

## Inhibition of respiration by light in *Coffea arabica*

Indira Pereira de Oliveira<sup>1</sup> , João Paulo Rodrigues Alves Delfino Barbosa<sup>1</sup> , Scott Saleska<sup>2</sup> ,  
João Paulo Pennacchi<sup>1</sup> , Neill Prohaska<sup>2</sup> 

<sup>1</sup>Universidade Federal de Lavras/UFLA, Departamento de Biologia, Setor de Fisiologia Vegetal, Lavras, MG, Brasil

<sup>2</sup>The University of Arizona, Department of Ecology and Evolutionary Biology, Tucson, Arizona, Estados Unidos

Contact authors: ind\_pereira@outlook.com; jp.barbosa@ufla.br; saleska@arizona.edu; jppennacchi@gmail.com; photosynthesis@gmail.com

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### ABSTRACT

Describing gas exchange between plants and the atmosphere is essential to improve current estimates of fluxes and carbon stocks. Light is directly related to gas exchange; when plants are under low light intensities, there is an increase in photosynthetic quantum yield and, consequently, in respiratory rate. That means there is an apparent inhibition of respiration under high light intensity, a phenomenon known as the Kok effect. Considering the effect of light and leaf age, the aim of this study was to describe the physiological responses of daytime gas exchange related to leaves of different ages in *Coffea arabica* L. cv. Catuaí Vermelho, seeking to identify leaf respiration inhibition by light. The experiment was conducted at the Universidade Federal de Lavras, Minas Gerais, Brazil. Leaves were measured using an infrared gas analyzer (IRGA; LI-6400XT, LI-COR, Lincoln, NE, USA) and the fluxes were measured to create a light response curve in “in situ”, thus obtaining photosynthetic parameters, used to estimate light and dark respiration, also the rate of inhibition of leaf respiration by light throughout the leaf age. The assimilation ( $A_{sat}$ ) and quantum efficiency did not differ among young, mature, and old leaves, showing that photosynthetic process was not affected by leaf age; however, light and dark respiration were higher in young and mature leaves than in old leaves. Inhibition of respiration by light was similar among leaf ages, assuming the occurrence of inhibition of respiration caused by light (the Kok effect).

**Key words:** Kok effect; quantum efficiency; gas exchange; leaf age.

### 1 INTRODUCTION

An increase in agricultural production, primarily in the context of environmental changes, depends on understanding the processes and factors that affect respiratory variation and photosynthetic parameters. Plant biomass production is related to these processes, and the difference between the uptake (photosynthesis) and release (respiration and photorespiration) of carbon dioxide in growing plants provides the net primary production, corresponding to production of plant substances minus the carbon released back into the atmosphere. It represents all the energy stored from photosynthesis minus the energy dissipated by respiration (Yin et al., 2011).

Respiration and photosynthesis are processes affected by several environmental factors, and radiation is one of the main ones. Improving knowledge of C assimilation processes and oxidative processes in the relation of plants to light improves understanding of ecosystem functioning and plant primary production (Wohlfahrt, 2015; Yin et al., 2011; Heskell; Tang, 2018).

Changes in gas exchange between plants and the atmosphere can occur due to the presence of light. Under low light intensities, near the light compensation point, there might be a break in the linear relationship in photosynthetic quantum yield, which increases according to an increase in respiratory rate. That suggests an apparent inhibition in the respiration rate under the incidence of light ranging from 0 to 1, where the closer to 1, the greater the degree of this inhibition (Gauthier et al., 2020). That phenomenon was first reported by Kok (1949),

based on measurements made on algae and, since then, has been observed in several plant species, the so-called “Kok effect” (Heskell, 2013; Tcherkez et al., 2017).

Understanding how the Kok effect influences carbon fluxes is important for including the variation in leaf respiration under light and photorespiration in estimates of the carbon cycle and the plant production cycle. Neglecting the effect of light on changes in respiratory rate may result in overestimation of gross primary production and respiration. Thus, it becomes necessary to evaluate and incorporate this variation in light respiration in plant gas exchange prediction models (Keenan et al., 2019).

To identify the inhibition of respiration by light in crops, *Coffea arabica* was chosen in this study because it is a woody and perennial plant, a model for many studies (Bote et al., 2018; Dubberstein et al., 2019). Coffee is one of the agricultural export products most traded in developing countries and is therefore a valuable source of income (International Coffee Organization - ICO, 2015); Brazil is the world's largest exporter at 22,089 million 60 kg bags of coffee beans in 2020, and *Coffea arabica* is the species most commercialized in Brazil and in the world (ICO, 2020).

The gas exchange characteristics of the coffee leaf may differ from other leaves, which requires caution when scaling estimates of leaf gas exchange. Coffee has low net  $CO_2$  assimilation ( $A$ ) rates, typically in the range of 4-11  $\mu mol\ m^{-2}\ s^{-1}$  at current natural atmospheric  $CO_2$  concentration and saturating light, which is in the lowest range recorded for plants. Decay in assimilation values has been associated with

diffusive (stomatal and mesophilic) limitations, rather than biochemical patterns (Damatta et al., 2007).

The saturation irradiance is relatively low in coffee leaves, ranging from about 300 to 700  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ , with the lowest values in shaded leaves. However, as many leaves are partially shaded within the coffee plant canopy, it may be that canopy photosynthesis would be saturated at irradiances higher than 700  $\mu\text{mol of photon m}^{-2} \text{s}^{-1}$  (Damatta, 2004; Damatta et al., 2007; Silva et al., 2004).

Details on gas exchanges between plants and the atmosphere can greatly contribute to coffee production, since, according to Santin et al. (2019), perennial species have particular biological aspects, with a long reproductive cycle and oscillation, showing different maturity and longevity periods. Occurrence of the Kok effect can impact the development of models, allowing the quantification of gain in biomass and, consequently, of the yield of the coffee crop under the effect of light.

Estimating light respiration using the Kok effect is an important practice, and the data obtained can contribute to predicting carbon cycle models at the leaf, field, and ecosystem scale. Thus, it is important to characterize the effect of leaf age in relation to the phenomenon of inhibition of plant respiration by light. It is important for understanding the carbon flux of coffee crops, their contribution as a carbon sink, and their yield. Furthermore, analyzing this phenomenon in coffee plants can provide basic information for carbon cycle studies in other species and in different ecosystems.

The aim of this study is to describe the physiological responses of light respiration related to leaf age in *Coffea arabica* plants, seeking to identify inhibition of the respiratory process under the effect of light in young, mature, and old leaves. The study was carried out on an Arabica coffee plantation to assess how leaf age impacts diurnal carbon fluxes. The aim of the study was to quantify diurnal carbon exchange in coffee to provide parameters for the light respiration model.

## 2 MATERIAL AND METHODS

The experiment was conducted at the Universidade Federal de Lavras (UFLA), Lavras-MG, Brazil, in a 4-year-old plantation of *Coffea arabica*, Catuaí vermelho variety. Under the climatic conditions of Lavras, MG, the average annual temperature is 19.3 °C and rainfall is 1530 mm (Köppen, 1948). The measurements of gas exchanges were carried out in a positive production period of the biennial season for the coffee plant, from November 2020 to June 2021, during the growing season. This season had an average temperature of 21 °C, relative humidity of 62%, and precipitation of 148 mm.

Three plants were selected for sampling, with measurement of gas exchanges, in which plagiotropic branches were chosen at random in the middle of the plants. Samples

were taken of leaves at two different ages, young leaves and mature leaves, in the first sampling set, with 3 curves at each level, for a total of 6 curves; leaves of 3 maturity levels, young, mature, and old, in the second sampling set, with 3 curves at each level, for a total of 9 curves; and young and mature leaves in the third sampling set, with 3 curves at each level, for a total of 6 curves, adding up to 21 curves over the time of the experiment.

Measurements were taken in the morning to early afternoon (8:00 to 13:00) in the northeast planting position, on sun, healthy, fully expanded leaves between the middle and distal plagiotropic branch parts. The branches were divided into proximal, middle, and distal parts. Young leaves were measured in the distal part, mature in the middle, and old in the proximal part. The number of curves applied is describing on Table 1.

**Table 1:** Number of A-PAR curves measured per sampling set.

Sampling set	Young leaf curves	Mature leaf curves	Old leaf curves	Total curves
1st	3	3	-	6
2nd	3	3	3	9
3rd	3	3	-	6
Total				$\Sigma = 21$

Gas exchanges were measured with the infrared gas analyzer (IRGA), LI-6400XT, LI-COR, Lincoln, NE, USA in “in situ” leaves, thus obtaining the net carbon assimilation rate (A,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), substomatal  $\text{CO}_2$  concentration ( $C_i$ ,  $\mu\text{mol mol}^{-1}$ ), stomatal conductance ( $g_s$ ,  $\text{mmol m}^{-2} \text{s}^{-1}$ ), and transpiration rate (E,  $\text{mmol m}^{-2} \text{s}^{-1}$ ) at each photosynthetically active radiation (PAR) point.

A-PAR curves were measured setting the ambient  $\text{CO}_2$  concentration at 400 ppm, temperature at 25 °C, and relative humidity at 60-80%. Different levels of PAR were applied, starting at the light level of 800  $\mu\text{mol m}^{-2} \text{s}^{-1}$  of photons to activate photosynthesis and obtain stable initial values. The PAR values were chosen from the literature (Heskel et al., 2018) and knowledge of the species’ physiological behavior, allowing optimization of the light curves. For that purpose, pre-tests were carried out following 7 different protocols to determine the best physiological response and optimization of the curve, e.g.

Protocol 6: 20 PAR levels (800, 500, 300, 150, 100, 88, 78, 70, 62, 55, 50, 45, 40, 36, 32, 28, 24, 19, 13, 7  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), with 5 repetitions, a minimum time of 50 seconds and a maximum of 90 seconds between the points taken, followed by turning off the RFA and analyzing the parameters in the dark for 40 minutes, with an interval of 120 seconds between the points taken. The leaf acclimatization time to start the curve was between 30 minutes and 1 hour.

From analysis of the A-PAR curves made as pre-tests, protocol 6 was initially chosen to be applied throughout the experiment in 3 plants chosen at random. However, due to the waiting time for the leaf to acclimatize in the chamber of the IRGA, the number of repetitions in each level of photosynthetically active radiation (PAR) was reduced, and this constitutes Protocol 7 used since then. Below is a model curve made in mature leaves referring to the first sampling set, obtained from Protocol 7, showing average values of 7 repetitions of each light level. Summary of protocols applied are described below (Table 2).

**Table 2:** Summary of protocols applied during the pre-test.

Protocol	PAR level	Repetition	Interval (second)	Dark time (minute)	Interval (second)
1	17	1	60-120	60	180
2	25	7	60-120	60	180
3	27	7	60-120	60	180
4	28	1	60-120	60	180
5	24	1	60-120	30	120
6	20	5	50-90	40	120
7	20	4	50-90	40	120

In this study, light respiration was estimated using the method of Kok (1949). A regression line was fitted between the identified “breakpoint” and the linear segment of the light response curve (about  $50 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR photons). The y-intercept of this regression is the estimated apparent light respiration rate (RL). Considering the changes in intercellular  $\text{CO}_2$  concentration ( $C_i$ ) that occur with decreasing light intensity (PAR), an adjustment of RL values was made with the regression described above with a constant value of  $C_i$ , using calculations described by Kirschbaum and Farquhar (1987).

Respiration inhibition (RI) was expressed as the proportion of light respiration to dark respiration ( $(\text{RE}-\text{RL}) / \text{RE}$ ), with values ranging from 0 to 1. A low value represents a high degree of inhibition, and a high value represents low inhibition (Heskel et al., 2018).

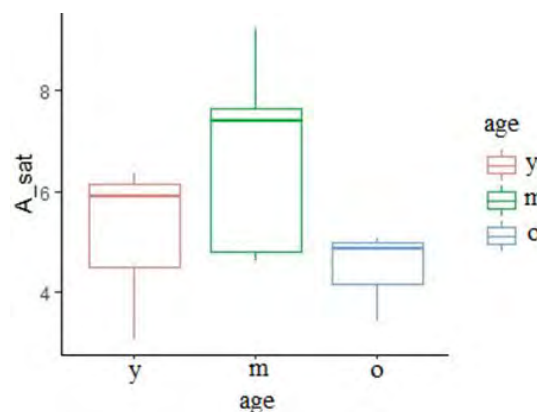
We estimated saturating light assimilation ( $A_{\text{sat}}$ ) and photosynthetic quantum efficiency related to the degree of inhibition of leaf respiration by light, and how this might vary throughout the leaf phenological cycle. Such parameters were calculated in each curve measured under a constant  $\text{CO}_2$  reference value of 400 ppm and a saturating light value of  $800 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR photons.

The data obtained correspond to assimilation in saturating light, quantum efficiency of photosynthesis, respiration rate in the dark (RE), respiration rate in the light (RL), and degree of inhibition of respiration in light (IR) calculated from each curve performed.

The experiment was conducted using a completely randomized design. Among the 21 previously chosen curves, only 11 were used to perform the statistical analyses after analyzing the quality of the curves. The measured and calculated variables were tested for normality before comparisons were made, and then ANOVA was applied to test for significant variance among leaf ages. The significance level of the statistical tests was preset at 5% (Gomes, 1973). All analyses were conducted using R software.

### 3 RESULTS

The net  $\text{CO}_2$  assimilation rate in saturating light ( $A_{\text{sat}}$ ,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), with the  $\text{CO}_2$  input maintained at the ambient value, is represented in Figure 1. The photosynthetic process was not affected by leaf age ( $p$ -value: 0.2176). Thus, assimilation values under saturating light did not show significant differences between young, mature, and old leaves, with values ranging from 4 to  $8 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ , which are the commonly rates found for the *Coffea arabica*. Quantum efficiency ( $\Phi_{\text{J}}$ ), which represents the fixed  $\text{CO}_2$  molar amount per mole of absorbed photon, did not differ among leaf ages (Figure 2,  $p$ -value: 0.3222).

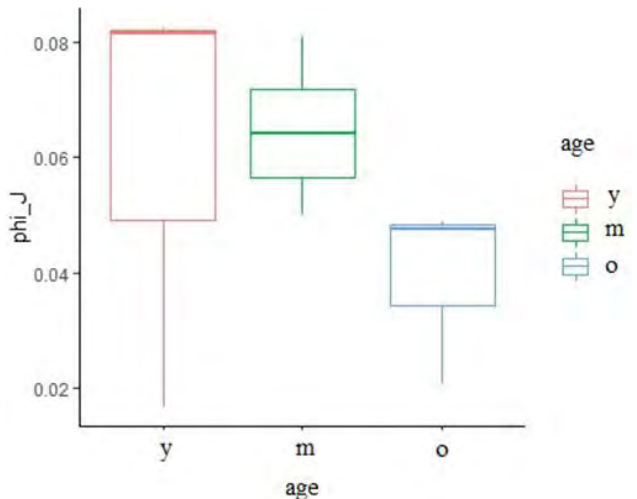


**Figure 1:** Assimilation ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) in saturating light of young (y), mature (m), and old (o) *C. arabica* leaves.

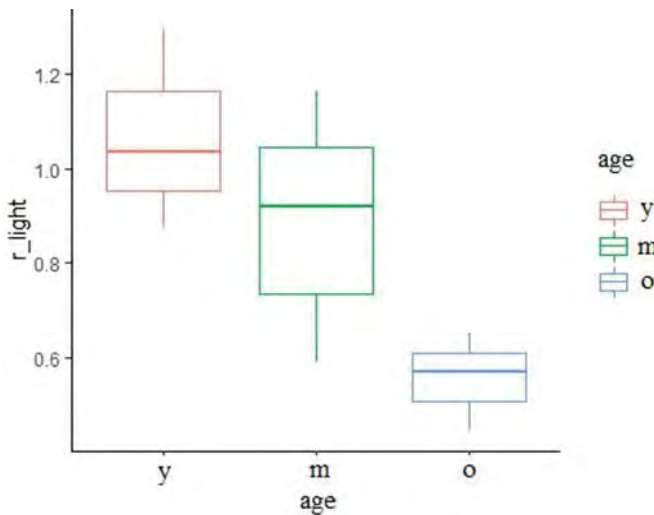
Light respiration (RL) showed significant differences among the three different leaf ages, with values ranging from 0.5 to  $1.3 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  (Figure 3,  $p$ -value: 0.0386), with the old leaves showing the lowest RL rates.

Old leaves showed less light respiration than young leaves did.  $P$ -value: 0.0386.

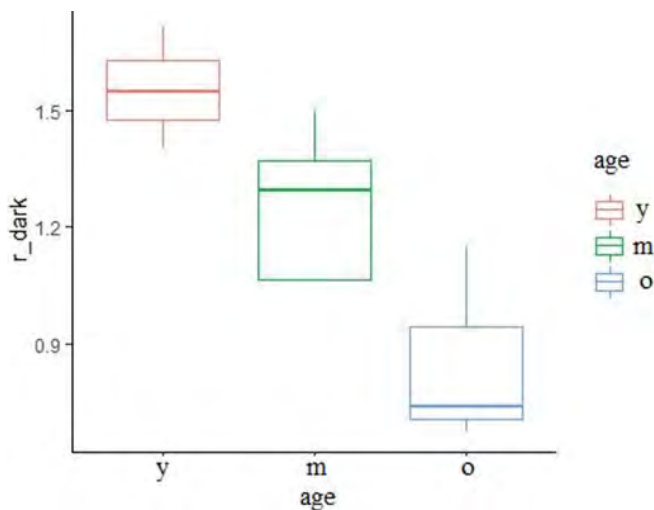
Following the same trend as light respiration, dark respiration had higher rates in young and mature leaves (Figure 4,  $p$ -value: 0.008671), differing from old leaves, which showed lower dark respiration (RD) rates. However, RD rates were higher than RL rates, with values exceeding  $1.5 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  in young leaves, shown that respiration was suppressed by light.



**Figure 2:** Quantum efficiency ( $\mu\text{mol CO}_2 \mu\text{mol}^{-1}$  photons) of young (y), mature (m), and old (o) *C. arabica* leaves.



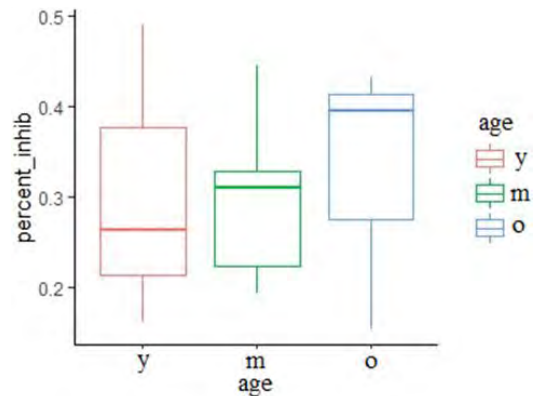
**Figure 3:** Light respiration ( $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$ ) of young (y), mature (m), and old (o) *C. arabica* leaves.



**Figure 4:** Dark respiration ( $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$ ) of young (y), mature (m), and old (o) *C. arabica* leaves.

Old leaves showed less respiration in the dark than young leaves did.  $P$ -value: 0.008671.

As RD and RL were measured in the same leaves over a relatively short period (< 24 hours), the data were used to investigate the relationship between RL and RD at the 3 different leaf ages. It was found that RL was significantly lower than RD. Respiration suppression was identified. Respiration is suppressed within a range of 10% to 50% by light (Figure 5,  $p$ -value: 0.9616), shown that light respiration cannot be assumed to have the same magnitude as dark respiration and not even as a fixed proportion. It is important to consider the potential differences in light inhibition between plant types.



**Figure 5:** Percentage of inhibition of respiration by light in young (y), mature (m), and old (o) *C. arabica* leaves.

There was no significant difference between young, mature, and old leaves regarding the percentage and inhibition of light respiration.  $P$ -value: 0.9616.

#### 4 DISCUSSION

We employed the Kok method (Kok, 1949), which have been widely used and cited in the literature, allows for comparisons between our results and those of other researcher, even if they studied other species and other factors, instead of the age of the plant.

The net  $\text{CO}_2$  assimilation rate in saturating light ( $A_{\text{sat}}$ ,  $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$ ) was measured in coffee leaves by Silva et al. (2004), who found mean values of 8.6  $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$  throughout the growing season; and Marias et al. (2017) found mean values of photosynthesis of 6.3 and 7.0  $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  for expanding and mature leaves, respectively.

In their study of a tropical evergreen forest over the growing season, which predominantly has expanding (young) leaves at the beginning of the season, fully expanded (mature) leaves at mid-season, and old leaves at the end, Heskell et al. (2018) did not find significant differences in  $A_{\text{sat}}$  or in other photosynthetic parameters. However, they observed differences in response to temperature and between leaves

grown at the top of the canopy and leaves in the middle of the canopy. Araujo et al. (2008), studying *Coffea arabica*, observed that the maximum CO<sub>2</sub> assimilation values in leaves at the top of the canopy and in the middle of the canopy were similar on all sides of the canopy.

The results showing similar assimilation rates among young, mature, and old leaves in this study are different from the results commonly found in coffee studies. Ghazali et al. (2020) studied leaf development through spectral analyses, evaluating the green level, chlorophyll, and water content, and observed that all the biochemical factors of leaves are reduced during their development, showing that coffee plant behavior is clearly defined by leaf age.

For coffee plants, a current year under the reproductive phase and the vegetative phase of the next year are simultaneous, and the growing coffee cherries are preferential sinks. Thus, the plant tends to increase the production of photoassimilates to supply the demands of the fruit in formation, as well as of the formation of vegetative buds (Mendonça et al., 2011). That high photoassimilate demand may explain the equal rates of assimilation in saturating light among young, mature, and old leaves.

Old leaves were sampled only in the middle of the growth period, in the fruit formation phases, which occur simultaneously with the vegetative growth of the cycle. That phase coincides with the period of greater photosynthetically active radiation (PAR), higher temperature, and greater rainfall, factors that positively affect photosynthetic capacity (Mendonça et al., 2011). That may explain the similarity of A<sub>sat</sub> from old leaves compared to mature and young leaves.

Silva et al. (2004) observed that during the 12-month season studied, 78% occurred in the warm and rainy season (October to March – active growth phase) and 22% during the cold and dry season (April to September – slow growth phase). That may indicate that in the active growth phase, mature leaves may have photosynthetic capacity like young and mature leaves, increasing carbon incorporation.

Albert et al. (2018) studied gas exchange in an evergreen tropical forest and observed that mature leaves showed a decrease in the assimilation rate throughout the growing season, and the photosynthesis parameters (carboxylation of the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase, electron transport rate, and use of triose phosphate) showed an age dependence, in which mature leaves performed better in these parameters than young and old leaves did. Wu et al. (2016), also studying an evergreen tropical forest, showed that the phase of greater photosynthetic capacity at the end of the dry season can be attributed to an increased quantity (demography) and quality (photosynthetic capacity per leaf area) of leaves, such as maturation time of the leaves as the consequence.

Quantum efficiency (Phi<sub>J</sub>) represents the fixed CO<sub>2</sub> molar amount per mole of absorbed photon, and in several

species, the average yield of C3-type plants ranges from 0.090 to 0.11, because in most plants, the average absorbance of the leaf ( $\alpha$ ) is 0.84 (Björkman; Demmig, 1987), and in this study, Phi<sub>J</sub> reached the range of 0.02 to 0.9. Araujo et al. (2008) did not observe any difference in quantum efficiency between the entire canopy and the low canopy leaves in any of the canopy positions.

Studying the perennial species *Eucalyptus pauciflora*, Way et al. (2015) showed that the RL had the highest rates in the summer, when most leaves were expanding (young leaves) or completely expanded (mature leaves), with an average of 6  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ . They observed that light respiration and temperature were positively correlated, starting with lower rates at the beginning of the day, reaching maximum values in the middle of the day, and decreasing in the late afternoon; and varied over the months of the study, corroborating the suggestions of Crous et al. (2012).

Atkin, Scheurwater and Pons (2006), Way et al. (2015), and Crous et al. (2012) found the same trend as in this study, with higher values for RD than RL; and they found that RD is also correlated with temperature, with higher rates under higher temperatures and which varied over the seasons.

Way et al. (2015) and Crous et al. (2012) observed higher rates of RD than rates of RL in the summer, which can be associated both with higher temperatures and with a greater number of expanding or fully expanded (young and mature) leaves.

Heskel and Tang (2018) observed higher dark and light respiration rates at the beginning of the growing season, which may be related to the abundance of young and old leaves. These RD and RL rates dropped significantly in the middle of the growing season (greater number of mature leaves) and increased again at the end of the season (greater number of old leaves). Within each set of measurement, leaves at the top of the canopy showed higher respiration rates than mid-canopy leaves.

Heskel and Tang (2018) found a positive relationship between RL and RD, without variation among the seasons of the study, canopy height, or measurement temperature. That contrasts with what was found in this study, which showed that RL was significantly smaller than RD.

In this study, dark respiration rates were higher than light respiration rates, assuming the occurrence of inhibition of respiration caused by light (the Kok effect). From that, the percentage of this inhibition was estimated for each phase of leaf development, and the Kok effect was identified, but no significant differences were found among the 3 ages. Therefore, it is understood that the inhibition of respiration under light occurs throughout the leaf phenological. These results may conflict with the results of Heskel and Tang. (2018), who found greater inhibition of respiration by light during the period of abundance of mature leaves (mid-growing season).

The rate of suppression of respiration was consistent with the average suppression often reported in the literature – approximately 30% (Crous et al., 2012; Heskell; Tang, 2018). Way et al. (2015) found the average seasonal rates of RL to be approximately 70% of the RD in plants growing at 25 °C. These authors found that the RL/RD ratio was related to leaf temperature increases in some cases and to decreases in others over the months of the growing season. It has been suggested that the decrease in inhibition at high temperatures may be associated with increased photorespiration, which increases the demand for tricarboxylic acid (TCA) cycle intermediates, thus stimulating RL. These results corroborate with Tcherkez (2008).

Atkin, Scheurwater and Pons (2006) studied plants growing under natural [CO<sub>2</sub>] and found that exposure to heat results in an increase in the degree of inhibition of respiration by light in leaves, with lower inhibition occurring in the cold; species of the genus *Plantago* showed higher inhibition of respiration as temperature increased (13, 20 and 27 °C), contrary to the results mentioned above, showing that RL should not be modeled as a constant fraction of RD.

Crous et al. (2012) observed inhibition of respiration by light in response to high concentrations of CO<sub>2</sub> and to water deficit and observed that high CO<sub>2</sub> interferes in inhibition, with a significant difference in the percentage of inhibition among the months studied and, in the H<sub>2</sub>O, and months studied interaction. The plants under water deficit showed greater inhibition (80%) than irrigated plants (50% inhibition) did for all months, except for the month of March, due to predominantly lower temperatures in that month in the study region. It is therefore understood that the summer drought increased the degree of inhibition by light in the leaf; but that may also be associated with leaf age, since in the summer there is a predominance of young and mature leaves in the canopy.

## 5 CONCLUSIONS

This is the first study on the Kok effect in coffee crops which was taken as a model of evergreen plants. The Kok effect has potential for application in agriculture and may be the starting point for creating technology aimed at increasing net primary production in plants.

The respiration estimated in this study were significantly higher in young leaves, however, photosynthesis and quantum efficiency did not differ among young, mature, and old leaves. The degree of light inhibition respiration did not significantly differ among leaf age, but it is significantly lower than dark respiration, showing the occurrence of the kok effect. Nevertheless, accurately predicting light respiration remains a challenge.

Thus, understanding the relationship of light in interaction with leaf age and how these factors affect plant respiration is important in increasing the development of crop

yield models. The results obtained will improve understanding of the growth of coffee plants and their production from gas exchange measurements. Moreover, provides information that can improve the models developed aimed at explaining the effects of climate on the yield of the coffee crops.

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## 7 AUTHORS' CONTRIBUTIONS

IPO wrote the manuscript and performed the experiment; JPP supervised the experiment and coedited the manuscript; NP conducted all statistical analyses; and JPRADB and SRS reviewed and approved the final version of the study.

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