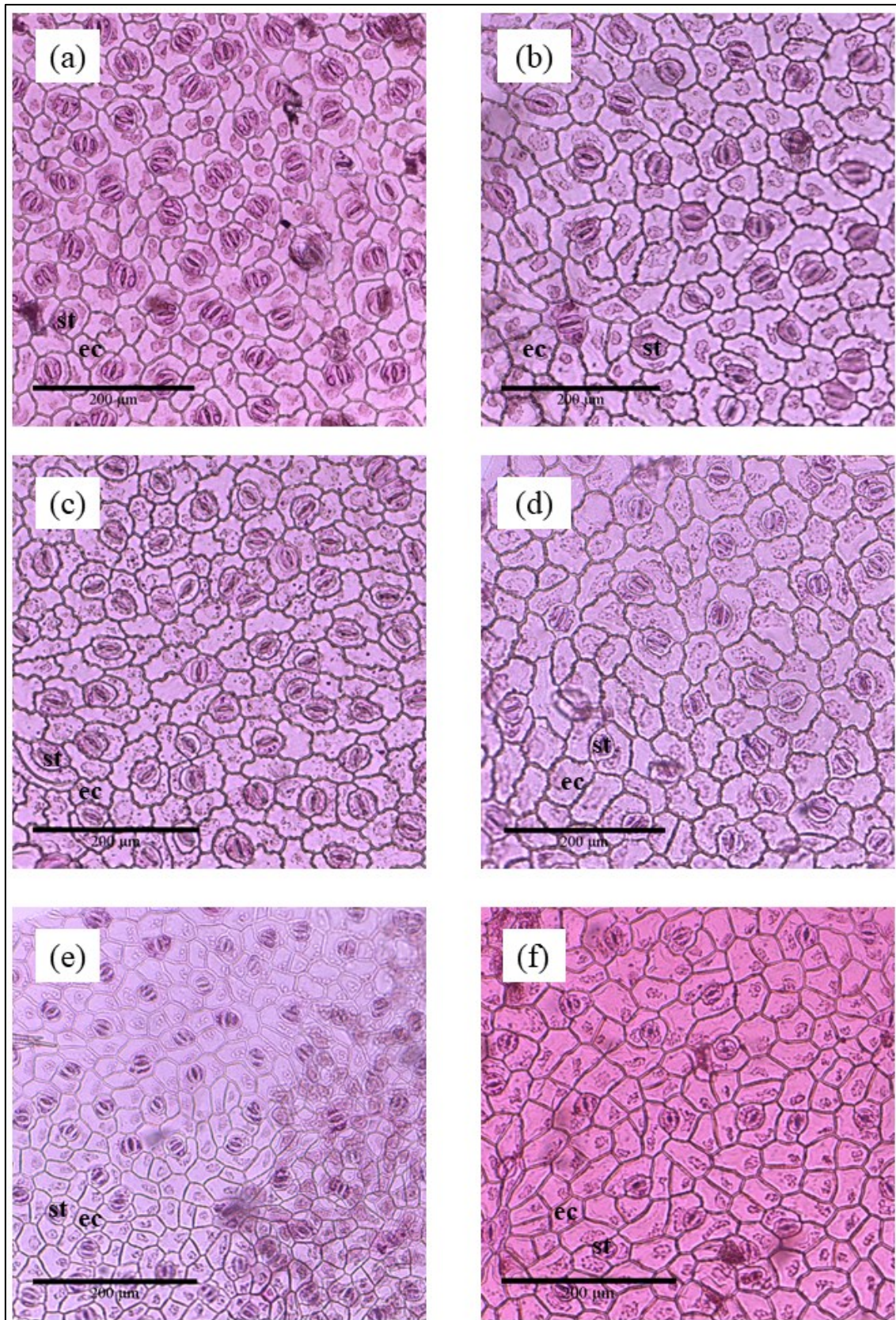
Figure 3. Example of stomatal structure and epidermis cells in abaxial surface of the coffee varieties growth in the sun (left column) and shade (right column) conditions. Catuaí Amarelo is represented by **(a)** and **(b)**; Mundo Novo is represented by **(c)** and **(d);** Conilon is represented by **(e)** and **(f)**. Letters inside the structures mean: **st**, stomatal; **ec**, epidermis cells. Scale bars = *200 µm*.
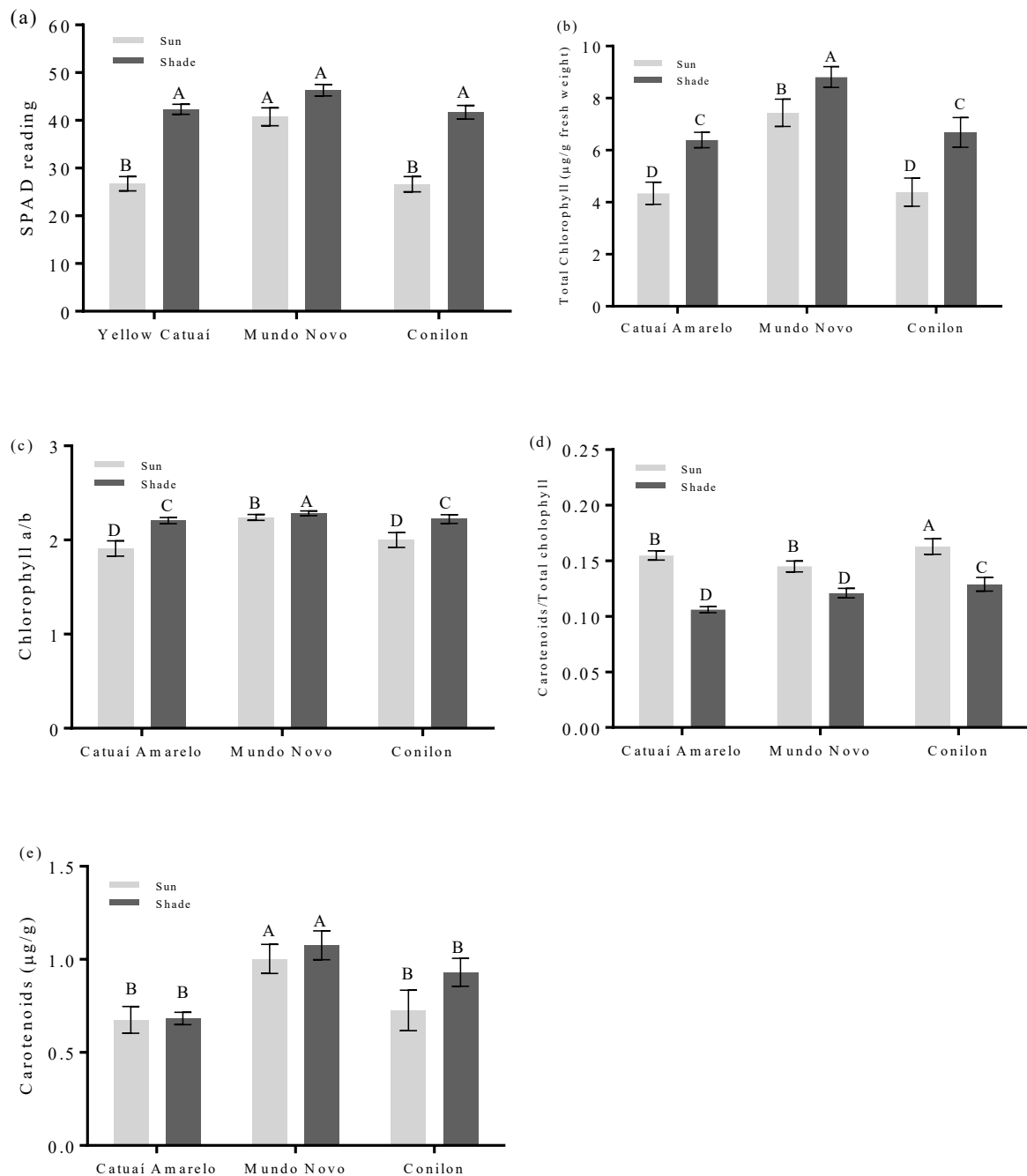
Figure 4. Result of SPAD index and DMSO pigments quantification are shown in the following graphs: SPAD readings **(a)**, Total Chlorophylls content **(b)**, Chlorophyll a/b ratio **(c)**, Chlorophyll/Carotenoid ratio **(d)** and Carotenoids content **(e)** in coffee varieties in each shading condition (sun and shade). Vertical bars are mean ± SE. Different letters in columns indicate statistically significant differences in that variety (n = 10, $p < 0.05$, ANOVA two-way and Tukey test).
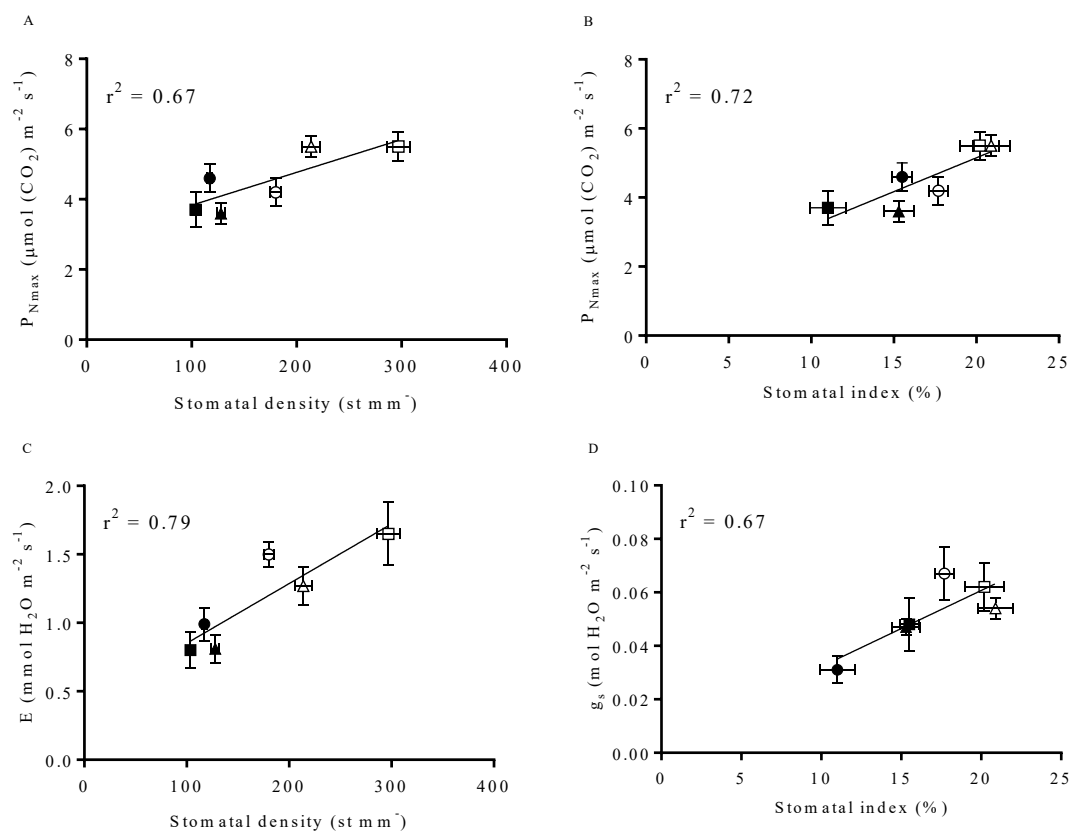
Figure 5. Relationship between stomatal traits and gas exchange. Significant relationships are shown for P_{Nmax} and SD (**A**), and P_{Nmax} and SI (**B**), E and SD (**C**) and gs and SD (**D**). Filled symbols represent the varieties in the shade and open symbols the varieties in the sun. Each symbol represents a variety, square (Conilon), triangle (Mundo Novo) and circle (Catuaí Amarelo).
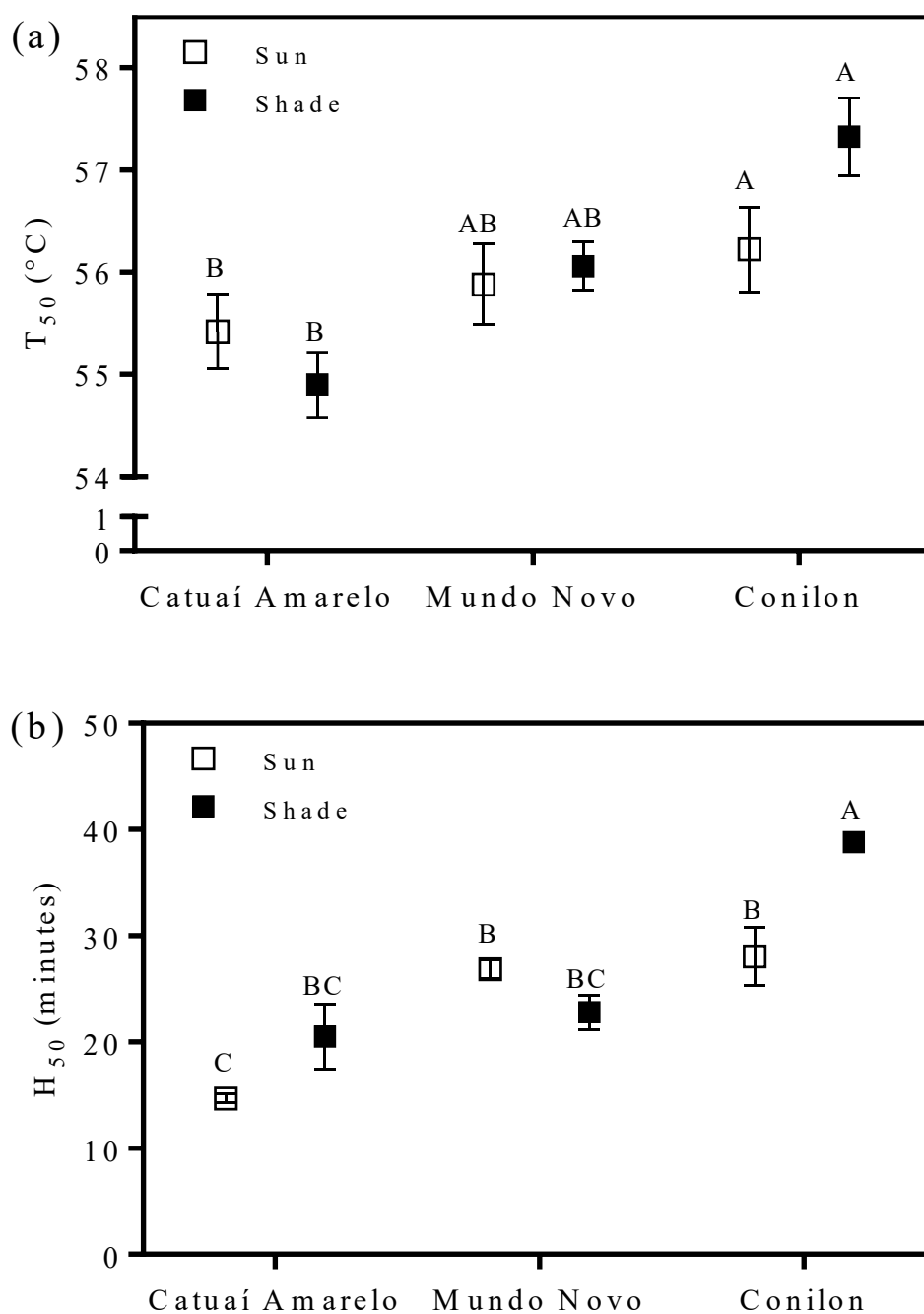
Figure 6. PSII thermotolerance of coffee varieties under different shading conditions, using two methodologies, ramping assay with results of T_{50} **(a)** and in static assay with results of H_{50} **(b)**. Means ± SE followed by the same uppercase letter do not differ significantly from one another for sun and shade treatments within varieties. (N = 5, $p < 0.05$, Two-way ANOVA and Tukey test).