eISSN: 1984-3909
Coffee Science, e161962, 2021

Doi: https://doi.org/10.25186/.v16i.1962

Leaf anatomy, physiology and vegetative growth of fertigated *Coffee arabica* L. trees after exposure to pruning

Daiane dos Santos Soares[1], Elisângela Aparecida da Silva[2], Milene Alves de Figueiredo Carvalho[3], Fernanda Aparecida Castro Pereira[4], Rubens José Guimarães[1]

[1]Universidade Federal de Lavras/UFLA, Departamento de Agricultura/DAG, Lavras, MG, Brasil
[2]Universidade do Estado de Minas Gerais/UEMG, Departamento de Ciências Agrárias e Biológicas/DCAB, Frutal, MG, Brasil
[3]Empresa Brasileira de Pesquisa Agropecuária/Embrapa Café, Brasília, DF, Brasil
[4]Universidade Federal de Lavras/UFLA, Departamento de Biologia/DBI, Lavras, MG, Brasil
Contact authors: soares.agronomia@gmail.com; elisangela.aparecida@uemg.br; milene.carvalho@embrapa.br; fernandacpereira01@gmail.com; rubensjg@ufla.br
Received in September 5, 2021 and approved in January 18, 2022

ABSTRACT

In coffee plants, fertigation can be an alternative way to minimize the negative effects exerted by drought and maximize fertilizer use efficiency. However, the fertilization recommendations for fertigated coffee trees are still not very specific, and the recommendations for rainfed crops are used. In addition, little is known about the nutritional requirements for fertigated coffee trees that have undergone the low *recepa* pruning treatment. Thus, the objective of this study was to evaluate the effects of different levels of nitrogen (N), phosphorus (P), and potassium (K) fertilizers on leaf anatomy, physiology, and vegetative growth of fertigated coffee trees (*Coffea arabica* L.) that have been under the low *recepa* pruning treatment. During the first five years of growth, the cultivar Topázio MG-1190 of the coffee crop received 10, 40, 70, 100, 130, and 160% of the fertilization levels recommended for the rainfed coffee crop. After this period, the crop was exposed to low *recepa* pruning. It was concluded that different doses of N, P, and K fertilizers modified the internal structure of coffee plant leaves, as well as physiological responses and plant growth; there was stronger vegetative growth, sharper leaf blade, greater thickness of spongy parenchyma, larger phloem area, and higher xylem relative hydraulic conductivity as the N, P, and K fertilizer levels in fertigated coffee (*Coffea arabica* L.) plants, which received the low *recepa* pruning treatment, increased. This knowledge can be used as a solid basis for main fertilization recommendations for fertigated coffee trees after exposure to the low *recepa* pruning treatment.

Key words: *Coffea arabica* L.; Fertigation; Mineral nutrition; Pruning.

1 INTRODUCTION

Brazil is the largest producer and exporter of coffee plants, with the total production at 63.08 million bags in 2020. The state of Minas Gerais is the main producer in Brazil, with 34.65 million bags only in the last harvest (Companhia Nacional de Abastecimento - CONAB, 2021). As a result of drought during critical phenological phases and therefore increased water demand, the irrigated coffee area has been significantly increasing in recent years. The irrigated coffee plantation area in Brazil is approximately 449.30 thousand hectares, with about 25% of the area using modern methods (Agência Nacional de Águas e Saneamento Básico - ANA, 2021).

Well-planned irrigation meets the constant need of crops for water, alleviating the problems caused by drought events, and therefore maximizing the efficiency of fertilizer use applied through fertigation (Dominghetti et al., 2014). Fertigation allows the controlled application of nutrients at regular time intervals, thus avoiding problems related to heterogeneous distribution and reducing nutrient loss through leaching (Coelho et al., 2018; Alemayehu; Asfaw; Tirfie, 2020).

The irrigation of coffee crops, as well as the plant biomass densification, favor the "closure" of coffee trees between planting rows, indicating the need for differentiated pruning management strategies (Moreira et al., 2004). Pruning is done when the coffee branches extend, invade, and overlap between the plant rows and also as plants age since the productivity tends to decrease (Carvalho et al., 2006). "Low *recepa*" pruning is the term for pruning the orthotropic branches at 30-40 cm above ground, which is performed in crops that have suffered severe damage to the aerial parts or have lost their potential productivity (Gonçalves, 1970; Miguel; Matiello; Almeida, 1986).

Coffee productivity is affected by several factors, including mineral nutrients, whose imbalances affect the plant metabolism and may also influence physiology and vegetative growth (Zhang et al., 2016) and leaf anatomy of the plant (Gama et al., 2017).

It is necessary to highlight the importance of leaf anatomical structure in coffee trees since it can play the leading role in the translocation of nutrients, thus developing adaptive traits such as changes in the thickness of palisade and spongy parenchyma and adjusting stomatal dimensions (Baliza et al., 2012; Rosolem; Leite, 2007; Nascimento et al., 2006). The changes in somatic characteristics, such as density, as well as polar diameter and functionality (polar and equatorial diameter ratios of stomata), can also occur based on the stomatal frequency in the leaf blade (Cock et al., 1987).

Photosynthesis is the driving force of reactions that take place in plants through a process called metabolism. Stomatal regulation is a key physiological process in coffee tress that is intrinsically linked to photosynthesis and provides information about adaptability, resilience, and growth capacity of plants (Martínez-Vilalta et al., 2014). Stomata allow the plant to quickly adapt to biotic and abiotic stimuli (Craparo et al., 2017) by regulating the gas exchange flow and is one of those important organs responsible for maintaining the balance in the plant.

According to Sobreira et al. (2011), irrigated crops exhibited stronger growth than crops grown under rainfed conditions, with additional nutrient requirements. This greater demand for N, P, and K nutrients was also demonstrated in fertigated crops compared to standard fertilization doses used for rainfed crops (118.33% and 122.61%, respectively) in a study conducted by Pinto et al. (2013) and Villela et al. (2015).

However, most of the fertilizer recommendations made for irrigated crops after the exposure to pruning treatment are similar to those for rainfed crops, which may not meet the plant needs. In this case, the recommendation made by Guimarães et al. (1999) for coffee trees that have undergone *recepa* pruning was adopted, with doses ranging from 20 to 60 g. plant^{-1} of N and 0 to 60 g. plant^{-1} of K. However, adjustments are needed in the case of fertigation.

However, there is still no consensus regarding the adequate fertilizer levels required for fertigated crops that received the *recepa* pruning treatment and also the interference of nutritional imbalances with anatomical structures and morphophysiological profiles of plants. Thus, the objective of the present study was to evaluate the effects of different levels of fertilizers, including nitrogen (N), phosphorus (P), and potassium (K) on leaf anatomy, physiology, and vegetative growth of fertigated coffee trees (*Coffea arabica* L.) that were subjected to low *recepa* pruning.

2 MATERIAL AND METHODS

The experiment was carried out in the Coffee Crop Sector, the Agriculture Department – DAG, of Universidade Federal de Lavras (UFLA), in Lavras - MG, (21°14’06” South latitude and 45°00’00” West latitude, with an average altitude of 910 meters). According to the Koppen climate classification, the climate of the region was characterized as Cwa, mesothermal, with mild summers and winter droughts (Alvares et al., 2013). The soil of the experimental area was classified as Dystroferric Red Latosol (Oxisol), with clay texture (Empresa Brasileira de Pesquisa Agropecuária - EMBRAPA, 2018).

The experiment was set up in March 2010, encompassing the seedlings of the Topázio MG-1190 cultivar of *Coffea arabica* L., planted at a spacing of 2.00 meters between the rows and 0.60 meters between the plants in the row. The fertilization levels used were 10, 40, 70, 100, 130, and 160% of the standard N, P, and K fertilizer rates recommended for rainfed coffee crops in Minas Gerais (Guimarães et al., 1999), based on the soil analysis results (Table 1).

Table 1: Soil chemical characteristics before receiving the treatments.

Depth*	0-20 cm	20-40 cm
pH (H_2O)	5.5	5.2
P- rem - (mg.L^{-1})	23.48	14.87
P - (mg.dm^{-3})	76.08	10.43
K - (mg.dm^{-3})	108	58
Ca - (cmolc.dm^{-3})	3.77	2.12
Mg - (cmolc.dm^{-3})	0.88	0.35
Al - (cmolc.dm^{-3})	0.20	0.40
H + Al - (cmolc.dm^{-3})	4.70	6.44
T - (cmolc.dm^{-3})	9.62	9.06
Mg - T%	9.12	3.89
Ca - T%	39.18	23.36
K - T%	2.87	1.64
V - (%)	51.2	28.9
m - (%)	2.23	7.76
M.O (dag.kg^{-1})	3.84	3.28

* P-Rem = the remnant phosphorus; (T) = cation exchange capacity at pH 7,0; V = base saturation index; m = aluminum saturation index; M.O. = soil organic matter.

After the harvest in August 2015, coffee trees were exposed to the low *recepa* pruning treatment, at 30 cm above the ground. The experiment was carried out in a randomized block design, with an experimental plot consisting of four blocks and six plants.

The application of nitrogen and potassium fertilizers was made through fertigation carried out in twelve equal parts, as suggested by Sobreira et al. (2011), while in the case of phosphorus, 50% of fertilizer doses were distributed in planting furrows, and the other 50% was applied through fertigation. Nutrient sources were urea (45% of N), purified mono ammonium phosphate (60% of P_2O_5 + 11% of N), and potassium nitrate (12% of N + 43% of K_2O). Micronutrients were only applied as foliar sprays as recommended by Guimarães et al. (1999) and without dose variations.

The fertigation system consisted of a central control unit encompassing a pumping system, screen and sand filter, fertilizer injector, pressure gauges, and connections, mainline of PN80 PVC pipes, PN40 PVC bypass lines, flexible polyethylene PN40 sidelines, drippers, and valves. The drippers with the 3,8 L hora^{-1} flow rate had the spacing of 30 cm in the plant row. Irrigation control was established by daily observation of climatological data, monitored by the UFLA Meteorological Station.

Evaluations of physiological characteristics like gas exchanges were carried out during four periods of March, July, and October 2016 and January 2017). However, anatomical and plant growth evaluations were made during two periods (March and October 2016). Climatic conditions such as atmospheric temperature (ºC), relative humidity (%), and precipitation (mm) from January 2016 to January 2017 are shown in Figure 1.

For the evaluation of gas exchange, the leaves fully expanded from the third node of plagiotropic branches from the middle third of the coffee trees were selected. An infrared gas analyzer (IRGA LICOR – 6400XT) was used to quantify photosynthesis (*A*-μmol CO_2 m^{-2} s^{-1}), stomatal conductance (gs - mol H_2O m^{-2} s^{-1}), and transpiration rate (*E* - mmol H_2O m^{-2} s^{-1}). Measures were taken in the morning between 9-11 a.m. with artificial lighting of 1000 μmol m^{-2} s^{-1}). The instantaneous water use efficiency (WUE) (μmol CO_2 $mmol^{-1}$ H_2O) was calculated using the *A*/ E ratio (Larcher, 2000).

For anatomical characterization, healthy and fully expanded leaves were collected from the third node of plagiotropic branches from the middle third of coffee trees and then fixed in a 70% alcohol solution (Johansen, 1940). Paradermal and transverse leaf sections were cut. The transverse sections of leaves were obtained using an LPC-type table microtome. All sections were clarified with a 50% sodium hypochlorite solution and washed three times in distilled water. The sections were stained with a safranin solution (1%) and Astra blue (0.1%) in a 7: 3 ratio (Kraus; Arduin, 1997) and then mounted on semi-permanent slides with 50% glycerol (v. v^{-1}) (Kraus; Arduin, 1997). Paradermal sections were obtained from the epidermis of the abaxial surface in the middle region of the leaf using a steel blade. Subsequently, the sections were clarified with a NaClO solution (50%), washed in distilled water, and stained with 1% safranin (Kraus; Arduin, 1997). The slides were mounted with 50% glycerin, observed, and then photographed under the optical microscope. Olympus BX-60 model, coupled to a Canon A630 digital camera, was employed to capture the images, evaluating three fields per slide.

The images were analyzed using the UTHSCSA-Imagetool software at the University of Texas Health Science Center at San Antonio, Texas, to develop the characteristics of the variables, including thickness of palisade parenchyma (PPA - μm), thickness of spongy parenchyma (PES - μm), leaf blade thickness (LIM - μm), phloem area (AF - $μm^2$), xylem area (AX - $μm^2$), xylem vessel frequency (FVX - vessels number/ mm^2), and relative hydraulic conductivity (CHR, according to Zimmermann, 1983). In paradermal sections, characteristics such as stomatal density (DEN) (the number of stomata per mm^2), the polar diameter of stomata (PD), equatorial diameter (ED) of stomata, and the ratio of stomata polar diameter/ equatorial diameter (PD/ ED) (highly correlated with stomatal function), were analyzed (Souza et al., 2010).

For plant growth analysis, the number of primary plagiotropic branches (counted from the orthotropic branch), the length of primary plagiotropic branches, plant height (cm), and stem diameter (mm) were examined.

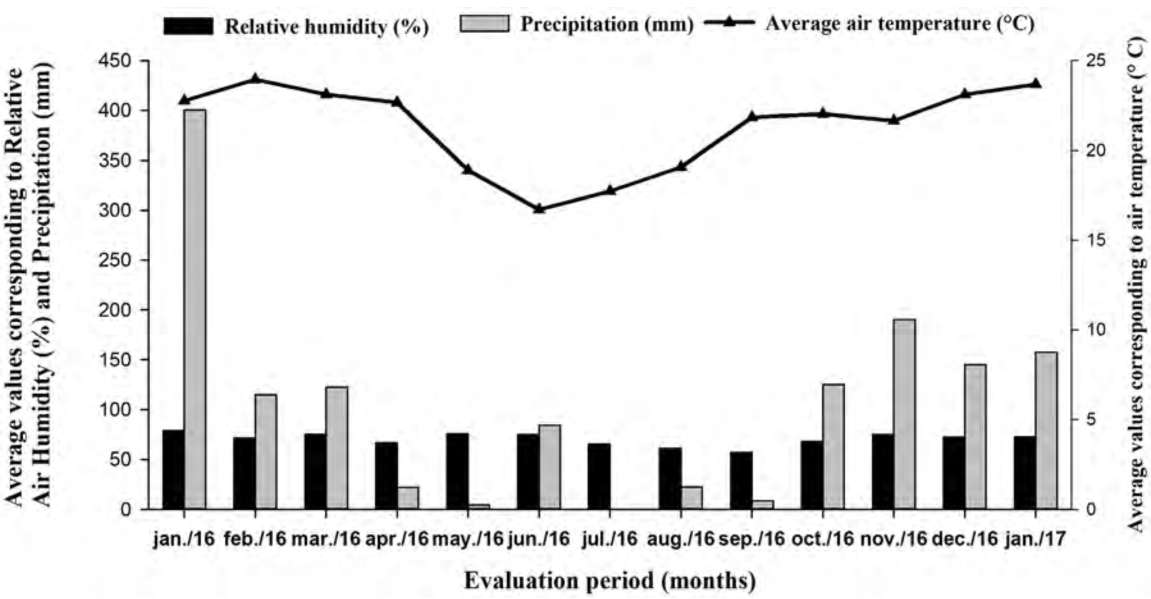

Figure 1: Mean values of atmospheric temperature (ºC), relative humidity (%), and precipitation (mm) from January 2016 to January 2017.

The data obtained were used to test the normality (Shapiro-Wilk) and homoscedasticity (Bartellet) (Snedecor; Cochran, 1989). Then, they were subjected to analysis of variance (ANOVA), and when significant differences were observed, the regression analysis was performed for variable doses. All statistical analyses were performed using the R software (R Core Team, 2020).

3 RESULTS

With regard to the anatomical variables, significant interactions ($p < 0.05$) were observed between the evaluated periods and N, P, and K levels for leaf blade, the thickness of palisade, and spongy parenchyma, phloem area, xylem vessels frequency, and relative hydraulic conductivity. Stomatal function (PD/ ED – the ratio of the polar and equatorial diameter of stomata) exhibited a significant interaction only between evaluations of March and N, P, and K doses, and a significant difference was observed for the xylem area only between fertilization levels, regardless of the periods evaluated.

The PD/ DE ratio was influenced by the fertilization levels in the evaluation carried out in March 2016, using the data set in the cubic regression model (Figure 2a), with the maximum value at the 100% fertilization level and the minimum value at the 70% fertilization level.

The data on leaf blade thickness (LIM) followed a linear trend in March and a quadratic trend in October (Figure 2b). In March, the maximum and minimum points (345.36 and 303.43 µm) were reached at the 160% and 10% fertilization levels, respectively, whereas, in October, the minimum point of 281.43 µm was observed at the 95% fertilization level. Except for the data obtained for 10 and 40% of standard fertilization rates, the values obtained for other fertilization levels were higher in March.

The data on the thickness of palisade parenchyma (PPA) indicated a quadratic trend for both the evaluated periods (Figure 2c). In March, the maximum value of 71.90 µm was recorded at the 78.87% fertilization level, while in October, the minimum value was found at the 126.10% fertilization level (49.95 µm). Except for the data obtained for 10 and 160% of standard fertilization levels, other values were found to be higher in March.

For the evaluation of the thickness of spongy parenchyma (PES), the linear model in March and the quadratic model in October were those that best represented the data (results) (Figure 2d). In March, the maximum value (230.32 µm) was obtained at the 160% fertilization level, while the minimum point (206.32 µm) was observed when the 10% fertilization level was applied. However, in October, the application of the 50.38% fertilization level resulted in the lowest value of 189.09 µm. The values obtained were higher in all March evaluations.

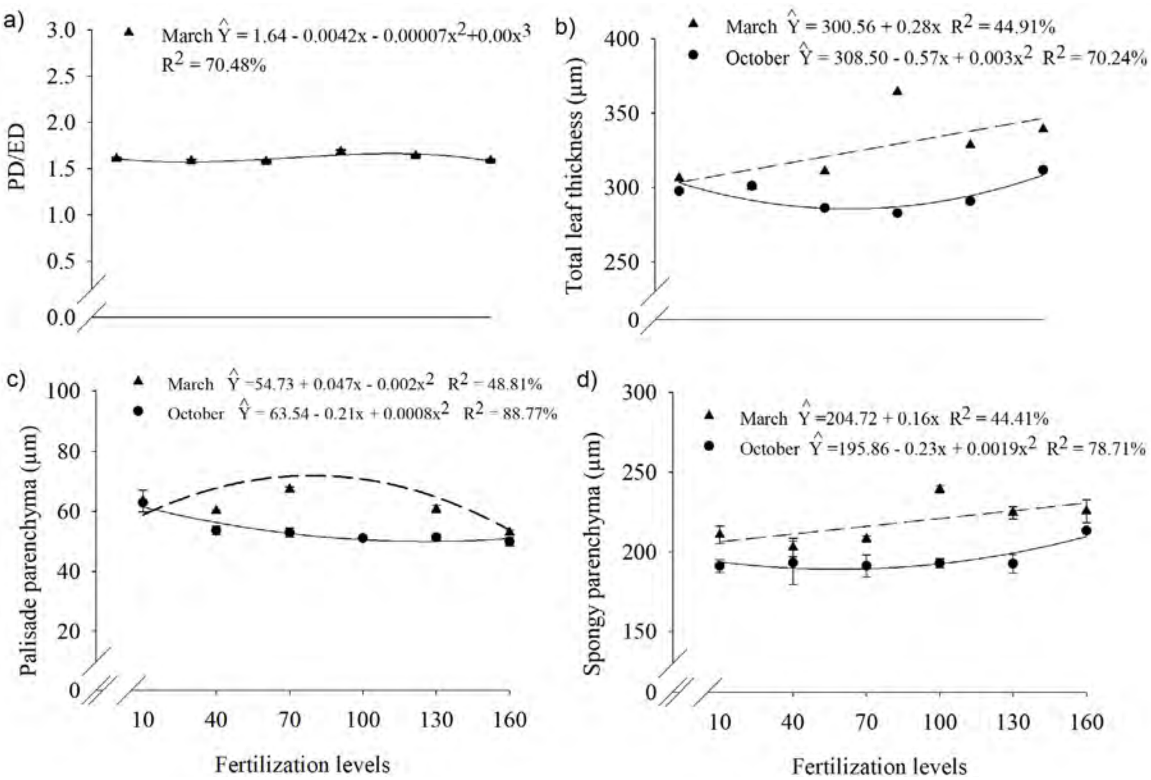

Figure 2: a) stomatal function (the PD/ ED ratio), b) total leaf thickness (LIM; µm), c) palisade thickness (PPA; µm), and d) spongy thickness (PES; µm) of irrigated coffee leaves under different regimes of NPK fertilization during two evaluated periods including Period 1: March 2016 and Period 2: October 2016.

The data on the phloem area (AF) presented a linear trend for both the evaluation periods (Figure 3a). In March, the maximum value (99056.65 μm^2) was obtained at the 160% fertilization level, while the minimum value of 66898.15 μm^2 was recorded at the 10% fertilization level. Similarly, in October, the highest and lowest values of phloem area were found at the 160% and 10% fertilization levels (87825.84 μm^2 and 65794.54 μm^2, respectively). The values obtained in March were higher in all treatments, and the difference between them increased when the higher fertilizer doses were applied to coffee trees.

For xylem vessel frequency (FVX), the quadratic behavior in the data of March was observed, while the data of October indicated a cubic behavior (Figure 3b). In March, the highest xylem vessel frequency (1749.42 vessels/ mm^2) was obtained at the 99.01% fertilization level. However, in October, the maximum value was obtained at the 47.64% fertilization level, while the minimum value was observed at the 125.84% rate. The values obtained were higher in all October evaluations.

For the xylem relative hydraulic conductivity (CHR), a variable related to the vessel caliber, the models that best fitted were linear in March and cubic in October (Figura 3c). In March, the highest value was at the 10% fertilization level (11.14 μm^4 10^6), whereas, in October, the maximum and minimum values were recorded at 128.07% and 52.60% fertilization levels, respectively.

The xylem area (AX) was influenced only by the fertilization levels, with the data showing a quadratic trend (Figure 3d). The largest area of the xylem (114586.70 μm^2) was obtained when N, P, and K were applied at the 122.06% level.

In relation to plant growth, a significant interaction ($P < 0.05$) was observed between the periods and N, P, and K fertilization levels for all the traits evaluated.

For plant height, the linear model best represented the results in these two evaluated periods (Figure 4a). In March, the maximum growth rate (78.04 cm) was obtained at the 160% fertilization level, while the minimum value of 61.53 cm was observed at the 10% fertilization level. Likewise, in October, the 160% fertilization level had the highest growth (110.78 cm), whereas the minimum value of 88.26 cm was recorded at the 10% fertilization level. As expected, the values obtained for the plant height were higher in all October evaluations, regardless of the fertilizer dose applied.

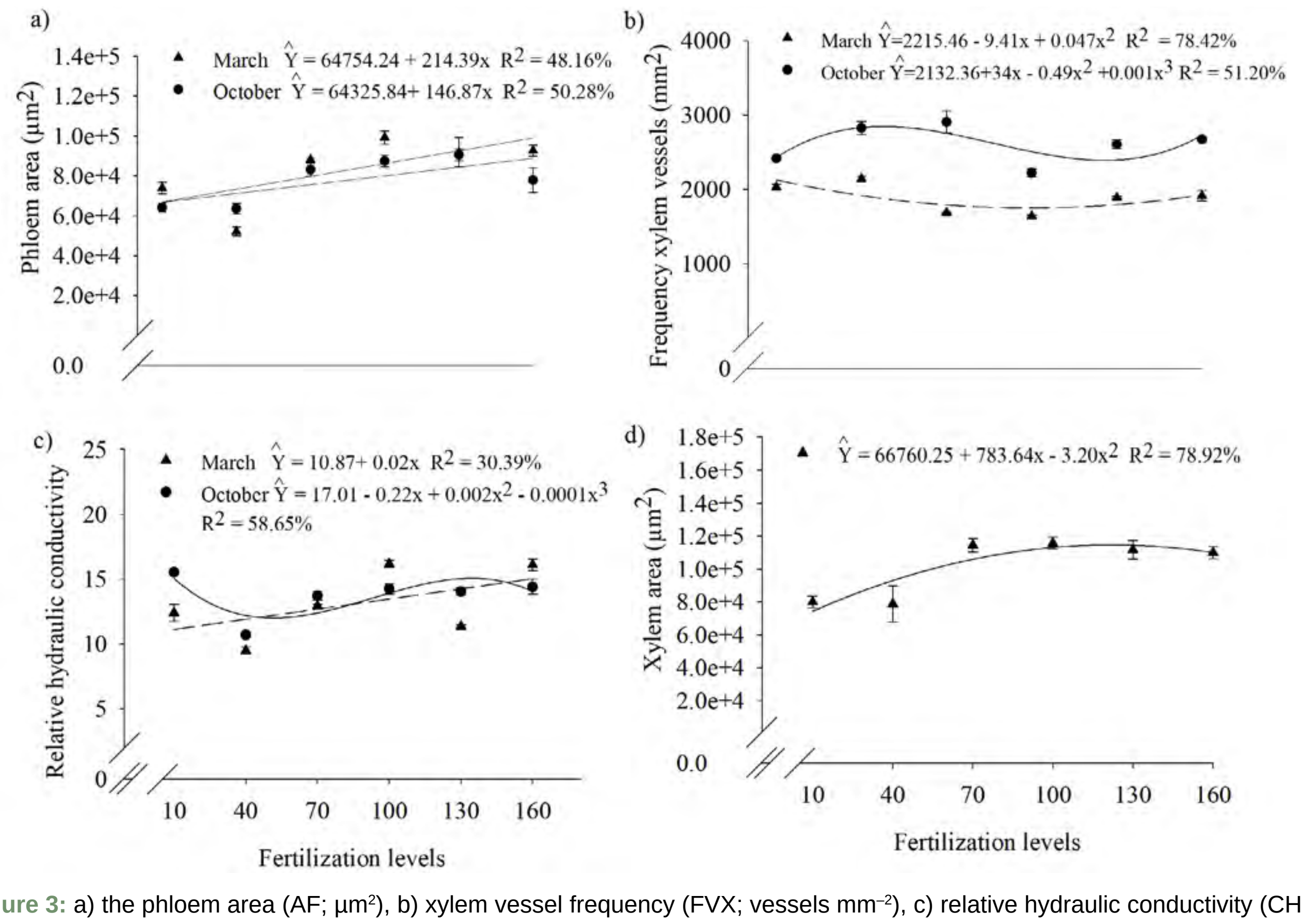

Figure 3: a) the phloem area (AF; μm^2), b) xylem vessel frequency (FVX; vessels mm^{-2}), c) relative hydraulic conductivity (CHR; μm^4 10^6), and d) xylem area (AX; μm^2) of irrigated coffee leaves under different levels of NPK fertilizers during two evaluated periods including Period 1: March 2016 and Period 2: October 2016.

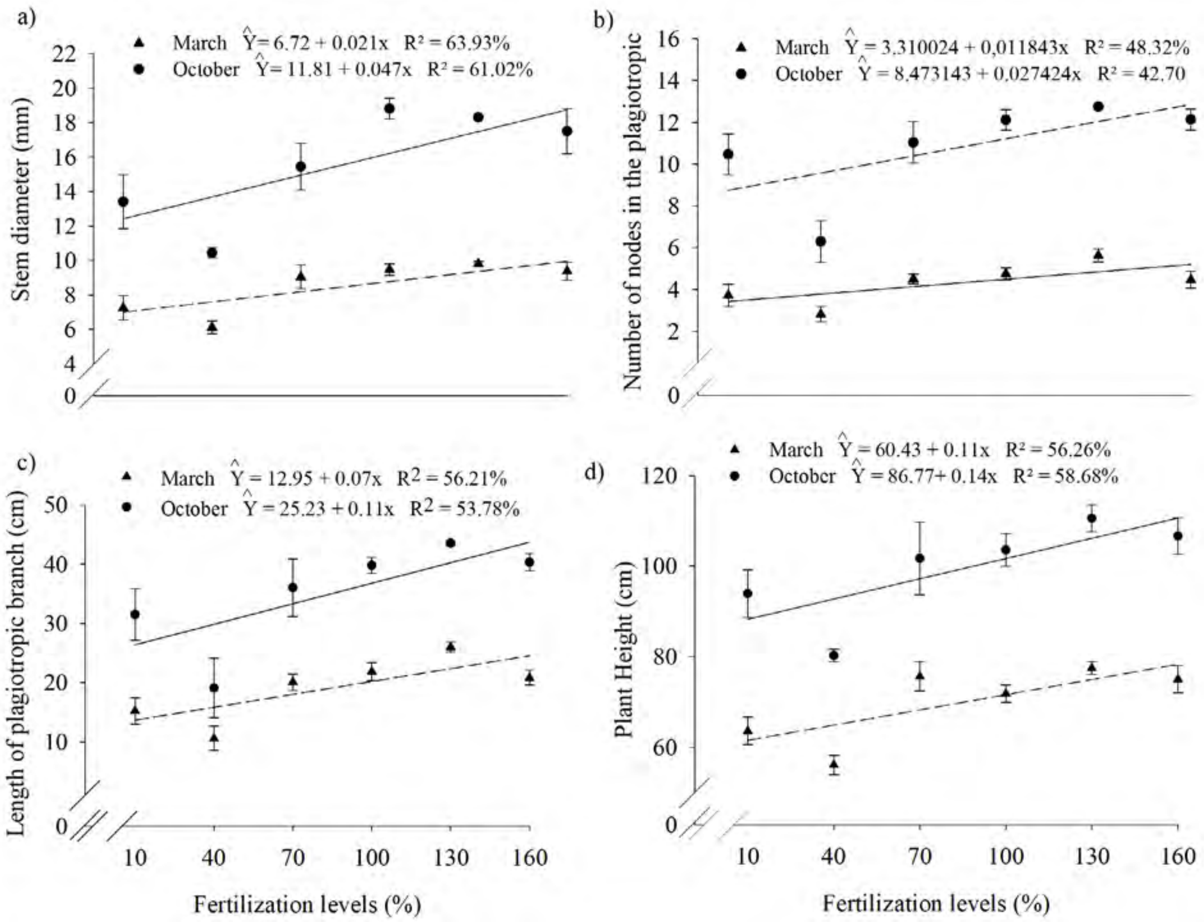

Figure 4: a) plant height (cm), b) stem diameter (mm), c) length of plagiotropic branches (cm), and d) the number of plagiotropic branches of irrigated coffee leaves under different levels of NPK fertilizers in two periods evaluated periods including Period 1: March 2016 and Period 2: October 2016.

For stem diameter, the linear behavior of the data for the evaluated periods was observed (Figure 4b). The highest growth in March (9.92 mm) was found at the 160% fertilization level, while the minimum value of 6.93 mm was obtained when the 10% fertilization level was applied. Moreover, in October, the application of the 160% fertilization level resulted in the maximum value of 19.35 mm and the minimum value of 12.28 mm at the 10% fertilization level. As expected, the values obtained for the stem diameter were higher in all October evaluations, regardless of the fertilization dose applied.

For the data obtained for plagiotropic branches, a statistical significance was observed, and the linear model was the one that better explained the plant growth in both periods (Figure 4c). In March, the plant had the highest and lowest growth rates of 24.16 and 13.68 cm when 160% and 10% fertilization levels were applied, respectively. In October, also, the maximum and minimum growth rates were observed at the 160% and 10% fertilization levels (44.44 cm and 26.39 cm, respectively). As expected, the length of plagiotropic branches was higher in all treatments of October, regardless of the fertilization dose applied.

The linear regression model best represented the data for the number of plagiotropic branches in both the evaluated periods (Figure 4d). The largest number of branches (7.43) was recorded at the 160% fertilization level, while the 10% fertilization level had a 5.98 number of branches. In October, similarly, the branches had the maximum number at the 160% fertilization level (16.09 branches), whereas, when the 10% fertilization level was used, the minimum number of branches was found (11.58). The number of plagiotropic branches, as expected, was higher in all evaluations of October, regardless of the applied fertilization dose.

With regard to physiological traits, significant interactions ($P < 0.05$) were observed between evaluated periods and fertilization levels for transpiration (*E*; mmol H_2O m^{-2} s^{-1}), stomatic conductance (gs; mol H_2O m^{-2} s^{-1}), and water use efficiency (*WUE*; µmol CO_2 $mmol^{-1}$ H_2O). Photosynthesis (*A*; µmol CO_2 m^{-2} s^{-1}) exhibited a significant difference only between fertilization levels, regardless of the evaluated period.

There was an interaction between fertilization levels and the evaluated periods for transpiration, with the observed quadratic behavior in the data of March 2016, but linear in July 2016 and cubic in January 2017. In October 2016, the interaction was not significant between the evaluated factors (Figure 5a). In March 2016, the lowest transpiration value (1.60 mmol H_2O m^{-2} s^{-1}) was at the 79.34% fertilization level, while, in July 2016, the minimum value of 0.65 mmol H_2O m^{-2} s^{-1} was observed at the 160% fertilization level. Furthermore, in January 2017, the minimum value based on the cubic regression model was detected at the 112.06% fertilization level (50.60%).

The quadratic regression model could provide the best fit to the stomatal conductance data in March and July 2016. In January 2017, the linear model could best explain the obtained data. With regard to the transpiration data in October, there were no significant interactions between factors (Figure 5b). In March 2016, the minimum stomatal conductance value (0.05 mol H_2O m^{-2} s^{-1}) was observed at the 97.54% fertilization level. In July 2016, however, the lowest stomatal conductance of 0.09 mol H_2O m^{-2} s^{-1} was found at the 106.58% fertilization level. The maximum value in January 2017 was obtained when the 10% fertilization level was applied (0.13 mol H_2O m^{-2} s^{-1}), while the minimum value was at the 160% fertilization level (0.08 mol H_2O m^{-2} s^{-1}).

For photosynthesis, data showed the statistical significance only between N, P, and K fertilization levels, regardless of the evaluated periods. A quadratic regression model was the one that best fitted the obtained data, with the minimum value of 8.37 µmol CO_2 m^{-2} s^{-1} at the 93.30% standard fertilization level (Figure 5c).

For water use efficiency, the quadratic regression model best explained the obtained data in March and July 2016. In October 2016 and January 2017, there was no significant interaction between the factors. (Figure 5d). In March 2016, the highest *QUE* value of 5.43 µmol CO_2 $mmol^{-1}$ H_2O was obtained with the application of the 93.26% standard fertilization level. In July 2016, however, when the 47.04% fertilization level was used, the lowest *WUE* value (4.46 µmol CO_2 $mmol^{-1}$ H_2O) was recorded.

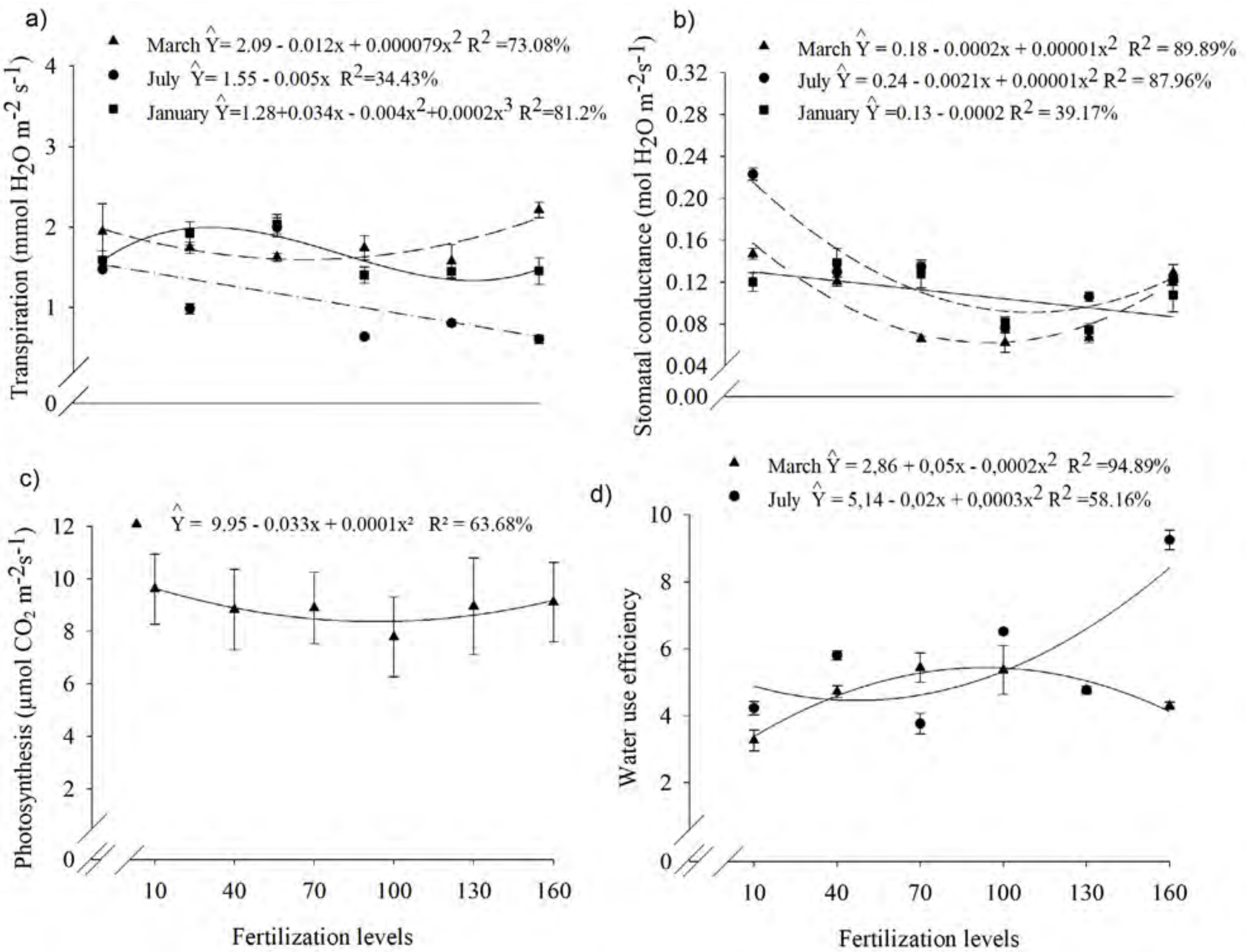

Figure 5: a) transpiration (mmol H_2O m^{-2} s^{-1}), b) stomatal conductance (mol H_2O m^{-2} s^{-1}), c) photosynthesis (µmol CO_2 m^{-2} s^{-1}), and d) water use efficiency (µmol CO_2 $mmol^{-1}$ H_2O) of the growth of irrigated coffee plants under different levels of NPK fertilizers in four evaluated periods including Period 1: March 2016, Period 2: July 2016, and Period 4: January 2017.

4 DISCUSSION

In March 2016, better stomatal function at the 100% standard fertilization level, the recommended level for rainfed coffee crops in Minas Gerais State, was observed (Guimarães et al., 1999). Considering the average climatic conditions (temperature and air relative humidity), as well as the monthly accumulated precipitation, for both anatomical evaluations (Figure 1), it is noted that for the time of leaf collection, the differences between these environmental conditions were slight. However, when exploring the trimester that preceded the leaf collection, in both periods, different climatic conditions and characteristics of rainy and dry periods were observed. These conditions may have influenced the stomatal dimensions (polar and equatorial diameters) and consequently the stomatal function. In addition to stress conditions, the environment and plant genotype can cause variations in stomatal behavior and dimensions (Oliveira; Miglioranza, 2014). According to Pillitteri and Dong (2013), environmental conditions can modify the density and size of stomata in response to different types of stresses.

In March 2016, variables such as leaf blade, the thickness of spongy parenchyma, phloem area, and xylem relative hydraulic conductivity followed the same pattern as the linear regression model in response to the stress. The maximum values were observed at the 160% fertilization level, indicating the increase in the values of these anatomical traits with the increase in N, P, and K levels. In soils with low or high fertility, there may be changes in conductive vessels with the great ability of the plant to transform raw sap into elaborated sap (Queiroz-Voltan et al., 2014).

Sridhar et al. (2007) observed the influence of the accumulation of nutrients like zinc and cadmium on the leaf thickness of barley, showing that nutritional imbalances can associate with the anatomical parameters of plant tissues. Increased thickness of leaf blade is considered as the mechanism of plant adaptation to high temperature and radiation (Baliza et al., 2012; Ribeiro et al., 2012). However, in the present study, the increased thickness of the leaf blade was due to the N, P, and K standard fertilization levels recommended by Guimarães et al. (1999). These characteristics favor the accumulation and storage of CO_2, which are necessary to carry out photosynthesis (Castanheira et al., 2016; Terashima et al., 2011).

Xylem relative hydraulic conductivity had lower values at N, P, and K fertilization levels below the standard recommendation levels in both the evaluated periods (10% level in March and 52.60% level in October 2016). Nutritional imbalances can affect the internal structure of plants, and thus changes in the differentiation of vessels may occur (Dickison, 2000). These variables are related to water transport in leaves, and the size of vessels affects xylem hydraulic conductivity. The thinner xylem vessel under unfavorable environmental conditions (nutritional deficiency or excess) can allow more efficient and safe water transport, improving hydraulic conductivity (Batista et al., 2010). Thus, the lowest CHR values obtained in this study at the 10% and 52.60% fertilization levels in March and October 2016, respectively, may have increased the efficiency of water transport in xylem vessels.

The increased palisade parenchyma thickness in March 2016 was observed at the 78.87% fertilization level. Gama et al. (2017), who studied the leaf anatomy of coffee plants at different fertilization levels, found an increase in the palisade parenchyma thickness when 70 to 130% of N, P, and K standard fertilization rates, recommended for rainfed coffee plants, were applied (Guimarães et al., 1999). The increase in palisade parenchyma thickness is related to the significant increase in the number of mesophyll cells per leaf, which can influence the physiological processes in plants (Voltan; Fahl; Carelli, 1992).

Most of the anatomical variables evaluated had higher values at the fertilization levels above the standard levels recommended for rainfed coffee plants (Guimarães et al., 1999). This indicates investment by plants in growing leaf structures in response to increased levels of fertilization, which may contribute to nutrient translocation in the coffee trees.

In the present study, the growth of fertigated coffee trees that had undergone pruning followed a linear trend in terms of plant height, stem diameter, and the number of plagiotropic branches, with maximum growth rates at the 160% N, P, and K fertilization levels for both the evaluated periods, indicating the demand of fertigated coffee crops for higher fertilization levels after the low *recepa* pruning treatment. Assis et al. (2015), who explored the critical ranges of nitrogen and potassium for crops in response to fertigated management, reported maximum growth rates with the application of fertilization levels of 194.81, 191.61, 185.04, 185.43, 192.28, and 189.59% of the reference fertilization levels proposed by Guimarães et al. (1999). Irrigated coffee crops had growth patterns different from those of non-irrigated crops (Sakai et al., 2015; Arantes; Faria; Resende, 2009; Sobreira et al., 2011), which caused changes in the nutritional requirements of plants. Rezende et al. (2010), who evaluated different standard NPK fertilization recommendation doses for the fertigated coffee crop during its growth and development, proposed by Matiello et al. (2020) under rainfed conditions, observed an initial increased plant height, reaching a maximum value of 109.4 cm at the 133.4% recommended NPK fertilization level. Pinto et al. (2013) and Villela et al. (2015), who studied fertigated coffee crops, found higher productivity when 118.33 and 122.61% of recommended NPK fertilization doses were applied to coffee trees, respectively. Costa et al. (2010) and Santinato and Fernandes (2012) stated that the nutritional requirements of irrigated coffee crops were 1.5 to 2.5 times more compared to those of non-irrigated crops during different stages of production.

Water availability increased the vegetative growth of coffee trees and probably also increased their nutritional requirements, and the maximum growth rates observed in the present study were obtained when the higher doses than the reference fertilization doses proposed by Guimarães et al. (1999) were applied. Arantes et al. (2006) analyzed the vegetative growth of pruned coffee trees at different water table depths, including 0, 40, 80, and 120% of the positive balance between evaporation and precipitation in the tank class A, and found that the 120% water depth provided 23% and 15% increase in plant height and canopy diameter, respectively, in relation to rainfed treatments. When evaluating the vegetative growth of coffee branches under different fertilization management regimes, Dubberstein et al. (2017) observed higher growth rates of the plagiotropic and orthotropic branches.

Stomatal regulation is a process intrinsically linked to photosynthesis, which allows the quick adaptation of the plant to varying biotic and abiotic stimuli (Craparo et al., 2017). The reduction in stomatal conductance is a mechanism for reducing transpiration (Shimazaki et al., 2007), which consequently reduces the influx of CO_2 into chloroplasts (Tatagiba; Pezzopane; Reis, 2015), causing reductions in photosynthetic rates. In the present study, in March 2016, the first evaluation after the low *recepa* pruning treatment, lower values of stomatal conductance, as well as lower transpiration rates were observed at the fertilization levels close to 100%. At the same fertilization level, regardless of the evaluated period, lower photosynthetic rates were also observed. Gama et al. (2017), who evaluated the same fertilization levels in fertigated coffee plants, did not observe any difference in gas exchange.

Different fertilization levels influenced the water use efficiency, with the behavior observed in March 2016 opposite to that in July 2016, confirmed by the quadratic regression model for transpiration in March 2016. In addition, in the case of photosynthesis, different behavior from that observed in July 2016, in which the increase in fertilization levels led to an increase in the rate of transpiration, was found. Among all the evaluated periods, the highest water use efficiency (9.24 µmol CO_2 $mmol^{-1}$ H_2O) was observed in July 2016 at 160% N, P, and K fertilization levels. The maintenance of photosynthetic rates in relation to lower *E* values is reflected in higher *WUE*.

This knowledge can be used as the solid basis for fertilization recommendations made for fertigated coffee crops that have undergone the low *recepa* pruning treatment.

5 CONCLUSION

The greater vegetative growth and spongy parenchyma thickness, as well as larger leaf blade portion and phloem area, and higher xylem relative hydraulic conductivity, were observed as the N, P, and K fertilization levels increased in fertigated coffee (*Coffea arabica* L.) plants that had received the low *recepa* pruning treatment.

6 AUTHORS' CONTRIBUTIONS

DSS wrote the manuscript and realized data analysis and interpretation, EAS realized data collection and analysis of plant material in the field and laboratory analysis and co-work the manuscript, MAFC realized data collection and analysis of plant material and co-work the manuscript, FACP realized the data analysis and statistical analysis, and RJG supervised the experiment and co-work the manuscript.

7 ACKNOWLEDGEMENTS

The authors would like to thank the Universidade Federal de Lavras and the Programa de Pós-Graduação em Agronomia/ Fitotecnia, and the Coordenação de Pessoal de Nível Superior (CAPES) for awarded scholarships. We also would like to express our gratitude to the Agência de Inovação do Café (InovaCafé) and the CNPq and the Consórcio Pesquisa Café for financial support.

8 REFERENCES

AGÊNCIA NACIONAL DE ÁGUAS E SANEAMENTO BÁSICO - ANA. **Atlas irrigação:** Uso da água na agricultura irrigada. 2 ed. Brasília, ANA, 2021, 130p.

ALEMAYEHU, Y. A.; ASFAW, S. L.; TIRFIE, T. A. Management options for coffee processing wastewater. A review. **Journal of Material Cycles and Waste Management**, 22(2):454-469, 2020.

ALVARES, C. A. et al. Köppen's climate classification map for Brazil. **Meteorologische Zeitschrift**, 22(6):711-728, 2013.

ARANTES, K. R. et al. Desenvolvimento do cafeeiro (*Coffea arabica* L.) podado sob irrigação. **Revista de Ciências Agro-Ambientais**, 4(1):75-86, 2006.

ARANTES, K. R.; FARIA, M. A.; REZENDE, F. C. Recuperação do cafeeiro (*Coffea arabica* L.) após recepa, submetido a diferentes lâminas de água e parcelamentos de adubação. **Acta Scientiarum Agronomy**, 31(2):313-319, 2009.

ASSIS, G. A. de et al. Critical ranges for leaf nitrogen and potassium levels in coffee fertigated at the production phase. **Revista Ciência Agronômica**, 46(1):126-134, 2015.

BALIZA, D. P. et al. Trocas gasosas e características estruturais adaptativas de cafeeiros cultivados em diferentes níveis de radiação. **Coffee Science**, 7(3):250-258, 2012.

BATISTA, L. A. et al. Anatomia foliar e potencial hídrico na tolerância de cultivares de café ao estresse hídrico. **Ciência Agronômica**, 41(3):475-481, 2010.

CARVALHO, G. R. et al. Avaliação de produtividade de progênies de cafeeiro em dois sistemas de plantio. **Ciência e Agrotecnologia**, 30(5):838-843, 2006.

CASTANHEIRA, D. T. et al. Potencial de utilização de características anatômicas e fisiológicas na seleção de progênies de cafeeiro. **Coffee Science**, 11(3):375-386, 2016.

COCK, J. H et al. C3-C4 intermediate photosynthetic characteristics of cassava (Manihot esculenta Crantz) - II. Initial products of 14 CO_2 fixation. **Photosynthesis Research**, 12:237-241, 1987.

COELHO, V. P. M. de. et al. Fertigation and growth regulator on coffee seedling production in tubes. **Pesquisa Agropecuária Tropical**, 48(4):350-357, 2018.

COMPANHIA NACIONAL DE ABASTECIMENTO - CONAB. **Acompanhamento de safra brasileira, café**: Primeiro levantamento, janeiro/ 2021. Available in: https://www.conab.gov.br/info-agro/safras/cafe. Access in: February,18,2021.

COSTA, A. R. et al. Número de ramos plagiotrópicos e produtividade de duas cultivares de cafeeiro utilizando irrigação por gotejamento. **Revista Ciência Agronômica**, 41(4):571-581, 2010.

CRAPARO, A. C. W. et al. Application of thermography for monitoring stomatal conductance of Coffea arabica under different shading systems. **Science of The Total Environment**, 609:755-763, 2017.

DICKISON, W. C. **Intgrative plant anatomy**. San Diego. Academic Press. 2000. 533p.

DOMINGHETTI, A. W. et al. Doses de fósforo e irrigação na nutrição foliar do cafeeiro. **Revista Brasileira de Engenharia Agrícola e Ambiental**, 18(12):1235-1240, 2014.

DUBBERSTEIN, D. et al. Influência da adubação no crescimento vegetativo de cafeeiros. **Coffee Science**, 12(2):197-206, 2017.

EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA - EMBRAPA. **Sistema brasileiro de classificação de solos**. 5 ed. Brasília: Embrapa, 2018, 356p.

GAMA, T. C. P. D. et al. Anatomia foliar, fisiologia e produtividade de cafeeiros em diferentes níveis de adubação. **Coffee Science**, 12(1):42-48, 2017.

GONÇALVES, J. C. **Fechamento e poda dos cafezais**. Campinas: CATE, 1970. 30p.

GUIMARÃES, P. T. G. et al. Cafeeiro. In: RIBEIRO, A. C.; GUIMARÃES, P. T. G.; ALVAREZ-VENEGAS, V. H. (Ed.). **Recomendações para o uso de corretivos e fertilizantes em Minas Gerais**: 5a aproximação. Viçosa, MG: CFSEMG, p. 289-302, 1999.

JOHANSEN, D. A. **Plant microtechnique.** 2a ed. New York: McGraw Hill, 1940. 523p.

KRAUS, J. E.; ARDUIN, M. **Manual básico de métodos em morfologia vegetal**. Rio de Janeiro: EDUR, 1997. 319p.

LARCHER, W. **Ecofisiologia vegetal**. RiMa Editora, São Carlos. 2000. 531p.

MARTÍNEZ-VILALTA, J. et al. A new look at water transport regulation in plants. **New phytologist**, 204(1):105-115, 2014.

MATIELLO, J. B. et al. **Cultura de café no Brasil**: Manual de recomendações. São Paulo: MAPA/PROCAFE, 2020. 716p.

MIGUEL, A. E.; MATIELLO, J. B.; ALMEIDA, S. R. Espaçamento e condução do cafeeiro. IN: ASSOCIAÇÃO BRASILEIRA PARA A PESQUISA POTASSA E DO FOSFATO. **Cultura do cafeeiro**: Fatores que afetam a produtividade. Piracicaba, p.303-322, 1986.

MOREIRA, R. C. et al. Espaçamentos para cafeeiro (*Coffea arabica* L.) com e sem o emprego de irrigação. **Acta Scientiarum. Agronomy**, 26(1):73-78, 2004.

NASCIMENTO, E. A. et al. Alterações morfofisiológicas em folhas de cafeeiro (*Coffea arabica L.*) consorciado com seringueira (*Hevea brasiliensis* Muell. Arg.). **Ciência Rural**, 36(3):852-857, 2006.

OLIVEIRA, E. C.; MIGLIORANZA, E. Density and stomata distribution in cassava Manihot esculenta Crantz IAC 576-70. **Scientia Agropecuaria**, 5(3):135-140, 2014.

PILLITTERI, L. J.; DONG, J. Stomatal development in arabidopsis. The Arabidopsis Book. American Society of Plant Biologists, 11: e0162: 1-26, 2013.

PINTO, C. G. et al. Faixas críticas de teores foliares de nitrogênio, fósforo e potássio para o cafeeiro (*Coffea arabica* L.) fertirrigado para o primeiro ano pós-plantio. **Coffee Science**, 8 (4):530-538, 2013.

QUEIROZ-VOLTAN, R. B. et al. Caracterização da anatomia foliar de cafeeiros arábica em diferentes períodos sazonais. **Revista Biotemas**, 27(4):1-10, 2014.

R CORE TEAM. **R:** A language and environment for statistical computing. **R** Foundation for Statistical Computing. 2020. Vienna, Austria. Available in: <http://www.R-project.org>. Access in: January 25, 2022.

REZENDE, R. et al. Crescimento inicial de duas cultivares de cafeeiro em diferentes regimes hídricos e dosagens de fertirrigação. **Engenharia Agrícola**, 30(3):447-458, 2010.

RIBEIRO, M. N. O. de. et al. Anatomia foliar de mandioca em função do potencial para tolerância à diferentes condições ambientais. **Revista Ciência Agronômica**, 43(2):354-361, 2012.

ROSOLEM, C. A.; LEITE, V. M. Coffee leaf and stem anatomy under boron deficiency. **Revista Brasileira de Ciência do Solo,** 31(3):477-483, 2007.

SAKAI, E. et al. Coffee productivity and root systems in cultivation schemes with different population arrangements and with and without drip irrigation. **Agricultural water management**, 148:16-23, 2015.

SANTINATO, R.; FERNANDES, A. L. T. **Cultivo do cafeeiro irrigado por gotejamento**. Uberaba - MG. 2012. 388p.

SHIMAZAKI, K. I. et al. Light regulation of stomatal movement. **Annual Review of Plant Biology**, 58(1):219-247, 2007.

SNEDECOR, G. W.; COCHRAN, W. G. **Statistical methods.** Eighth Edition, Iowa State University Press: Ames, 1989.491p.

SOBREIRA, F. M. et al. Adubação nitrogenada e potássica de cafeeiro fertirrigado na fase de formação em plantio adensado. **Pesquisa Agropecuária Brasileira**, 46(1):9-16, 2011.

SOUZA, T. C. de et al. Leaf plasticity in successive selection cycles of 'Saracura' maize in response to periodic soil flooding. **Pesquisa Agropecuária Brasileira, Brasília**, 45(1):16-24, 2010.

SRIDHAR, B. M. et al. Effects of Zn and Cd accumulation on structural and physiological characteristics of barley plants. **Brazilian Journal of Plant Physiology**, 19(1):15-22, 2007.

TATAGIBA, S. D.; PEZZOPANE, J. E. M.; REIS, E. F. Fotossíntese em Eucalyptus sob diferentes condições edafoclimáticas. **Engenharia na Agricultura**, 23(4):336-345, 2015.

TERASHIMA, I. et al. Leaf functional anatomy in relation to photosynthesis. **Plant Physiology**, 155(1):108-116, 2011.

VILLELA, G. M. et al. Faixas críticas de teores foliares de macronutrientes primários para cafeeiros fertirrigados em formação. **Coffee Science**, 10(3):271-279, 2015.

VOLTAN, R. B. Q.; FAHL, J. I.; CARELLI, M. L. C. Variação na anatomia foliar de cafeeiro submetidos a diferentes intensidades luminosas. **Revista Brasileira de Fisiologia Vegetal**, 4(2):99- 105, 1992.

ZHANG, Z. X. et al. Effects of fertilization on the growth, photosynthesis, and biomass accumulation in juvenile plants of three coffee (*Coffea arabica* L.) cultivars. **Photosynthetica**, 55(1):134-143 2016.

ZIMMERMANN, M. H. **Xylem structure and the ascent of sap**. Springer-Verlag, Berlin, Germany, 1983. 143p.