



Content of Photosynthetic Pigments and Leaf Gas Exchanges of Young Coffee Plants under Light Restriction and Treated with Paclobutrazol

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Authors' contributions

This work was carried out in collaboration among all authors. Author AFFR developed the study, participated in the collection of data and the accomplishment of the statistical analysis, of the writing of the manuscript and carried out the bibliographic research. Author SNM. guided the first author during the development of the study, and participated in performing the statistical analysis and writing of the manuscript. The other authors assisted in the conduction of the experiment, data collection and analysis. All authors read and approved the final manuscript.

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ABSTRACT

The use of shading and paclobutrazol in coffee plants can be an important cultivation strategy to mitigate the negative effects of high solar radiation and atmospheric temperature. Therefore, the levels of photosynthetic pigments and foliar gas exchanges of young coffee plants submitted to doses of paclobutrazol were evaluated, in environments with artificial light restriction. Five experiments were performed: one in full sunlight and four in artificially shaded environments with black polyethylene meshes at 20%, 40%, 60% and 80% levels of light restriction. In each of these

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environments, an experiment was carried out, consisting of five treatments, defined by the application of paclobutrazol via substrate, at doses of 0, 10, 20, 30 and 40 mg of active ingredient per plant. Joint analysis of experiments and analysis of variance of the regression were made, for the study of levels of shading and doses of paclobutrazol. The light restriction optimized the photosynthetic apparatus of the plants, mainly at levels close to 60%, and considerably favored leaf gas exchanges of arabica coffee. The application of paclobutrazol in the studied dosages resulted in little or no effect on photosynthetic pigment contents and did not influence leaf gas exchanges of coffee plants.

Keywords: *Coffea arabica L.*; shading; triazole; physiological changes.

1. INTRODUCTION

In several farming regions of Brazil, cultivated plants are constantly exposed to climatic adversities that limit their initial establishment in the field, negatively reflecting the yield potential. Among these, intense solar radiation, high temperatures, and low rainfall are limiting factors.

When plants are exposed to light energy higher than that required by photosynthesis, there may be energy imbalance that results in photoinhibition. This may promote the biosynthesis of reactive oxygen species and, consequently, cause oxidative stress. Photoinhibition may also be a result of photophysical parameters, which include response to light intensity or wavelength [1-2].

Coffee is a native species of understory regions and therefore is considered as a shade plant [3], with low point of light saturation. Thus, shading of coffee plantations may be an alternative cultivation method to mitigate negative effects of direct exposure to the sun, in order to favor the initial establishment of the crop and optimize its development in subsequent stages.

In general, coffee tree presents low rates of assimilation of CO₂ (A) when compared to other tropical trees. Shading may favor certain environmental factors, such as temperature attenuation and reduction of water vapor pressure deficit, in order to benefit the gas exchange of coffee plants [4-5].

However, existing information on the effects of shading on gas exchange of coffee plants is contrasting and depends on the level of light restriction [6-7].

In general, comparison to full sunlight exposure, leaves under shading contain greater amount of chlorophyll per reaction center, more developed antenna complexes, smaller ratio between

chlorophyll *a* and *b*, and lower content of carotenoids [8-9].

Plant growth regulators, especially inhibitors of biosynthesis of gibberellins, have been applied to the traditional management of high technological standards of crops. These products can influence various aspects of plant metabolism, both morphologically and physiologically, reducing susceptibility to biotic and abiotic stresses [10]. This capacity of modulation provided to the plants has substantial importance in face of the climatic adversities verified in cropping environments.

Paclobutrazol (PBZ) [(2*RS*, 3*RS*)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-pentan-3-ol] is triazole capable of inhibiting cytochrome P450 dependent mono-oxygenases and, consequently, biosynthesis of gibberellins [11]. The changes in plant hormonal balance caused by triazole, such as elevated levels of cytokinins and abscisic acid, can interfere with foliar gas exchange and photosynthetic pigment content [12-15].

Several studies have demonstrated the ability of paclobutrazol to mitigate the damage caused by abiotic stresses [16-21], including high temperature stress [11,21].

The effects of this growth regulator have variations according to dosage, phenological stage, and form of application [22]. For coffee plants, however, the knowledge about such technology is still incipient, and there is a need for systematic and analytical studies on the subject.

Therefore, the use of shading and the application of paclobutrazol in coffee plants is an important strategy to minimize negative factors related to high solar radiation index and elevation of atmospheric temperature. The objective of this study was to evaluate the levels of

photosynthetic pigments and foliar gas exchange of young coffee plants submitted to doses of paclobutrazol in environments with artificial light restriction.

2. MATERIALS AND METHODS

The experiments were conducted at the Universidade Estadual do Sudoeste da Bahia, Vitória da Conquista Campus, Brazil, between January and May 2017. The experimental area is located at 14° 53' 05" S and 40° 48' 00" W, at 852 meters of altitude. The climate of the municipality, according to Köppen-Geiger climatic classification, is of Cwa (tropical of altitude) type, with mean annual temperature of 20.2°C and a mean annual precipitation of 733.9 mm [23]. The meteorological data obtained during the period of tests can be observed in Fig. 1.

Coffea arabica L. 'Catuai Red IAC 144' seedlings were obtained in an accredited nursery. When they had four pairs of mature leaves (approximately five months old), they were individually transplanted to pots with a capacity of 20 dm³ (32.5 cm high x 34.5 cm higher diameter and 22 cm lower diameter).

Containers were filled with mix of soil (typical Eutrophic YELLOW LATOSOLO) and humus, in the ratio 9: 1, and homogenized through sieve of 5 mm. The chemical analysis of the soil used in

the mixture showed the following results: pH (H₂O): 5.4; P: 2.0 mg dm⁻³; K⁺: 0.23 cmol_c dm⁻³; Ca²⁺: 2.2 cmol dm⁻³; Mg²⁺: 0.8 cmol_c dm⁻³; Al³⁺: 0.1 cmol_c dm⁻³; H⁺: 2.7 cmol_c dm⁻³. Liming and fertilization of the substrate were carried out based on soil chemical analysis, and according to the technical recommendation of the Soil Fertility Commission of the State of Minas Gerais [24].

Immediately after transplanting, the pots were placed in different environments, with 0% (full sun), 20%, 40%, 60% and 80% of artificial light restriction. Shaded environments (4 meters wide x 8 meters long x 2 meters high) were obtained through black polyethylene meshes. In each environment (shaded and in full sun) an experiment was conducted, totaling five experiments.

Each experiment (0%, 20%, 40%, 60% and 80% of light restriction) consisted of five treatments, defined by the application of different doses of paclobutrazol via substrate (0, 10, 20, 30 and 40 mg of active ingredient per plant). A completely randomized design was used, with four replications, totaling 20 plots. Each experimental unit consisted of a pot containing a coffee plant. For analysis of leaf gas exchanges, readings were made in blocks, with four replications, due to variations occurred during the evaluation period, from 8:00 a.m. to 12:00 p.m.

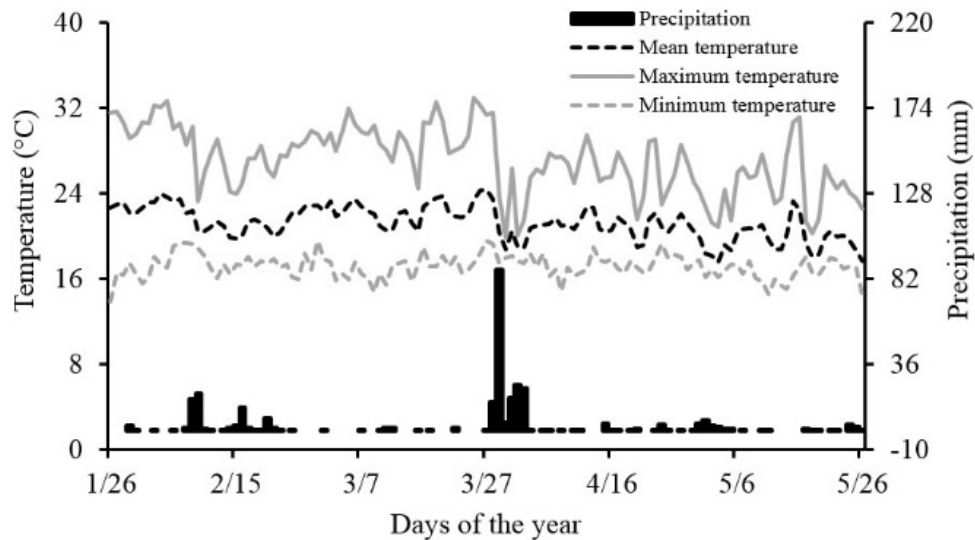


Fig. 1. Meteorological data recorded in the automatic meteorological station of the Universidade Estadual do Sudoeste da Bahia, Brazil, during the experimental period (INMET)

Applications of paclobutrazol were carried out at 18 days after transplanting of seedlings, with the commercial product Cultar 250 SC® (250 g i.a. L⁻¹ of paclobutrazol), and volume of solution of 200 mL per plant, applied directly to the substrate.

Management of weeds and pests was performed according to the occurrence along the experiment conduction. All plants were irrigated every two days, with water volume determined by the gravimetric method (a control pot for each experiment), in which these containers were saturated with water, with subsequent gravimetric drainage until constant weight. First, plant pot of each experiment was weighed to obtain the initial mass (IM). Every two days, the control pots were weighed again, obtaining the final mass (FM). The volume of water (V) to be applied at the date of each water replenishment, in liters, was determined by the difference between the two masses, through the equation: $V = IM - FM$, with masses being expressed in kilograms.

At 100 days after application (DAA) of paclobutrazol, SPAD (Soil Plant Analysis Development) index and photosynthetic pigment content were evaluated. The intensity of green color of leaf (SPAD index) was determined using a portable chlorophyllometer (SPAD 502, MINOLTA, Japan), with readings at three points of the first fully expanded leaf, from the apex of the plant, and then the average.

The extraction of photosynthetic pigments was performed according to the modified methodology of [25], by eliminating the stages of maceration and centrifugation of the discs, described by [26]. The first fully expanded leaf of each plant was collected, from which 10 leaf discs of six millimeters of diameter were removed, with the aid of manual leaf disc extractor. The material was immediately weighed on analytical balance and filled into aluminum-coated test tubes containing 20 mL of 80% acetone (v/v). This procedure was performed in an environment without direct incidence of light. The tubes were then capped, sealed with plastic film, and kept in the dark for 48 hours to extract the pigments.

After this period, absorbance readings of the extracts were performed in spectrophotometer (700 Plus, Femto, Brazil), at wavelengths of 663 nm, 646 nm and 470 nm. For the calibration of the spectrophotometer, 80% acetone (v/v) was used as "blank". Concentrations ($\mu\text{g mL}^{-1}$) of

extract) of *a*, *b*, and total chlorophyll, and carotenoids were calculated using specific equations for each pigment [27]. Depending on the mass of each sample and the volume of acetone used, the values were converted and the pigment content expressed as mg g^{-1} of fresh leaf matter.

At 99 DAA of paclobutrazol, leaf gas exchanges were evaluated. These evaluations were performed on the same leaf used for the other physiological analyzes, using an infrared gas analyzer (IRGA), LCPro, ADC, UK coupled to an actinic light source of $1000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ of photosynthetically active radiation.

Rate of CO₂ assimilation (*A*, $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), transpiration rate (*E*, $\text{mmol water vapor m}^{-2} \text{ s}^{-1}$), stomatal conductance (*g_s*, $\text{mol m}^{-2} \text{ s}^{-1}$), and the internal CO₂ concentration in the leaf (*C_i*, $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ air}$). Carboxylation efficiency (*A/C_i*) was calculated by the ratio of CO₂ assimilation rate to internal CO₂ concentration in the leaf.

Data were submitted to normality tests (Lilliefors) and homogeneity of variances (Cochran). After the analysis of variance of each experiment (each level of light restriction) was carried out, joint analysis of experiments was performed, respecting for each variable relation between mean squares of residue less than or equal to 1:7, according to [28]. When joint analysis presented significance ($p < 0.05$), regression analysis was performed for the study of paclobutrazol doses and levels of shading. The regression models were defined based on the significance ($p < 0.05$), the highest coefficient of determination (*R*²) and the biological response for each characteristic studied. For statistical analysis, the program Statistical and Genetic Analysis System (SAEG), version 9.1 was used.

3. RESULTS AND DISCUSSION

Light restriction was the factor with the greatest impact on the variables related to photosynthetic pigments (content of chlorophyll *a*, *b*, and total, carotenoid content, and chlorophyll *a:b* ratio), and intensity of green color in the leaf. For chlorophyll *a* and total content, there was interaction between the studied factors (levels of light restriction and doses of paclobutrazol). The environment with 60% of light restriction was not grouped for the analysis of the parameters chlorophyll *b* content and carotenoid content (Table 1).

For the unfolding of interaction between the factors, a cubic model for the relationship between chlorophyll *a* content and light restriction levels (LR) in coffee plants treated with 0 and 30 mg of paclobutrazol (PBZ) was delineated. For the coffee plants submitted to 10, 20 and 40 mg of the regulator, a linear model was established increasing as a function of levels of shading (Fig. 2A).

Chlorophyll *a* content of plants not treated with PBZ (0 mg) was lower than treatment in full sun at levels below 33.6% of LR. From this level, the values were higher than the control, with an estimated maximum content of 2.34 mg g⁻¹ of chlorophyll *a* (65.7% LR). The maximum levels of chlorophyll *a* estimated for the treatments with 10, 20 and 40 mg of PBZ (2.45, 2.43 and 2.34 mg g⁻¹, respectively), remained close to the estimated maximum value for coffee plants without regulator application.

On the other hand, in coffee plants treated with 30 mg of PBZ, the effect of shading on increasing chlorophyll *a* content was potentiated. There was an expressive increase in the content of this pigment promoted by shading at levels above 17.2%, with an estimated maximum value of 3.09 mg g⁻¹ of chlorophyll *a* (121.75% higher than the full sun treatment), at the level of 60.4% of LR.

It was not possible to delineate a mathematical model to express the relationship between the chlorophyll *a* content and the PBZ doses of coffee plants conducted under levels of 0, 20, 40 and 80% of LR. A cubic model was designed to express the effect of PBZ doses on the chlorophyll *a* content of coffee plants kept under 60% of light restriction. The values were higher than the control (without PBZ application) at

doses higher than 17.7 mg of the regulator per plant, with an estimated maximum value for the dose of 31.0 mg PBZ (2.82 mg g⁻¹) (Fig. 2B).

For the unfolding of interaction between LR levels and PBZ doses, in the evaluation of the total chlorophyll content, a similar trend was observed for chlorophyll *a* (Figs. 2C and 2D). Maximum levels of total chlorophyll as a function of LR levels were estimated at 2.85, 3.00, 2.97, 4.19 and 2.87 mg g⁻¹ of fresh matter for plants treated with 0, 10, 20, 30 and 40 mg of PBZ, respectively. As with chlorophyll *a*, coffee plants treated with 30 mg of PBZ via soil showed a more significant increase in total chlorophyll content as a function of shade levels compared to other doses (Fig. 2C). For this treatment, maximum total chlorophyll content (4.19 mg g⁻¹), estimated at 60.4% of LR level, was approximately 2.5 times higher than the treatment in full sun.

As a strategy to increase the efficiency of light absorption processes, plants grown under lower radiation levels tend to have higher density of light-picking complexes when compared to plants kept in full sunlight [29]. In addition, the increase in chlorophyll content under low light conditions may be associated, in part, with higher nitrogen allocation to photosystems [30].

The total chlorophyll content of coffee plants conducted under 60% of LR, as a function of doses of PBZ applied, was higher than the control at dosages above 16.5 mg of the inhibitor, with a maximum point estimated for 30.6 mg of PBZ. Any mathematical model among those studied expressed the effect of PBZ treatment on the total chlorophyll content of coffee plants kept under 0, 20, 40 and 80% shading (Fig. 2D).

Table 1. Analysis of variance summary and coefficients of variation (CV) of leaf greening (SPAD), chlorophyll *a* content (Chl *a*), total chlorophyll content (Chl *a+b*), ratio of chlorophyll *a* to *b* (Chl *a:b*), chlorophyll *b* content (Chl *b*) and carotenoid content (Car) of *Coffea arabica* L. 'Catuaí Vermelho IAC 144' plants submitted to different light restriction levels (LR) and paclobutrazol doses (D), evaluated at 100 days after the application of the regulator. Vitória da Conquista - BA, 2017

| Mean Squares | | | | | | | | |
|--------------|----|--------------------|-------------------|-------------------|-------------------|----|----------------------|---------------------|
| SV | df | SPAD | Chl <i>a</i> | Chl <i>a+b</i> | Chl <i>a:b</i> | df | Chl <i>b</i> | Car |
| LR | 4 | 391.1** | 3.2** | 5.9** | 4.0 | 3 | 0.2915** | 0.042 |
| D | 4 | 97.5 ^{ns} | 0.2 ^{ns} | 0.5 ^{ns} | 0.3 ^{ns} | 4 | 0.0008 ^{ns} | 0.003 ^{ns} |
| LR*D | 16 | 63.7 ^{ns} | 0.3 | 0.6** | 1.0 ^{ns} | 12 | 0.0137 ^{ns} | 0.011 ^{ns} |
| Wn | 75 | 66.1 | 0.1 | 0.3 | 0.7 | 60 | 0.0085 | 0.011 |
| CV (%) | | 12.3 | 18.5 | 20.5 | 17.6 | | 23.5 | 25.5 |

^{ns}, * e **: non-significant, significant by "F" test at 5% and 1% of probability, respectively.

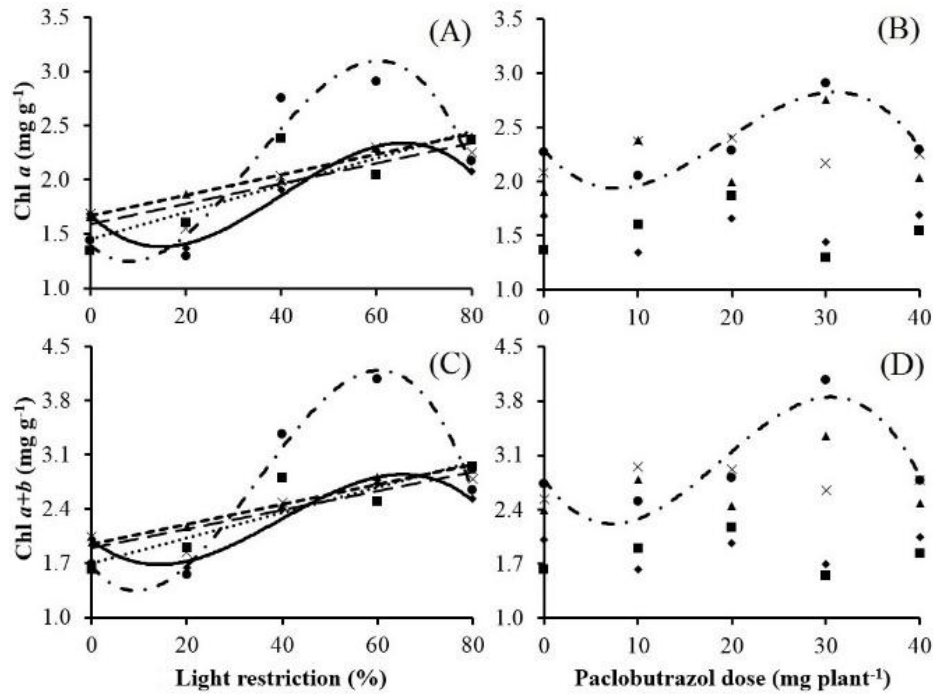


Fig. 2. Chlorophyll a and total chlorophyll content in leaves of coffee plants (*Coffea arabica* L. 'Catuai Vermelho IAC 144') in response to different light restriction levels (LR) and paclobutrazol doses (D), at 100 days after the application of the regulator. (A, B) chlorophyll a content (Chl a): (A) ♦0 mg - $\hat{Y}^* = 1.67811 - 0.0428795X + 0.00176607X^2 - 0.0000146094X^3$ ($R^2 = 0.9874$); ■10 mg - $\hat{Y}^{} = 1.451 + 0.0124625X$ ($R^2 = 0.7246$); ▲20 mg - $\hat{Y}^{**} = 1.664 + 0.0095125X$ ($R^2 = 0.9839$); ●30 mg - $\hat{Y}^{**} = 1.39532 - 0.0378676X + 0.00264821X^2 - 0.0000257552X^3$ ($R^2 = 0.9276$); x40 mg - $\hat{Y}^{**} = 1.592 + 0.0093125X$ ($R^2 = 0.7836$). (B) ♦0%; ■20%; ▲40%; ●60% - $\hat{Y}^{**} = 2.29157 - 0.0999643X + 0.00811786X^2 - 0.00014X^3$ ($R^2 = 0.9199$); x80%. (C, D) total chlorophyll content (Chl a+b): (C) ♦0 mg - $\hat{Y}^* = 1.99146 - 0.0472693X + 0.00204464X^2 - 0.0000171615X^3$ ($R^2 = 0.9584$); ■10 mg - $\hat{Y}^{**} = 1.706 + 0.016225X$ ($R^2 = 0.8051$); ▲20 mg - $\hat{Y}^{**} = 1.952 + 0.0127625X$ ($R^2 = 0.9797$); ●30 mg - $\hat{Y}^{**} = 1.66129 - 0.0719911X + 0.00446161X^2 - 0.0000426562X^3$ ($R^2 = 0.9875$); x40 mg - $\hat{Y}^{**} = 1.8975 + 0.0122X$ ($R^2 = 0.7984$). (D) ♦0%; ■20%; ▲40%; ●60% - $\hat{Y}^{**} = 2.78125 - 0.171187X + 0.0146375X^2 - 0.000258125X^3$ ($R^2 = 0.8583$); x80%. * e **: significant by regression analysis at 5% e 1% of probability, respectively**

Treatment with PBZ may result in increases cytokinin levels [14]. It is known that elevation in cytokinin levels can accelerate chloroplast differentiation and chlorophyll biosynthesis, and maintain the integrity of this molecule [31]. Working with *Solenostemon rotundifolius*, [32] observed that PBZ treatment resulted in higher number of chloroplasts per cell unit in the leaves when compared to the control treatment.

It was observed increase in SPAD index of arabica coffee plants, due to the increase in shading levels, with maximum value (68.99) estimated for the level of 62.7% of LR (Fig. 3A). There is positive correlation between SPAD index and chlorophyll content in leaves of

different plant species [33-36]. Therefore, the increase observed in the SPAD index in this study was associated with higher chlorophyll content in leaves of the shaded plants (Fig. 2C).

Generally, treatment with PBZ provides higher SPAD index in plants. This fact is commonly associated with the increase of chlorophyll content, or the higher number of chloroplasts per unit of leaf area, in response to increases of leaf thickness and decrease of leaf area [32, 13]. However, for the present study, there was no effect of the PBZ doses applied via soil, on the coffee plants SPAD index (Fig. 3B).

It should be noted that SPAD index is based on a unit of green light reflectance area, while the chlorophyll content determined in the present work was defined based on the mass of the leaf blade. Due to the existence of an impact intensity differential of PBZ on leaf morphology (area reduction and thickness increase) and chloroplast metabolism (chlorophyll synthesis, movement, distribution, and anatomy of these plastids), different associations between these effects may interfere with the intensity of the green color of the leaf.

There was a tendency for linear increase of chlorophyll *b* content as a function of the increase in LR levels (Fig. 3C). [29] associated the decrease of the chlorophyll *b* content in *Illicium floridanum* cultivated in full sun to the degradation of this pigment by the excess of irradiation. In addition, the higher development of LHCI and LHCII antenna complexes in shaded plants [8] may be associated with this response.

A linear decreasing effect was observed for the relationship between chlorophyll *a* and *b* ratio (Chl *a:b*) and LR levels (Fig. 3E). In general, the size of the antenna complexes (LHCI and LHCII) of plants increases under low irradiation, while under high irradiation, it is reduced to avoid overexcitation of the photosystems [8]. It is well-known that photosystems only contain chlorophyll *a*, while antenna complexes present both chlorophyll *a* and *b* [9]. Thus, increase in LHCI and II complexes in shaded plants may result in lower chlorophyll *a:b* ratio when compared to sun leaves.

Under shading conditions, the environment under the canopy of shading plants is enriched with green light, as this is the predominant wavelength in the light transmitted and reflected by leaves. Chlorophyll *b* shows the maximum absorption peak closest to green wavelength, compared to chlorophyll *a*. Therefore, the reduction of chlorophyll *a:b* ratio is an important strategy to increase the use of the predominant green light, which affects the leaves of shaded plants [37].

A quadratic model for the relationship between carotenoid content of coffee plants and the levels of shading was delineated. The conduction of coffee plants in shaded environments resulted in higher levels of carotenoids, with maximum value estimated at 49.3% of LR (Fig. 3G). Generally, very intense solar radiation induces elevation of carotenoid levels, since this pigment is involved

in protecting the damage caused by excessive light [9], which was not observed in the present study.

However, the effect verified in the present study corroborates with [3], who observed higher carotenoid content in arabica coffee leaves conducted under 85% of light restriction, when compared to those grown in full sun.

The higher carotenoid content in shaded coffee plants observed in this work may have occurred due to the greater amount of light absorption complexes per unit of leaf area in these plants [29], which has carotenoids as components of the complex antenna.

The content of chlorophyll *b*, chlorophyll *a:b* ratio and carotenoid content of coffee plants were not altered by the application of paclobutrazol (Figs. 3D, 3F, and 3H). According to [38], the treatment with triazoles can increase abscisic acid and cytokinins, resulting in increase in chlorophyll and carotenoid contents in leaves. However, for the present work, the dosages of PBZ used were not effective in inducing such changes.

It should be emphasized again that the anatomical effect induced by PBZ in increasing thickness and reducing leaf area may interfere with pigment contents when considering the quantification based on the mass of the leaf blade.

Light restriction influenced all the characteristics related to leaf gas exchange, with the exception of internal CO₂ concentration in the substamatic chamber. However, no characteristics were affected by PBZ doses or the interaction between LR and PBZ (Table 2).

Often, limitations of leaf gas exchange in coffee plants are strictly associated with the sensitivity of stomata to the increase in the vapor pressure deficit between leaf and atmosphere [39, 5]. Air temperature attenuation is an important environmental change promoted by shade cultivation [4], and may reduce the above limitations [40].

A quadratic model was designed to express stomatal conductance (g_s) response of coffee plants as a function of the levels of light restriction. Initially, it is observed a slight decrease of the values up to the level of 15.3% of shading (3.5% lower than the control). However, the increase was more expressive

from the 30.5% of LR level, with maximum g_s at 80% of shading, 58.93% higher than the full sun treatment (Fig. 4A).

Shading provides a modification in the microclimate of the growing environment, in order to decrease wind speed and leaf

temperature, and increase relative humidity of the air. This results in a reduction in vapor pressure deficit and, therefore, reduces stomatal limitations of coffee trees [40]. The highest values of g_s in coffee plants conducted under higher levels of light restriction in this study were associated with this fact.

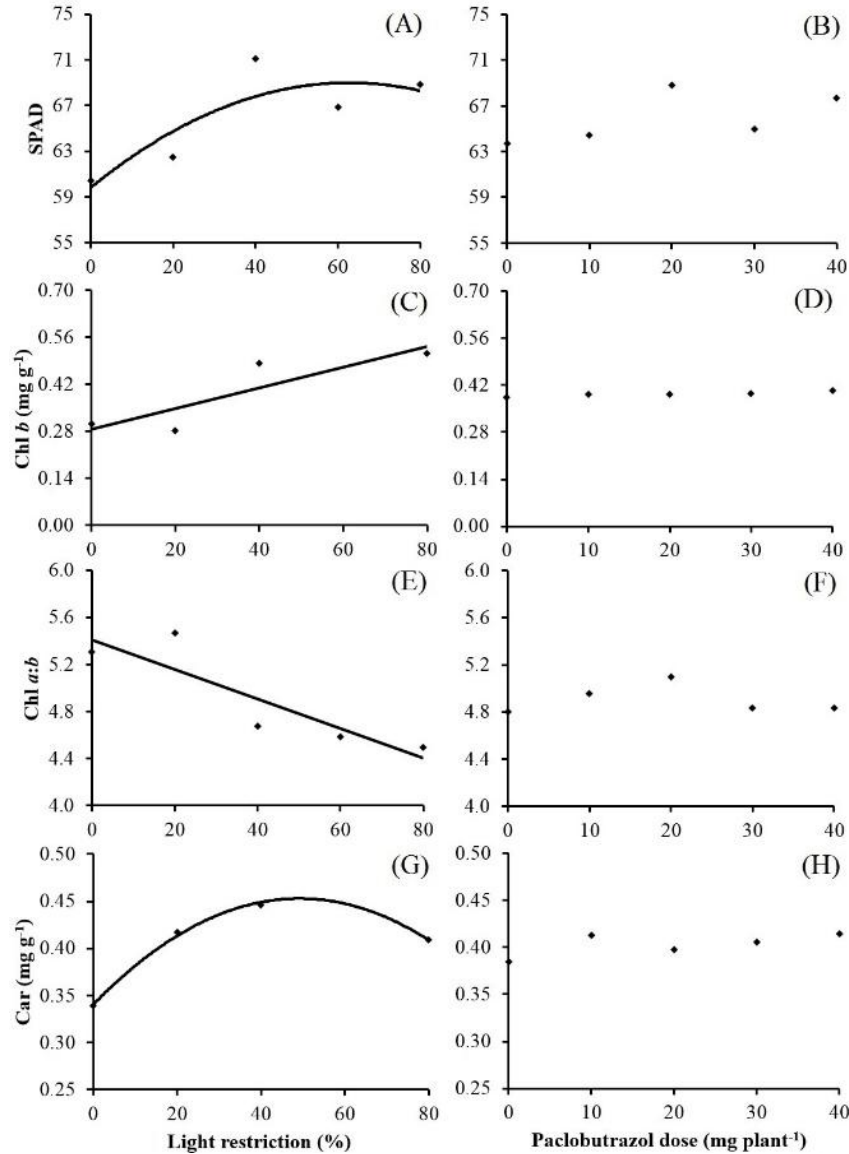


Fig. 3. SPAD index, chlorophyll b content, ratio between chlorophyll a e b, and carotenoid content in leaves of coffee plants (*Coffea arabica* L. 'Catuai Vermelho IAC 144') in response to different light restriction levels (LR) and paclobutrazol doses (D), at 100 days after the application of the regulator. (A, B) SPAD index (SPAD): (A) $\hat{Y}^{} = 59.8123 + 0.292836X - 0.00233598X^2$ ($R^2 = 0.7305$); (C, D) chlorophyll b content (Chl b): (C) $\hat{Y}^{**} = 0.2856 + 0.00307571X$ ($R^2 = 0.7583$); (E, F) ratio of chlorophyll a to b (Chl a:b): (E) $\hat{Y}^{**} = 5.4074 - 0.01257X$ ($R^2 = 0.7853$); (G, H) carotenoid content (Car): (G) $\hat{Y}^* = 0.339864 + 0.00457841X - 0.0000464205X^2$ ($R^2 = 0.9959$). * e **: significant by regression analysis at 5% e 1% of probability, respectively**

Direct relationship between the increases in light restriction levels and the potential net assimilation rate of CO₂ (*A*) and transpiration rate (*E*) of the coffee plants were verified. The elevation of *A* and *E* values in 73.04 and 43.27%, respectively, was observed for the highest levels of shading (Figs. 4C and 4E).

Stomatal conductance is the main limiting factor of the photosynthetic rate in plants grown in full sun [40], a fact that may be associated to the increase in *A* values of shaded coffee plants, since *g_s* was also elevated under these conditions.

It is worth mentioning that the increase of *A* under light restriction was similar to the increase in the content of photosynthetic pigments under these conditions. Thus, the higher content of chlorophylls and carotenoids (Figs. 2A, 2C, 3C and 3G) may also have contributed to the increase in photosynthetic rates.

The increase of *E* observed in the shaded coffee plants was related to the higher values of *g_s* (less leaf stomatal resistance), since this process results mainly from the diffusion of water vapor through stomatal opening.

Higher values of *g_s* and *E* were verified in coffee plants conducted under artificial light restriction, compared to coffee plants grown in full sun [6]. Higher photosynthetic rates in shaded coffee plants were verified by [41], compared to plants conducted without light restriction.

In the present work, although the shaded coffee plants presented less resistance to gas diffusion, internal CO₂ concentration (*C_i*) was not altered by light restriction levels (Fig. 4G). [7] also

observed no difference between the *C_i* of shaded coffee trees and full sun.

On the other hand, carboxylation efficiency (*A/C_i*) showed a tendency of linear increase as a function of the increase of shading levels (Fig. 4I). This parameter was elevated up to 75.9% at the level of 80% of shading, compared to the control treatment (full sun). This result was related, in part, to the temperature attenuation in shaded environments.

Ribulose-1,5-bisphosphate-carboxylase/oxygenase (rubisco) enzyme present in chloroplasts can catalyze both photosynthesis and photorespiration. The rates of each of these processes depend on the activity of rubisco as carboxylase or oxygenase, and they are modified by the environmental conditions [9].

Although the activity of the enzyme as carboxylase increases with temperature, the affinity of rubisco by CO₂, as well as the solubility of CO₂, decrease. This results in increases in photorespiratory activity at higher temperatures and, consequently, lower carboxylation efficiency [42].

On this way, increases in the carbon assimilation rate due to the light restriction, associated to a constant *C_i* between the treatments, resulted in higher carboxylation efficiency in shaded coffee plants.

PBZ treatment can alter several aspects of leaf gas exchange in many species [12, 43-44]. In the present study, however, PBZ application via soil did not influence any of the parameters related to gas exchange of coffee plants (Figs. 4B, 4D, 4F, 4H and 4J).

Table 2. Analysis of variance summary and coefficients of variation (CV) of stomatal conductance (*g_s*), net CO₂ assimilation rate (*A*), transpiration rate (*E*), internal CO₂ concentration (*C_i*), and carboxylation efficiency (*A/C_i*) of *Coffea arabica* L. 'Catuaí Vermelho IAC 144' plants submitted to different light restriction levels (LR) and paclobutrazol doses (D), evaluated at 99 days after the application of the regulator. Vitória da Conquista – BA, 2017

| SV | df | MEAN SQUARES | | | | |
|--------|----|----------------------|--------------------|--------------------|----------------------|------------------------|
| | | <i>g_s</i> | <i>A</i> | <i>E</i> | <i>C_i</i> | <i>A/C_i</i> |
| LR | 4 | 0,1488** | 37,93** | 2,38** | 622,66 ^{ns} | 0,000428** |
| D | 4 | 0,0431 ^{ns} | 5,07 ^{ns} | 0,11 ^{ns} | 254,59 ^{ns} | 0,000061 ^{ns} |
| LR*D | 16 | 0,0229 ^{ns} | 3,89 ^{ns} | 0,30 ^{ns} | 399,76 ^{ns} | 0,000049 ^{ns} |
| BL | 3 | 0,5144** | 11,86* | 0,99* | 3100,54** | 0,000076 ^{ns} |
| Wn | 72 | 0,0357 | 3,25 | 0,32 | 266,98 | 0,000036 |
| CV (%) | | 60,76 | 27,84 | 24,49 | 5,31 | 28,34 |

^{ns}, * e **: non-significant, significant by "F" test at 5% and 1% of probability, respectively.

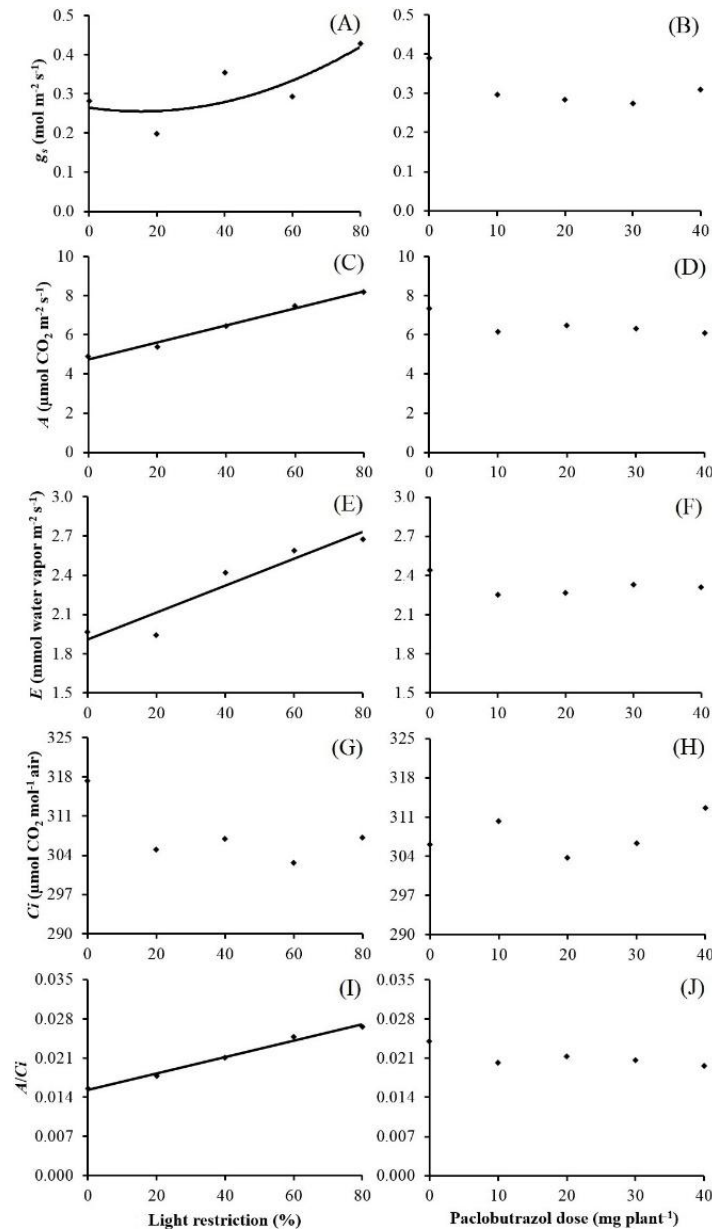


Fig. 4. Leaf gas exchanges of coffee plants (*Coffea arabica* L. 'Catuaí Vermelho IAC 144') in response to different light restriction levels (LR) and paclobutrazol doses (D), at 99 days after the application of the regulator. (A, B) stomatal conductance (g_s): (A) $\hat{Y}^* = 0,2644 - 0,0012025X + 0,000039375X^2$ ($R^2 = 0,6289$); (C, D) net CO_2 assimilation rate (A): (C) $\hat{Y}^{**} = 4,7421 + 0,043295X$ ($R^2 = 0,9884$); (E, F) transpiration rate (E): (E) $\hat{Y}^{**} = 1,9066 + 0,0103125X$ ($R^2 = 0,8940$); (G, H) internal CO_2 concentration (C_i); (I, J) carboxylation efficiency (A/C_i): (I) $\hat{Y}^{**} = 0,015343 + 0,000145525X$ ($R^2 = 0,9892$). * e **: significant by regression analysis at 5% e 1% of probability, respectively

According to Jaleel et al. [12], the increase in abscisic acid contents resulting from triazole application may result in partial stomatal closure and reduction in the transpiration rate of treated plants. On the other hand, PBZ application did

not alter stomatal conductance in coffee plants [45].

The effect of PBZ on increasing [44] or reducing [46] the rate of CO_2 assimilation is modulated by

dosage and form of application. In coffee plants, [45] found that application of lower concentrations of PBZ via leaf yielded higher photosynthetic rates and carboxylation efficiency, while higher concentrations restricted both processes.

The absence of the effect of PBZ on leaf gas exchanges of coffee plants, in this work, was possibly due to the fact that the dosages studied were too low to alter these parameters.

4. CONCLUSION

Light restriction optimized the photosynthetic apparatus of the plants, mainly at levels close to 60%, and favored the leaf gas exchanges of arabica coffee in initial growth. The application of paclobutrazol in the dosages studied resulted in little or no effect on the levels of photosynthetic pigments, and did not influence the leaf gas changes of young arabica coffee plants.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Tyystjärvi E. Photoinhibition of photosystem II. *International Review of Cell and Molecular Biology*. 2013;300:243-303. DOI:10.1016/B978-0-12-405210-9.00007-2
2. Roach T, Krieger-Liszka A. Regulation of photosynthetic electron transport and photoinhibition. *Current Protein and Peptide Science*. 2014;15(4):351-62.
3. Cavatte PC, Oliveira AA, Morais LE, Martins SC, Sanglard LM, DaMatta FM. Could shading reduce the negative impacts of drought on coffee? A morphophysiological analysis. *Physiologia Plantarum*. 2012;144(2):111-22. DOI: 10.1111/j.1399-3054.2011.01525.x
4. DaMatta FM. Ecophysiological constraints on the production of shaded and unshaded coffee: A review. *Field Crops Research*. 2004;86(2-3):99-114. DOI: 10.1016/j.fcr.2003.09.001
5. Batista KD, Araújo WL, Antunes WC, Cavatte PC, Moraes GA, Martins SC et al. Photosynthetic limitations in coffee plants are chiefly governed by diffusive factors. *Trees*. 2012;26(2):459-68. DOI:10.1007/s00468-011-0606-2
6. Baliza DP, Cunha RL, Castro EM, Barbosa JPRAD, Pires MF, Gomes RA. Trocas gasosas e características estruturais adaptativas de cafeeiros cultivados em diferentes níveis de radiação. *Coffee Science*. 2012;7(3):250-58. Portuguese.
7. Martins SC, Galmés J, Cavatte PC, Pereira LF, Ventrella MC, DaMatta FM. 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*. 2014;9(4):e95571. DOI: 10.1371/journal.pone.0095571
8. Rochaix JD. Regulation and dynamics of the light-harvesting system. *Annual Review of Plant Biology*. 2014;65:287-309. DOI:10.1146/annurev-arplant-050213-040226
9. Taiz L, Zeiger E, Møller IM, Murphy A. *Fisiologia e desenvolvimento vegetal*. 6th ed. Porto Alegre: Artmed; 2017.
10. Rademacher W. Plant growth regulators: backgrounds and uses in plant production. *Journal of Plant Growth Regulation*. 2015;34(4):845-72. DOI:10.1007/s00344-015-9541-6
11. Baninasab B, Ghobadi C. Influence of paclobutrazol and application methods on high-temperature stress injury in cucumber seedlings. *Journal of Plant Growth Regulation*. 2011;30(2):213-19. DOI:10.1007/s00344-010-9188-2
12. Jaleel CA, Manivannan P, Sankar B, Kishorekumar A, Sankari S, Panneerselvam R. Paclobutrazol enhances photosynthesis and ajmalicine production in *Catharanthus roseus*. *Process Biochemistry*. 2007;42(11):1566-70. DOI:10.1016/j.procbio.2007.08.006

13. Temiz M, Cimen I, Karahan E. Effect of paclobutrazol on fiber quality of cotton (*Gossypium hirsutum* L.). Asian Journal of Chemistry. 2009;21(3):1990-94.
14. Burondkar MM, Upreti KK, Ambavane AR, Rajan S, Mahadik SG, Bhave SG. Hormonal changes during flowering in response to paclobutrazol application in mango cv. Alphonso under Konkan conditions. Indian Journal of Plant Physiology. 2016;21(3):306-11. DOI: 10.1007/s40502-016-0236-1
15. Moura FB, Vieira MRS, Simões, AN, Silva SL, Medeiros DC, Paes RA et al. Participation of cytokinin on gas exchange and antioxidant enzymes activities. Indian Journal of Plant Physiology. 2017;22(1): 16-29. DOI:10.1007/s40502-017-0283-2
16. Zhou Z, Ma H, Liang K, Huang G, Pinyopusarerk K. Improved tolerance of teak (*Tectona grandis* Lf) seedlings to low-temperature stress by the combined effect of arbuscular mycorrhiza and paclobutrazol. Journal of Plant Growth Regulation. 2012;31(3):427-35. DOI:10.1007/s00344-011-9252-6
17. Sankar B, Gopinathan P, Karthiashwaran K, Somasundaram R. Biochemical content variation in *Arachis hypogaea* under drought stress with or without paclobutrazol and ABA. Journal of Ecobiotechnology. 2014;6:9-14.
18. Abbadi A, Shekari F, Mustafavi SH. Effect of paclobutrazol and salicylic acid on antioxidants enzyme activity in drought stress in wheat. Idesia. 2015;33:5-13.
19. Moradi S, Baninasab B, Gholami M, Ghobadi C. Paclobutrazol application enhances antioxidant enzyme activities in pomegranate plants affected by cold stress. The Journal of Horticultural Science and Biotechnology. 2016;92(1):65-71. DOI:10.1080/14620316.2016.1224605
20. Yadav DK, Hemantaranjan A. Mitigating effects of paclobutrazol on flooding stress damage by shifting biochemical and antioxidant defense mechanisms in mungbean (*Vigna radiata* L.) at pre-flowering stage. Legume Research. 2017;40(3):453-61. DOI:10.18805/lr.v0i0.7593
21. Still JR, Pill WG. Growth and stress tolerance of tomato seedlings (*Lycopersicon esculentum* Mill.) in response to seed treatment with paclobutrazol. The Journal of Horticultural Science and Biotechnology. 2004;79(2): 197-203.
22. Benett KSS, Faria Junior MJDA, Benett CGS, Seleguini A, Lemos OL. Utilização de paclobutrazol na produção de mudas de tomateiro. Comunicata Scientiae. 2014;5(2):164-69. Portuguese.
23. Superintendência de Estudos Econômicos e sociais da Bahia. Estatísticas dos municípios baianos. Salvador: SEI; 2013.
24. Ribeiro AC, Guimarães PTG, Alvarez VVH, editors. Recomendação para o uso de corretivos e fertilizantes em Minas Gerais: 5ª aproximação. Viçosa: Comissão de Fertilidade do Solo do Estado de Minas Gerais; 1999.
25. Arnon DI. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiology. 1949;24(1):1-15.
26. Barbieri Junior É, Rossiello ROP, Morenz MJF, Ribeiro RC. Comparação de métodos diretos de extração e quantificação dos teores de clorofilas em folhas do capim-Tifton 85. Ciência Rural. 2010;40(3). Portuguese.
27. Wellburn AR. 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. 1994;144(3):307-13. DOI:10.1016/S0176-1617(11)81192-2
28. Banzatto DA, Kronka SDN. Experimentação agrícola. Jaboticabal: Funep; 2006.
29. Griffin JJ, Ranney TG, Pharr DM. Photosynthesis, chlorophyll fluorescence, and carbohydrate content of *Illicium taxa* grown under varied irradiance. Journal of the American Society for Horticultural Science. 2004;129(1):46-53.
30. Gong WZ, Jiang CD, Wu YS, Chen HH, Liu WY, Yang WY. Tolerance vs. avoidance: two strategies of soybean (*Glycine max*) seedlings in response to shade in intercropping. Photosynthetica. 2015;53(2):259-68. DOI: 10.1007/s11099-015-0103-8
31. Fletcher RA, Gilley A, Sankhla N, Davis TD. Triazoles as plant growth regulators and stress protectants. In: Janick J, editor. Horticultural Reviews. 24th ed. Oxford: John Wiley & Sons; 2000. DOI: 10.1002/9780470650776.ch3
32. Kishorekumar A, Jaleel CA, Manivannan P, Sankar B, Sridharan R, Somasundaram

- R et al. Differential effects of hexaconazole and paclobutrazol on the foliage characteristics of Chinese potato (*Solenostemon rotundifolius* Poir., JK Morton). *Acta Biologica Szegediensis*. 2006;50(3-4):127-29.
33. Amarante CVT, Steffens CA, Zanardi OZ, Alves EO. Quantificação de clorofilas em folhas de macieiras 'Royal Gala' e 'Fuji' com métodos ópticos não-destrutivos. *Revista Brasileira de Fruticultura*. 2008;30(3):590-95. Portuguese.
34. Marengo RA, Antezana-Vera SA, Nascimento HCS. Relationship between specific leaf area, leaf thickness, leaf water content and SPAD-502 readings in six Amazonian tree species. *Photosynthetica*. 2009;47(2):184-90. DOI: 10.1007/s11099-009-0031-6
35. Reis AR, Favarin JL, Malavolta E, Júnior JL, Moraes MF. Photosynthesis, chlorophylls, and SPAD readings in coffee leaves in relation to nitrogen supply. *Communications in Soil Science and Plant Analysis*. 2009;40(9-10):1512-28. DOI:10.1080/00103620902820373
36. Mielke MS, Schaffer B, Li C. Use of a SPAD meter to estimate chlorophyll content in *Eugenia uniflora* L. leaves as affected by contrasting light environments and soil flooding. *Photosynthetica*. 2010;48(3):332-38. DOI: 10.1007/s11099-010-0043-2
37. Whatley JM, Whatley FR. *A luz e a vida das plantas*. São Paulo: EPU-EDUSP; 1982.
38. Kishorekumar A, Jaleel CA, Manivannan P, Sankar B, Sridharan R, Panneerselvam R. Comparative effects of different triazole compounds on growth, photosynthetic pigments and carbohydrate metabolism of *Solenostemon rotundifolius*. *Colloids and Surfaces B: Biointerfaces*. 2007;60(2):207-12. DOI: 10.1016/j.colsurfb.2007.06.008
39. Chaves AR, Ten-Caten A, Pinheiro HA, Ribeiro A, DaMatta FM. Seasonal changes in photoprotective mechanisms of leaves from shaded and unshaded field-grown coffee (*Coffea arabica* L.) trees. *Trees*. 2008;22(3):351-61. DOI:10.1007/s00468-007-0190-7
40. Franck N, Vaast P. Limitation of coffee leaf photosynthesis by stomatal conductance and light availability under different shade levels. *Trees*. 2009;23(4):761-69. DOI:10.1007/s00468-009-0318-z
41. Pompelli MF, Pompelli GM, Cabrini EC, Arruda EC, Ventrella MC, DaMatta FM. Leaf anatomy, ultrastructure and plasticity of *Coffea arabica* L. in response to light and nitrogen availability. *Biotemas*. 2012; 25(4):13-28. DOI:10.5007/2175-7925.2012v25n4p13
42. Mathur S, Agrawal D, Jajoo A. Photosynthesis: Response to high temperature stress. *Journal of Photochemistry and Photobiology B: Biology*. 2014;137:116-26. DOI:10.1016/j.jphotobiol.2014.01.010
43. Li Q, Deng M, Chen J, Henny RJ. Effects of light intensity and paclobutrazol on growth and interior performance of *Pachira aquatica* Aubl. *HortScience*. 2009;44(5): 1291-95.
44. Mohan R, Vyas D, Bhat HA, Kaur TD, Dhar A. Exploring possibilities of induction of water stress tolerance in mulberry in rainfed condition by application of paclobutrazol. *Journal of Global Biosciences*. 2015;4(9):3301-10.
45. d'Arêde LO, Matsumoto SN, Santos JL, Viana AES, Silva PAR. Morfofisiologia do crescimento vegetativo inicial de cafeeiros arabica submetidos a aplicação via foliar de paclobutrazol. *Coffee Science*. 2017;12(4):451-62. Portuguese
46. Harmath J, Schmidt G, Forrai M, Szabó V. Influence of some growth retardants on growth, transpiration rate and CO₂ fixation of *Caryopteris incana* 'Heavenly Blue'. *Folia Oecologica*. 2014;41(1):24-33.

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