PYRACLOSTROBIN PRESERVES PHOTOSYNTHESIS IN ARABICA COFFEE PLANTS SUBJECTED TO WATER DEFICIT

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ABSTRACT

The objective of this study was to investigate the effect of pyraclostrobin on the photosynthetic performance of rabica coffee plants subjected or not to a water deficit, using the parameter of gas exchange (net CO_2 assimilation, stomatal conductance, transpiration rate, and internal CO_2 concentration and nocturnal respiration), chlorophyll fluorescence *a* parameters (minimum fluorescence, maximum fluorescence, maximum quantum yield of photosystem II, effective quantum yield of PSII, quantum yield of regulated energy dissipation and quantum yield dissipation non-regulated) as well as the concentrations of chloroplast pigments. In the plants maintained without water deficit, pyraclostrobin did not cause any alteration on the parameters of chlorophyll *a* fluorescence; however, it contributed to an increase in the level of chlorophyll *a* + *b*, CO_2 assimilation and CO_2 influx for the carboxylation sites of the stroma. Decreases in nocturnal respiration in plants treated with pyraclostrobin, submitted or not to water deficit seems to be a common strategy in reducing energy waste in the maintenance metabolism. Under water deficit, pyraclostrobin contributed to increase the photochemical yield, enabling plants to effectively prevent the capture, use and dissipation of light energy.

PIRACLOSTROBINA PRESERVA A FOTOSSÍNTESE EM PLANTAS DE CAFÉ ARÁBICA SUBMETIDAS AO DÉFICIT HÍDRICO

RESUMO

Objetivou-se com este estudo investigar o efeito da piraclostrobina no desempenho fotossintético de plantas de café arábica submetidas ou não ao déficit hídrico, utilizando para isso, os parâmetros de trocas gasosas (assimilação líquida de CO_2, condutância estomática, taxa de transpiração, concentração interna de CO_2 e respiração noturna), os parâmetros de fluorescência da clorofila *a* (fluorescência mínima, fluorescência máxima, eficiência quântica máxima do fotossistema II, rendimento da fotoquímica, rendimento da dissipação de energia regulada e o rendimento das perdas de dissipação não regulada), bem como as concentrações de pigmentos cloroplastídicos. Nas plantas mantidas sem déficit hídrico, a piraclostrobina não causou alteração sobre os parâmetros de fluorescência da clorofila *a*, no entanto, foi verificado acréscimos no conteúdo de clorofila *a* + *b*, na assimilação do carbono e no influxo de CO_2 para os sítios de carboxilação do estroma. Decréscimos na respiração noturna nas plantas tratadas com piraclostrobina, submetidas ou não ao déficit hídrico parece ser uma estratégia comum em diminuir o desperdício de energia no metabolismo de manutenção. Sob déficit hídrico, a piraclostrobina contribuiu para o incremento do rendimento fotoquímico, capacitando as plantas em prevenir, de forma efetiva, a captura, o uso e dissipação a energia luminosa.

INTRODUCTION

Coffee (*Coffea arabica* L. and *Coffea canephora* Pierre ex A. Froehner) is a crop of recognized economic and social importance due to its production volume, domestic consumption, share in export tariffs, and capacity for generating employment and income. It is the second most traded commodity in the world, falling a little short behind oil products (SAKIYAMA et al., 2015).

Despite its importance, the production of coffee has been affected by climatic conditions, particularly due to the occurrence of increasingly prolonged periods of drought as a consequence of the global warming phenomenon (DAMATTA et al., 2010). Among abiotic factors, the water deficit is the main environmental factor that restricts the growth and productivity of agricultural crops (CHAVES et al., 2009). In the cultivation of coffee, very dry periods can reduce productivity by up to 80% (DAMATTA et al., 2010).

In the search for alternatives to mitigate situations caused by abiotic stresses (e.g., water deficits), studies have found beneficial effects of the molecules from a particular group of fungicides. Among them, we highlight pyraclostrobin, which is a molecule belonging to the strobilurin group (BALARDIN et al., 2011; BARBOSA et al., 2011; FAGAN et al., 2010; JÚNIOR et al., 2013; LIMA et al., 2012). In coffee cultivation, pyraclostrobin, which is the principal commercial strobilurin, is used to combat two fungal diseases, rust and cercosporiosis, which are caused by *Hemileia vastatrix* and *Cercospora coffeicola*, respectively.

In addition to their fungicidal actions, strobilurins can act positively on the physiology of plants in the absence of pathogenic agents (DEBONA et al., 2016, 2016; KÖEHLE et al., 2003). Such benefits include an increase in the rate of photosynthesis (FAGAN et al., 2010), in the activity of the nitrate reductase enzyme, which is a key enzyme involved in nitrogen assimilation (FAGAN et al., 2010; KÖEHLE et al., 2003) and in the productivity (WISE & MUELLER, 2011). It also reduces the mitochondrial respiration (Fagan et al., 2010) and the rate of ethylene synthesis, which can prevent the degradation of the chlorophyll and delay leaf senescence (GROSSMANN & RETZLAFF, 1997; KÖEHLE et al., 2003), which in turn may promote the accumulation of dry biomass (FAGAN et al., 2010; MARTINAZZO et al., 2015). Additionally, strobilurins may also increment the concentration of abscisic acid and improve water-use efficiency (GROSSMANN & RETZLAFF, 1997; KÖEHLE et al., 2003).

Despite the knowledge of the physiological effects caused by strobilurin in plants, no studies have been conducted regarding the use of this molecule in relation to the photosynthesis of coffee plants up to now. Moreover, the effects of pyraclostrobin on plants subjected to water deficit remain unclear. Therefore, the following hypotheses were considered in this study: I) pyraclostrobin improves the photosynthetic yield of Arabica coffee plants, and II) pyraclostrobin may preserve the damage to the photosynthetic apparatus caused by the water deficit. To address these matters, detailed assessments were performed for gas exchanges, chlorophyll *a* fluorescence and photosynthetic pigments to investigate the effects of pyraclostrobin on the photosynthetic performance of Arabica coffee plants subjected or not to water deficit.

MATERIALS AND METHODS

The experiment was conducted in a climate-controlled greenhouse in the experimental area of the Center for Studies and Diffusion of Technology in Forests, Water Resources, and Sustainable Agriculture (Núcleo de Estudos e Difusão de Tecnologia em Florestas, Recursos Hídricos e Agricultura Sustentável - NEDTEC) of the Agricultural Sciences Centre at the Federal University of Espírito Santo (Centro de Ciências Agrárias da Universidade Federal do Espírito Santo - CCA-UFES), located in the municipality of Jerônimo Monteiro, situated at latitude 20°47'25″S, longitude 41°23'48″W and an altitude of 120 m.

This study used Arabica coffee (*Coffea arabica* L.) seeds of Catuaí Vermelho/IAC 144 cultivar for the production of the seedlings, obtained from the Institute for Research Institute and Rural Extension of Espírito Santo (Instituto Capixaba de Pesquisa e Extensão Rural - INCAPER), in Venda Nova do Imigrante, ES, Brazil. The seedlings were grown in

perforated black polyethylene bags and exhibited the usual dimensions of coffee seedlings (0.15 x 0.25 m). After the bedding was prepared, two seeds were sown per plastic bag. The seeds were covered with substrate to maintain moisture during germination. After germination of the seeds, only one seedling was retained in each plastic bag. During the first 20 days of development, the seedlings were kept in beds in the nursery with 50% shading. The seedlings were subsequently selected for uniformity and transplanted to 14-dm^3 pots. The pots had 1-cm diameter circular holes at the bottom, which allowed aeration of the roots and drainage of the excess of water. For the entire experimental period, the seedlings were suspended at a height of approximately 1 m, on benches with 2 m in length and 1.20 m in width.

After transplantation to the pots, the seedlings were grown in a greenhouse, maintaining the content of the substrate moisture near field capacity (FC) for 130 days. After that, the treatments with the different water availability began with 100% and 30% available water (AW) for 100 days. The experiment lasted 230 days.

The application of pyraclostrobin under the leaves of the plants was done in only one step, 85 days after the start of the treatments, while the different water availabilities were maintained in the substrate; that is, application was performed at 185 days in the experiment period. For the application of pyraclostrobin, a 20-L capacity backpack sprayer with a fan-type nozzle was used. Three concentrations of pyraclostrobin were applied: 0, 0.7 and 1.4 g/L, from the dilution of the concentrated commercial product Comet® (250 g/L or 25.0% m/v) produced by BASF S.A. The concentration of pyraclostrobin recommended by the company was 0.6 to 0.8 g/L. For the plants in the treatment with no pyraclostrobin (0 g/L), distilled water was applied with a sprayer.

The substrate used to fill the pots consisted of red-yellow latosol (70%), washed sand (20%) and fermented bovine manure (10%), broken down and passed through a 2.0-mm sieve to obtain fine air-dried soil. A particle size analysis of the substrate was performed, and the clayey-sandy textural classification was obtained.

The need for the application of chemical fertilizers and corrective agents was based on the chemical analysis of the substrate content: pH $(H_2O) = 7.3$; P = 35, K = 100 and Na = 2 (mg dm^{-3}); Ca = 2.8, Mg = 1.2, Al = 0 and H + Al = 0 (cmol$_c$ dm^{-3}); C = 3.1 and OM = 5.3 (g kg^{-1}). During the planting, fertilization and correction of the soil acidity were not necessary. Four topdressing fertilizations were applied at 45-day intervals, until the end of the experiment, as recommended by Prezotti et al. (2007).

To determine the depth of irrigation, samples of the crushed and previously sieved substrate were saturated for 12 h (EMBRAPA, 1997) and then taken to a Richards pressure chamber with a porous plate for stabilization. The samples were held under pressure for three days, and the gravimetric moisture (U) was subsequently determined, which corresponded to 0.010 Mpa and 1.5 MPa for field capacity and permanent wilting point, respectively.

The volumetric moisture (θ) for each of the pressure used in the experiment was obtained from the product of the gravimetric moisture and soil density θ = U.Ds. The mean values for the calculation of the water levels, which resulted from three replicates for soil density, gravimetric moisture at the field capacity and gravimetric moisture at the permanent wilting point, were 1.04 g cm^{-3}, 23% and 14%, respectively.

The available water (AW) found in the study was 9.4%, which was calculated through the expression AW = FC - PWP, using the volumetric moisture values obtained for the field capacity (FC) at 23.9%, which was determined at the pressure of 0.010 MPa, and the permanent wilting point (PWP) at 14.5%, determined at a pressure of 1.5 MPa. The mean values of volumetric moisture and the pressure used were fit by the mathematical model proposed by Vangenuchten (1980), with the Soil Retention Curves (SWRC) software, version 2.0.

To establish water availability, two water depths were used, defined from the total avaialbe water, with values of 100% and 30% (EMBRAPA, 1997). The irrigation control was done using the gravimetric method (daily weighing of the pots), by adding water until the weight of the pots reached the previous value determined at each depth, considering the weight of the soil and the water (DARDENGO et al., 2010). Weights of the pots

were adjusted at 40-day intervals, until the end of the experiment, considering the total fresh weight obtained by the plants during the growth.

An automatic meteorological station was installed inside each climate-controlled greenhouse to obtain microclimate data. CS500 sensors (Vaisala, United States) for air temperature and relative air humidity were attached to the stations. The data were collected and stored by a CR10x datalogger model (Campbell Scientific, United States of America). The reading time was 10 s, and the mean data were stored every 15 minutes. During the experimental period, the average temperature was 23°C and the average relative air humidity recorded was 85%.

At 230 days of the experimentat, samples of leaf tissue were collected from the external part of the upper third of the plants of each replicate per treatment to allow determination of the concentration of the photosynthetic pigments. The samples were kept in liquid nitrogen while being collected and then stored at -80 °C for later analysis. Chlorophyll *a*, chlorophyll *b* and carotenoids were extracted in 80% acetone, and their concentrations were estimated in accordance with Tatagiba et al. (2016). The whole procedure was performed indoors under green light.

At 15 days before the end of the experiment, the gas exchange parameters were evaluated using a portable open-flow infrared gas exchange system (LI-6400XT, LI-COR, Lincoln, NE, USA). The net carbon assimilation (A), the stomatal conductance to water vapour (g_s), the transpiration rate (E), the internal CO_2 concentration (C_i) and the nocturnal respiration (R) were evaluated in fully expanded leaves from the upper third of the plants of each replicate per treatment at 1, 7 and 14 days after application of the pyraclostrobin. The measurements for A, g_s, E and C_i were taken between 9:00 a.m. and 10:30 a.m., a time during which A had its maximum value under the photosynthetically active artificial radiation of 1,000 μmol of photons m^{-2} s^{-1}. The measurements of R, in turn, were performed between 10:00 p.m. and 11:30 p.m. During the evaluations, 400 μmol of CO_2 mol^{-1} of air was used at the level of the leaves. All the measurements were performed at 25°C, and the vapour pressure deficit was maintained at approximately 1.0 kPa.

Before dawn, minimum fluorescence (F_0) was obtained through excitation of the leaf tissues by means of low intensity modulated red light (0.03

μmol photons m^{-2} s^{-1}), when all the FS II reaction centres were "open." Maximum fluorescence (F_m) was obtained by applying a pulse of 0.8 s of saturating actinic light (2400 μmol photons m^{-2} s^{-1}), when all the FS II reaction centres were "closed." From these initial measurements, the maximum efficiency at which the light is absorbed by FS II was estimated through the maximum quantum efficiency of the photochemistry, by determining the ratio: $F_v/F_m = [(F_m - F_0)/F_m]$. The leaf tissues were subsequently exposed to actinic irradiation (530 μmol m^{-2} s^{-1}) for 120 s, in order to obtain the steady-state fluorescence yield (F_s). A pulse of saturating white light (2400 μmol m^{-2} s^{-1}, 0.8 s) was then applied to achieve the maximum light-adapted fluorescence (F_m'). The initial light-adapted fluorescence (F_0') was estimated in accordance with Oxborough (2004). Following the calculations formulated by Kramer et al. (2004), the energy absorbed by FS II was determined for the following three dissipation components of the quantum yield: the photochemical yield [Y(II) = (F_m' - F_s)/F_m'], the yield induced by the regulated dissipation process [Y(NPQ) = (F_s/F_m') – (F_s/F_m)], and the yield of non-regulated energy dissipation [Y(NO) = F_s/F_m] (KRAUSE & WEIS, 1991).

The evaluations of the chlorophyll *a* fluorescence parameters were performed on the same leaves and using the same day-interval at which the gas exchanges were evaluated.

Two experiments were carried out. The first experiment was highlighted in a 2 × 3 factorial scheme, with six replications, consisting of two water levels (100% and 30%, denominated -WD and +WD, that is, without water deficit and water deficit, respectively) and three combinations of pyraclostrobin (0, 0.7 and 1.4 g/L), arrangements in a completely randomized experimental design, which was used to aid in the concentration of chloroplast pigments.

Another experiment was carried out in a 2 × 3 × 3 factorial scheme, in a completely randomized design, consisting of two water levels, three piracy bases and three sampling times (1, 7 and 14 days after application of pyraclostrobin), with six applications evaluated as gaseous exchanges and chlorophyll *a* fluorescence parameters.

Each experimental unit consisted of a plastic bag containing a plant and all experiments were repeated once. After verification of the variance homogeneity by the Cochran test, data for chloroplast pigment concentration, gas exchange

parameters and chlorophyll fluorescence were combined. The tests were compared by the test of Tukey (P ≤ 0.05) using version 5.1 of the SISVAR® software.

RESULTS AND DISCUSSIONS

Significant decreases of 18% and 17% for chlorophyll *a + b* were found in the plants subjected to -WD at the concentrations of 0 and 0.7 g/L, respectively, compared to the plants with the concentration of 1.4 g/L, which indicated that the increase in the concentration of pyraclostrobin contributed effectively to an increase in the level of the chlorophylls (Figure 1A).
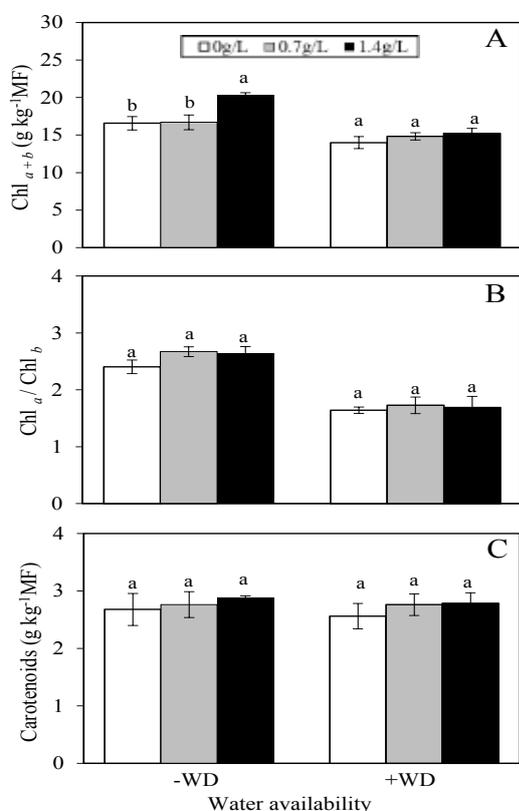

Figure 1. (a) Chlorophyll *a + b*, (b) chlorophyll *a/b* ratio and (c) carotenoids in the leaves of *Coffea arabica* L. subjected to different concentrations of pyraclostrobin and 100% (plants without water deficit, -WD) or 30% (plants with water deficit, +WD) of available water. Means followed by the same letter in the columns do not differ from each other by the test of Tukey (P ≥ 0.05). Bars at each point in the graphs represent the standard error of the mean.

Similar results were found by Júnior et al. (2013), who studied the effect of strobilurins on the growth and physiology of Catuaí 144 coffee tree, in which they observed significant increases in the SPAD indices in the leaves to which pyraclostrobin had been applied. The SPAD index reflects the relative chlorophyll content and is measured based on a certain amount of light emitted by the instrument and reflected by the leaf (SALLA et al., 2007). Plants that receive pyraclostrobin may increase the production of cytokinins, which in turn would increase the green effect and may lead to less degradation of chlorophylls and carotenoids (QUIRINO et al., 2000).

By contrast, the increase in the concentration of pyraclostrobin in the plants subjected to the +WD did not have the same effect, thus demonstrating that under dry conditions, the molecule did not promote significant increases in the level of the chlorophylls (Figure 1A).

Neither the chlorophyll *a/b* ratio nor the carotenoids showed significant differences, regardless of the level of water to which the plants were subjected (-WD and +WD, Figures 1B and 1C, respectively), which shows that the application of pyraclostrobin did not affect the balance of chlorophylls *a* and *b* or the level of carotenoids in the photosynthetic apparatus of the coffee tree. Neither Lima et al. (2012) with banana trees nor Debona et al. (2016) with rice found significant differences in the levels of chlorophyll *b* and carotenoids in plants treated with pyraclostrobin.

The technique used for fluorescence imaging of chlorophyll *a* provides a detailed analysis in the way the plant responds to changes, for example, at the application of pyraclostrobin while under cultivation (ROLFE & SCHOLES, 2010). This technique provides unique parameters that map the alterations associated with the photochemical phase of the photosynthesis in the leaves (KRAMER et al., 2004). However, for the plants subjected to -WD, no significant differences were observed between the means for any of the chlorophyll *a* fluorescence parameters

studied during the days after the application of pyraclostrobin, demonstrating that under conditions of adequate water availability (-WD), this molecule did not contribute to the increase in the photochemical yield of the coffee tree (Figures 2A, B, C, D, E and F).

In water-deficit conditions (+WD), the chlorophyll *a* fluorescence parameters showed the importance of pyraclostrobin supply as a strategy for reducing the damage to the photochemical yield caused by water stress (Figure 3).

The increment in the concentration of pyraclostrobin to 1.4 g/L, which was observed in the plants subjected to +WD, was sufficient to promote significant increases in the values of F_0 and F_m relative to the plants in which the molecule was not applied (0 g/L), during the days of evaluation, indicating that pyraclostrobin can

contribute to the efficiency of the excitation energy in the light-collecting antenna and reduce water-deficit damage to the FSII reaction centres (Figures 3A and B). In spite of this significant increase in the mean values of F_0 and F_m, neither the plants treated with pyraclostrobin (1.4 g/L) nor the untreated ones (0 g/L) showed significant differences in the means of the F_v/F_m values, which indicates that the *C. arabica* plants exposed to drought conditions maintained the integrity of the D1 protein of the FS II and were not affected by photo-inhibition (TAIZ & ZEIGER, 2013) (Figure 3C). The maximum quantum efficiency of thephotochemistry (F_v/F_m) has been widely used to compare leaf tissues under stress conditions (ROLFE & SCHOLES, 2010; ROUSSEAU et al., 2013). This proportion is close to or slightly above 0.8 in mature and stress-free leaf tissue;
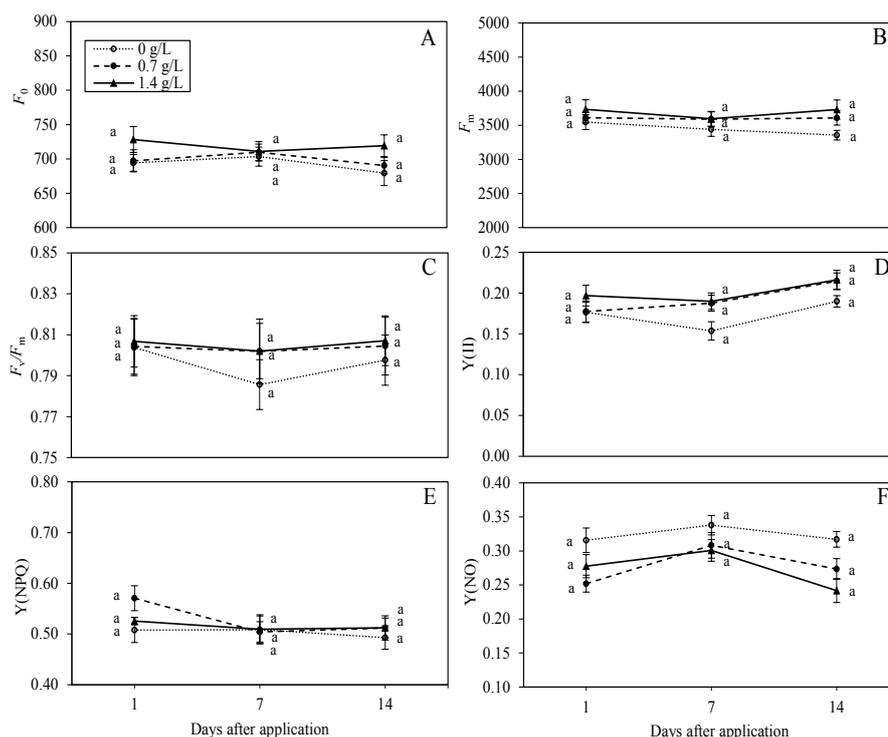

Figure 2. (A) Minimum fluorescence (F_0), (B) maximum fluorescence (F_m), (C) maximum quantum yield of the FS II (F_v/F_m), (D) effective quantum yield of the FSII Y(II), (E) quantum yield of the regulated dissipation Y(NPQ), and (F) quantum yield of the non-regulated dissipation Y(NO), in leaves of *Coffea arabica* L. subjected to different concentrations of pyraclostrobin, with 100% of available water (plants without water deficit, -WD). Means followed by the same letter at each sampling period (days after application of pyraclostrobin) do not differ from each other by the test of Tukey (P ≥ 0.05). Bars at each data point indicate the standard error of the mean.
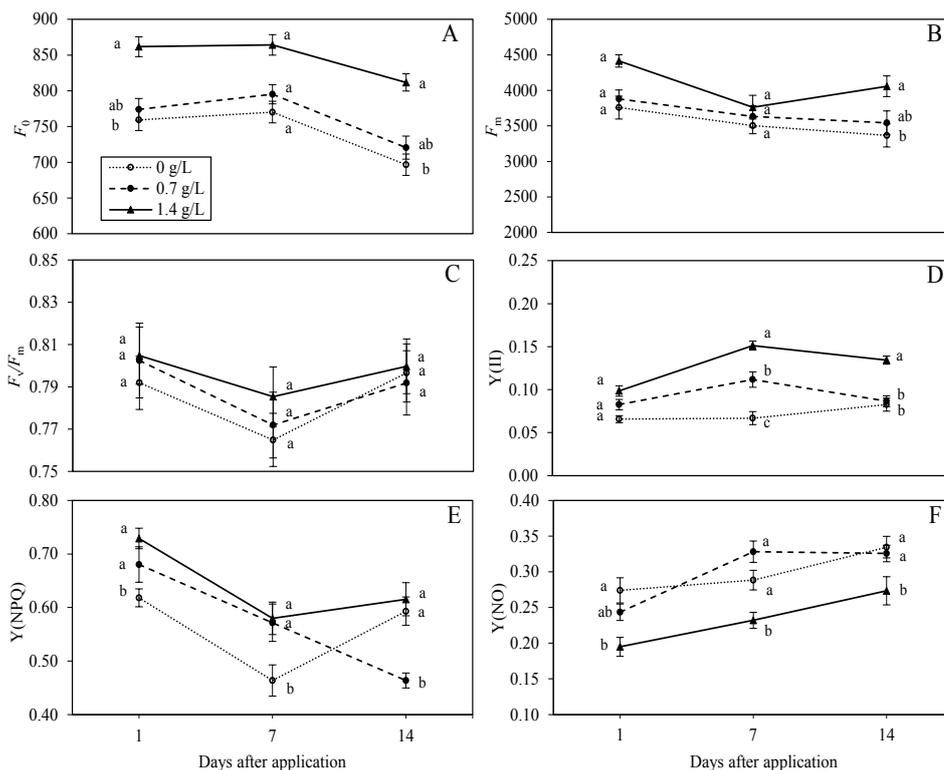
Figure 3. (A) Minimum fluorescence (F_0), (B) maximum fluorescence (F_m), (C) maximum quantum yield of the FS II (F_v/F_m), (D) effective quantum yield of the FSII Y(II), (E) quantum yield of the regulated dissipation Y(NPQ) and (F) quantum yield of the non-regulated dissipation Y(NO), in leaves of *Coffea arabica* L. subjected to different concentrations of pyraclostrobin, with 30% available water (plants with water deficit, +WD). Means followed by the same letter at each sampling period (days after application of pyraclostrobin) do not differ from each other by the test of Tukey ($P \geq 0.05$). Bars at each data point in the graphs represent the standard error of the mean.

however, the proportion decreases progressively as the damage in the FS II reaction centers increase (KRAUSE & WEIS, 1991).

Debona et al. (2016) also did not find significant differences in the F_v/F_m ratio in plants of rice, treated or not with pyraclostrobin. Previous studies have reported high stability in the photochemical efficiency of the FS II when faced with water deficiency in *C. canephora* (DAMATTA et al., 1997) and *C. arabica* (CAVATTE et al., 2012).

In contrast to the results found for F_v/F_m, the photochemical [Y(II)] and non-photochemical [Y(NPQ) and Y(NO)] yields showed significant alterations with an increase in the pyraclostrobin concentration in the plants subjected to +WD (Figure 3D). Significant increases were observed in the mean values of Y(II) in the plants treated

with 1.4 g/L, compared to the plants treated with 0 g/L pyraclostrobin 56% at 7 days and 36% at 14 days of evaluation, therefore, indicating a greater absorption and use of the light by the photochemical apparatus, which is likely to result in an enhancement in the rate of electron transport through the photosystems (KRAUSE & WEIS, 1991) (Figure 3D). Additionally, the significant increases observed in the means of Y(II), 27% and 35% at 7 and 14 days, respectively, after the application of pyraclostrobin in the plants treated with 1.4 g/L, compared to the plants treated with 0.7 g/L, show that the increase in the concentration of the molecule had contributed positively to the photochemical yield of the FS II (Figure 3D). This finding also provides an indication that the use of pyraclostrobin in plants under water deficit may

direct a greater amount of energy in the form of nicotinamide adenine dinucleotide phosphate (NADPH) and adenosine triphosphate (ATP) from thylakoid reactions to the carboxylation reactions of CO_2 that occur in the stroma of the chloroplasts (KRAMER et al., 2004).

In turn, the significant increases observed in Y(NPQ) in the plants treated with 0.7 and 1.4 g/L, compared to the plants that were not treated with pyraclostrobin (0 g/L), suggest that the molecule contributed to greater dissipation of the excitation energy in the form of heat, indicating, therefore, that it acts under water-deficit conditions, promoting the dissipation of non-photochemical energy to offset the dissipation of photochemical energy (Figure 3E). Additionally, significant reductions observed in the Y(NO) during the evaluation days for the plants treated with 1.4 g/L pyraclostrobin, in comparison with the plants that were not treated with this molecule (0 g/L) (Figure 3F), confirm that the supply of pyraclostrobin to the plants at a higher concentration contributed effectively to the dissipation of energy lost either constitutively in the FS II antennas or by the fluorescence, thus promoting a further energy dissipation for the non-photochemical events. However, under water-deficit conditions, the excitation energy was directed to both photochemical and non-photochemical events in an efficient way. Collectively, these responses are consistent with the incremented use of the photochemical excitation energy for resulting in higher rates of *A*.

Although the pyraclostrobin did not act on the photochemical yield of the plants subjected to -WD, significant increases in *A* of 22% and 23% at 7 and 14 days of evaluation in the plants treated with 1.4 g/L pyraclostrobin, in relation to the untreated plants (0 g/L), make clear the importance of this molecule in the increase in CO_2 assimilation in *C. arabica* (Figure 4A).

Similar results were also obtained by Fagan et al. (2010) with soybeans; Jadoski et al., (2015) with beans; and Debona et al. (2016) with rice, which confirm that pyraclostrobin contributes effectively to increases in *A*. Additionally, it is

important to mention that the g_s alone would not explain the significant changes in *A* in the leaves of the plants subjected to -WD and treated with pyraclostrobin. In general, a greater opening of the stomata was observed in the leaves of the plants treated with 1.4 g/L pyraclostrobin, which in turn may have guaranteed the maintenance of higher *A* values (Figure 4B). According to Grossmann & Retzlaff (1997), pyraclostrobin increases the photosynthesis rate of plants by altering the CO_2 compensation point, which favours the absorption of carbon dioxide as opposed to release by respiration. The greater stomatal opening shown by the plants subjected to -WD and treated with pyraclostrobin, may have contributed to significant *E* values obtained at 7 and 14 days of evaluation (Figure 4C). For beans, Jadoski et al. (2015) also found significant increases in *E* in the plants that received pyraclostrobin under water conditions suitable for development. Furthermore, the plants subjected to -WD and treated with pyraclostrobin showed significant reductions in C_i at 7 and 14 days of evaluation, when compared to the plants that were not treated with this molecule (0 g/L) (see Figure 4D). If the C_i decreases in the plants treated with pyraclostrobin, the CO_2 reaching the cells of the mesophyll is fixed more efficiently in the carboxylation phase of the ribulose 1,5-bisphosphate carboxylase oxygenase (Rubisco) when compared to the untreated plants, thus contributing to the increase in values of *A*.

Significant decreases in *R* were found on days 1 and 7 of the evaluation in the leaves of the plants subjected to -WD and treated with pyraclostrobin at concentrations of 0.7 and 1.4 g/L, when compared to the plants that were not treated with pyraclostrobin (Figure 4E). According to Köehle et al. (2003), in addition to acting directly on the electron transport chain, pyraclostrobin may act as a moderator of biotic and abiotic stresses since it participates in the metabolism of nitric acid and stress hormones, as it is the case with abscisic acid. Thus, the decreases in *R* in the plants treated with pyraclostrobin may be an important factor for reducing the energy waste of plants in the maintenance metabolism, which may increase the
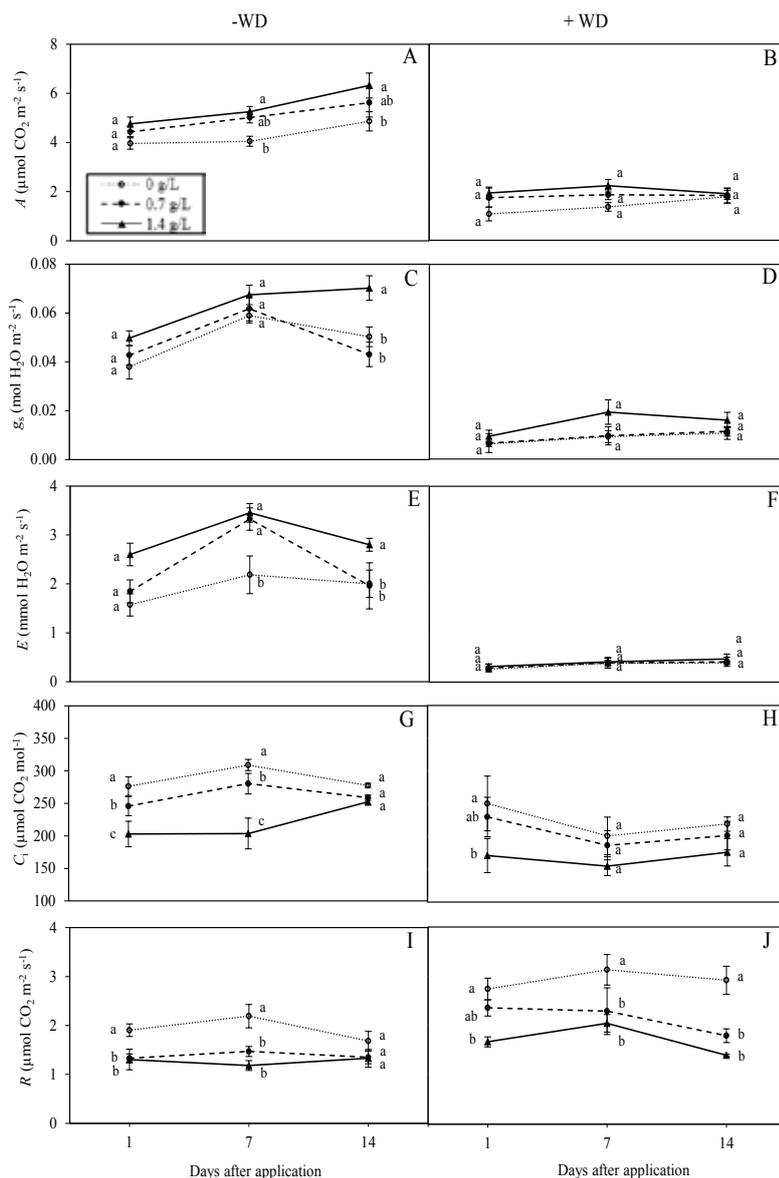
Figure 4. (A and B) Net CO_2 (A), (C and D) stomatal conductance (g_s), (E and F) transpiration rate (E), (G and H) internal concentration of CO_2 (C_i), and (I and J) nocturnal respiration (R) in leaves of *Coffea arabica* L. subjected to different concentrations of pyraclostrobin, with either 100% (plants without water deficit, -WD) or 30% (plants with water deficit, +WD) of available water. Means followed by the same letter at each sampling time (days after application of pyraclostrobin) do not differ from each other by the test of Tukey ($P \geq 0.05$). Bars at each data point in the graphs represent the standard error of the mean.

net photosynthesis rate and directly promote the production of plant biomass.

Finally, in the plants subjected to +WD, no significant differences were observed in the A, g_s or E in the plants treated or not with pyraclostrobin (Figures 4 F, G, and H). For C_i, significant differences were only found between the means of the treatments on the first day of the evaluation

(Figure 4I).

Similar to the plants subjected to -WD, the plants subjected to +WD and treated with pyraclostrobin at the concentration of 1.4 g/L, showed significant decreases in R of 39, 35, and 52% at 1, 7, and 14 days of evaluation, respectively, in relation to the untreated plants. When comparing the plants that had 0.7 g/L pyraclostrobin applied with the plants

not treated with this molecule, significant reductions of 27% and 38% were found in *R*. Therefore, the higher concentration of pyraclostrobin, decreased more effectively the respiration of the plants exposed to the water deficit.

CONCLUSIONS

- In the plants subjected to -WD, the pyraclostrobin promoted an increase in the level of chlorophyll *a* + *b* and *A*.

- In the plants subjected to +WD, the pyraclostrobin contributed to an increase in the photochemical yield, which was associated with increases in F_0, F_m, Y(II) and Y(NPQ) as well as a decrease in Y(NO).

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