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Looking for adjustments to severe drought in coffee: Lessons of a rainfall exclusion plot in the Southern Brazil

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ABSTRACT

Rainfall exclusion experiments allow us assessing the effects of environmental stresses such as long-term water limitations on both leaf and canopy structural traits. This work aimed to evaluate how leaf anatomical traits and canopy development of productive coffee trees change when submitted to more dry conditions in the southern region of Minas Gerais - Brazil. The experimental plots have been set up in a plantation area in which is growing *Coffea arabica* L. cv. Mundo Novo- IAC 379-19, in a completely randomized arrangement, composed by three treatments: Control (C) - no gutter system; Control plus roof (Ĉ) and Exclusion system (E). Leaf anatomical and canopy traits were determined within a year at the end of each season: *late Spring- 2015 (Sp), and late Summer (Su), late Autumn (Au) and late Winter (Wi) - 2016.* During the studied period the rainfall exclusion condition led to a reduction in the relative water content (RWC) of leaves. In the leaf-level, as the dry condition increase, the leaves invested in thicker cuticles, reduced xylem and phloem areas and smaller stomata, especially with the rainfall exclusion. In the canopy-level, there was a remarkable reduction in leaf area index (LAI) especially in the winter as a strategy of reduction of transpiratory area, when the availability of water decreased around 25%. In a context of reduced water availability due to effects of climate change, coffee trees may be able to present modifications at both levels, to cope with the effects of this abiotic stress.

Key words: Adaptation strategies; *Coffea arabica* L.; Ecophysiology; Leaf anatomy; Seasonal patterns.

1 INTRODUCTION

Coffee production is strongly controlled by climatic conditions. Climate changes have become a fact that drastically affects most coffee-producing regions around the world and Brazil (Camargo, 2010; Läderach et al., 2017). Coffee, particularly arabica (*Coffea arabica* L.), is highly sensitive to climate change and global projections indicate a reduction in the area suitable for its production due to rising temperatures and altered rainfall patterns (DaMatta et al., 2018). As result, studies seek to understand the negative impacts of climate change on coffee plants, with an emphasis on anatomical, morphological, physiological, and genetic adjustments that may allow the acclimatization, especially to repeated drought events (Batista et al.,2021).

Drought is a climatic phenomenon that occurs due to insufficient rainfall, leading over time to stress due to insufficient amount of water in the soil and/or atmosphere. The predicted increase in the frequency and severity of drought episodes, as well as warming, result in several physiological effects on plants (DaMatta et al., 2018). These effects may occur at different time and space scales to maintain carbon assimilation and reduce transpiration, maximizing the water use efficiency. At the leaf-level, functional traits related to the structure can respond to changes in the environment, influencing physiological functions (Poorter et al., 2009). Thus, it is common to observe changes in leaf thickness, specific leaf area, and investment in vascular tissues, in addition to stomatal characteristics, such as density and polar and equatorial diameters (Tribulato et al., 2019). At the canopy-level, the leaf area index can change according to the availability of water in the soil, which further can influence crop productivity (Pfeifer et al., 2018.

Therefore, the effect of environmental stresses such as drought on the leaf functional traits and canopy structure can be relevant to understanding the responses that plants exhibit during development, which facilitates the maintenance of leaf function during exposure to stressors (Baird et al., 2017). In coffee, studies show that drought affects a hierarchical scale ranging from the leaves to the canopy-level so that photosynthesis is restricted, transpiration increases and there is a decline in the production of coffee beans (DaMatta; Ramalho, 2006; Zambolim et al., 2006; DaMatta et al., 2018; Guedes et al., 2018; Dubberstein et al., 2020). However, many of them have focused on plants grown in pots and in greenhouse conditions, presenting limitations due to the restricted growth of roots, the difference between the chemical and physical properties of the substrates in relation to the natural soil, besides the humidity and temperature characteristics of the greenhouse (DaMatta; Ramalho, 2006). With that, there is a need for studies in field conditions using permanent plots of plants in growth and production, which consider the environmental variation and in which the effect of drought can be assessed, through total or partial rain exclusion.

Thus, the importance of rain exclusion experiments is emphasized, given that even under suitable conditions for the growth and development of the crop, they manage to induce a temporal and small- or large-scale water restriction, allowing the analysis of aspects such as hydraulic adjustment (Tomasella et al., 2018), the production of wood from forest trees (Moser et al., 2014) and the physiological responses/ strategies to soil drying (Martin-StPaul et al., 2013). Such experiments allow evaluating the anatomical, morphological, and metabolic adjustments of plants, optimizing the availability of water to keep a positive carbon balance and maximize the water use efficiency, in the face of different conditions of duration and intensity of drought and the subsequent water stress. The aim of this study was to evaluate, within a year, leaf anatomical and canopy characteristics of productive coffee trees (*Coffea arabica* L. cv. Mundo Novo-IAC 379-19) submitted to water restriction through partial rainfall exclusion. We hypothesized that coffee trees would change leaf- (e.g., xylem and phloem area, leaf thickness) and canopy-level (e.g., leaf area index) traits in order to adjust their physiological activities, especially in the rain exclusion treatment during the drier season.

2 MATERIAL AND METHODS

2.1 Experimental area and Plant material

This study was conducted in a coffee crop area located close to the campus of the Federal University of Lavras (UFLA), Lavras (21º13’40’’S and 44º57’50’’W GRW), Minas Gerais, Brazil. According to the Köppen climate classification map for Brazil (Alvares et al., 2013), the characteristic climate of the region, classified as Cwb, shows a climatic seasonality where the winter is cold and dry and summer is hot and humid. The region has an annual mean temperature of 19.4 °C and an average annual rainfall of 1500 mm.

The rain season extends from October to March, with the dry season between April and September. According to the climatological normal of the period between 1961 to 1990 presented by INMET (2020) for the region, in this dry period, a negative water balance prevailed in most months, with higher values of evapotranspiration in relation to precipitation, especially in the dry winter months of June, July and August, the period that precedes the beginning of the rainy season. The soil is a Dystrophic Red Latosol. The variety analyzed was *Coffea arabica* L. cultivar Mundo Novo- IAC 379-19. The coffee trees were planted in January of 2011, being that at the beginning of the experimental period, they were approximately 2.2 m tall and 2.0 m crown diameter.

2.2 Rainfall exclusion system and Environmental conditions

Three rainfall exclusion plots were installed in a coffee experimental area in April 2015. The exclusion system was composed of six eucalyptus posts of 3.2 m of height, three on each side, where fixed woody beams with three metallic supports on the top, to support the gutters, built from translucid polypropylene tile 0.4 m wide and 6.0 m long (Figure 1).

The total rain exclusion area was 4.8 m² and the total plot area was 114 m². This system was projected to exclude approximately 25% of the rain in the plots, based on the IPCC report that projects a reduction of around 20% of rainfall for Brazilian southeastern areas (IPCC, 2014). For each treatment, there were selected 15 coffee trees, totaling 45 plants; however, to reduce limiting boundary effects, a smaller number of plants were sampled from the center of each plot and the number of repetitions for each variable is described below in the *Experimental Design.*

Figure 1: The infrastructure of the rain exclusion system: translucent polyethylene trough above the coffee planting lines. (A) Channeling rainwater from the gutter to a PVC pipe (B) PVC pipe directed to a 200-liter reservoir.

The environmental conditions were recorded throughout the experimental period. The rainfall depth (mm) was measured by a pluviometer type Ville de Paris positioned at two meters above the soil. Daily air temperature (°C) and air relative humidity (%) were measured by three thermohygrometer (Extect Instruments model RHT10) installed in each treatment sampling every 30 min. The daily air temperature information was processed to calculate the minimum mean and maximum temperatures (Tmin, Tmean, and Tmax, respectively). The soil water potential (Ψ- MPa) was determined weekly by nine tensiometers (SondaTerra®) installed at 0.30 m deep in each experimental plot. The total experimental period was from September 2015 to September 2016. Tmin, Tmax, relative humidity (RH), and wind speed (WS), in addition to geolocation, were used to calculate Potential evapotranspiration (PET, in mm) for each month according to FAO Penman-Monteith equation through the ETo Calculator software developed by the FAO Land and Water Division (Allen et al., 1998; FAO, 2019). Also, the water balance was calculated as the subtraction between precipitation and ETo, monthly.

2.3 Water relations, specific leaf area, and leaf area index

Within each season, every 15 days, completely expanded leaves were collected at 9:00 am from the middle-third part of eight coffee trees per treatment. Leaf relative water content (RWC) was determined according to Matos et al., 2010. Leaves were collected, placed in a plastic bag, and kept refrigerated until reach the laboratory. Six disks with a 1.5 cm diameter were cut and weighted to obtain the fresh mass weight (FM). The same disks were incubated in 10 mL of distilled water in the refrigerator for 24 h. After that, turgid mass (TM) was taken. The samples were oven dried at 68 ºC for 48 h to obtain dry mass (DM). RWC was calculated using: RWC = [(FM - DM) / (TM - DM)]. x 100%. The same six disks were used to calculate specific leaf area (SLA- $cm^2.g^{-1}$ dry mass), which was estimated by the area and the dry mass: SLA = $(\pi R^2) / (6 \times DMW)$, where R is the radius of the disk.

The leaf area index (LAI- $m^2.m^{-2}$) of the coffee trees was estimated according to Barbosa et al., 2012. In each collection, every 15 days, biometric assessments were carried out on five trees per treatment, in five different positions in the canopy of the plant collecting data on the height from ground level, the total length of plagiotropic branches, and length of the leafless region in the canopy.

2.4 Leaf anatomy

For leaf anatomy characterization, the five most young, completely expanded and sun-exposed leaves from five coffee trees per treatment were fixed in 70% formaldehyde, glacial acetic acid, and ethanol (FAA) for 48 h and then transferred to 50% Ethanol (Johansen, 1940). Handmade paradermal, from the abaxial epidermis side, and cross sections were taken from the media region of the leaf using a double-edged razor. The sections were clarified with 50% sodium hypochlorite, stained with 1% Safranin and Safrablau (0.1% Astra Blue and 1% Safranin), for paradermal and cross sections, respectively, and then placed in slides with 50% glycerin. The slides were photographed in light microscope (Zeiss Scope AX10) coupled with a digital camera (Canon Powershot G10).

As cited above, leaves were collected from five plants per treatment (one leaf per plant, five leaves per treatment) and three slides were mounted for each repetition, totaling 15 slides per treatment. For each treatment, there was measured 15 observational fields from different slides. The images were analyzed using the software UTHSCSA-Imagetool®. The anatomical characteristics evaluated were xylem, phloem and midrib area (μm²), the thickness of cuticle, the adaxial and abaxial surface of epidermis, and mesophyll (μm), stomatal density (cm²), polar (PD- μm) and equatorial diameter (ED- μm) of stomata, and PD/ED ratio. We also present the ratios of the xylem and phloem area in relation to the total area of the midrib (XA/RA ratio and PA/RA ratio).

2.5 Experimental Design

The mean values of RWC, SLA, and LAI were calculated from data obtained every 15 days within each season. On the other hand, leaf anatomy characteristics were determined at the end of each season, being performed in December of 2015, late Spring- 2015 (**Sp-15**); in March of 2016, late Summer- 2016 (**Su- 16**); in June of 2016, late Autumn- 2016 (**Au- 16**); and in September of 2016, late Winter- 2016 (**Wi- 16**). The experimental design was completely randomized, with eight replicates (RWC and SLA) and five replicates (LAI and leaf anatomy characteristics), and composed by three treatments: i) Control (C) - no gutter system; ii) System with no rainfall exclusion - perforated gutter system that allows the water of the rain to go through the gutters (Control plus roof - Ĉ); and iii) System with rainfall exclusion - gutter system that collect nearly ¼ of the rainfall (Exclusion - E); and four seasons: i) Sp-15; ii) Su-16; iii) Au- 16; and iv) Wi- 16.

2.6 Statistical analyses

All statistical analyzes were conducted using the RStudio statistical software (Version 1.2.5033 © 2009-2019 RStudio, Inc.). The significance (F-tests) of main effects and interactions between treatment factors was assessed by analysis of variance (ANOVA). Assessment of residuals showed that the data satisfy the assumptions of the analysis on the raw scale, so it was not necessary to transform. Pairs of means of most interest were assessed and separated by the standard error of the difference between means (SED) and the Fisher's protected least significant difference (LSD) test at $P < 0.05$. Additionally, all anatomical variables evaluated were correlated with the climatic characteristics of the region, using a Pearson statistical correlation analysis ($p < 0.05$), to find the relationship that best explains the interaction between anatomical adaptation strategies and the climate (Temperature, precipitation, relative humidity, wind speed, water balance, and soil water potential). All anatomical variables and the characteristics of the canopy were also correlated with RWC values to deepen the relationship between leaf water status and anatomical/canopy changes.

3 RESULTS

3.1 Climate conditions and the effects of the rainfall exclusion

In terms of climatic characterization, during the experimental period, the greatest rainfall ranged from November to March, covering the late spring of 2015 and the summer of 2016. This period was characterized by a positive water balance in which precipitation was higher than potential evapotranspiration. The dry season ranged from April to September when the rains resumed, covering the fall and winter of 2016. This time, in turn, it was observed a negative water balance in which precipitation was less than evapotranspiration (Figure 2a). The highest temperatures were recorded during the summer (on average 29.3 °C), while the lowest occurred in the transition between autumn and winter, in the months of June and July (on average 13.1 °C) (Table 1).

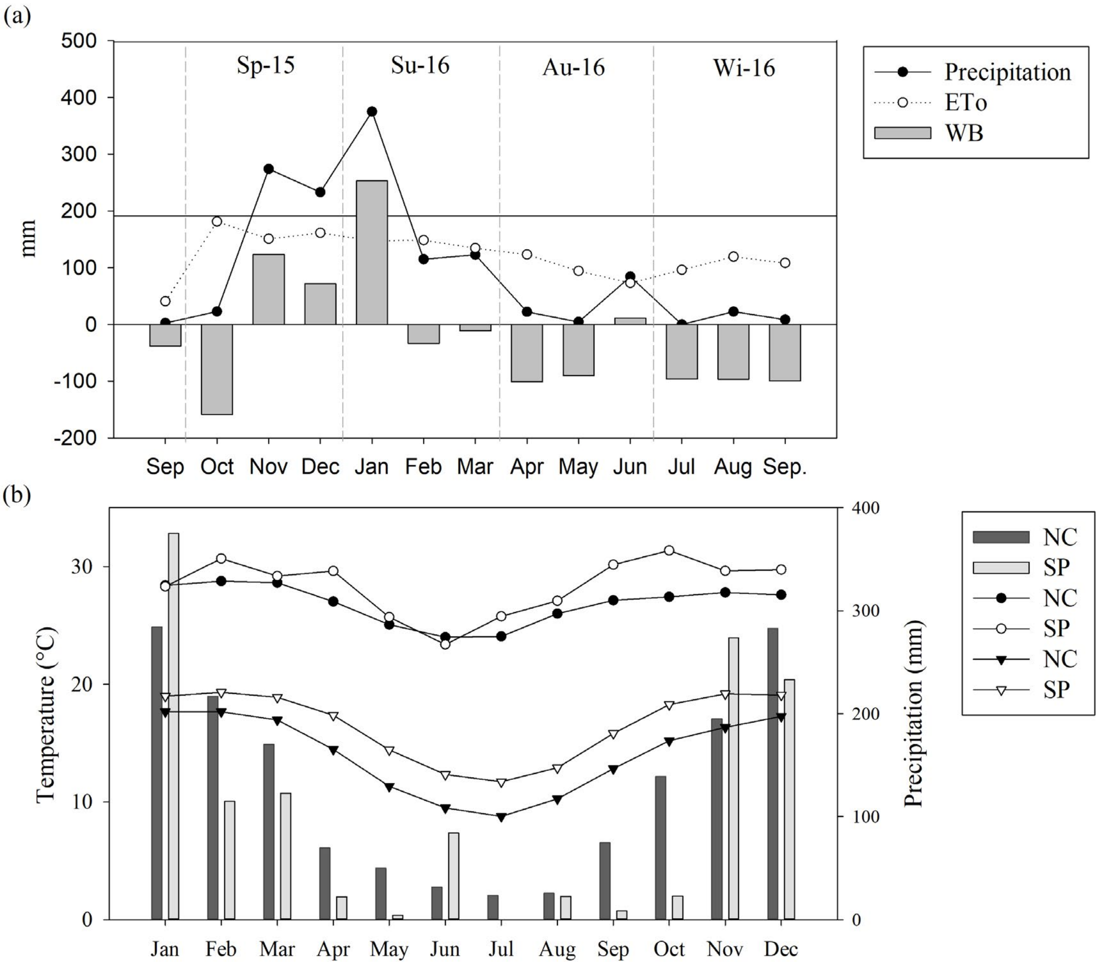


Figure 2: Climatic characterization of the period and region under study (a) Water balance (WB - gray bars) being considered as precipitation (black circles) minus evapotranspiration (white circles). (b) Intra-annual variation in precipitation (light gray bars) and maximum (white circles) and minimum (white triangles) temperature during the study period (SP) in relation to the 1961-1990 climatological normal values (NC - dark gray bars, black circles, and black triangles). Each bar or symbol represents the average of the daily values for each month.

Table 1: Climatic characterization of the study period at UFLA.

Season	Prec	ETo	WB	Tmin	Tmean	Tmax	RH	SWP
	Mm	mm	Mm	°C	°C	°C	%	MPa
Sp15	496.6	481.6	15.0	18.77	23.58	30.34	69.77	-0.11
Su16	618.1	432.2	185.9	19.07	23.22	29.26	75.62	-0.15
Au16	166.4	310.9	-144.5	15.53	20.28	27.08	72.33	-0.31
Wi16	31.2	354.2	-323.0	13.11	19.05	26.89	62.95	-0.39

Prec: precipitation (mm); ETo: evapotranspiration (mm); WB: water balance (mm); Tmin: minimum temperature (°C); Tmean: mean temperature (°C); Tmax: maximum temperature (°C); RH: relative humidity (%) and SWP: soil water potential (MPa).

Considering the intra-annual variation, the evaluated period and site presents a similar trend as the climatological normal of the period between 1961 to 1990 (NC) presented by INMET (Figure 2b). The biggest variation from the normal is observed especially for the accumulated precipitation. On average, the rainfall values for the study period were 279.2 mm below NC values, with the largest differences in January (90.7 mm above NC) and February (101.9 mm below NC). In turn, on average, the maximum and minimum temperatures were higher than the NC temperatures by 1.5 °C and 2.5 °C respectively.

The soil water potential values followed the dynamics of precipitation. The spring and summer seasons in which there is greater precipitation presented the least negative values (-0.1 MPa). The potential value was progressively decreasing for autumn (-0.3 MPa) and winter (-0.4 MPa) when precipitation decreases (Figure 3). This variable was significantly influenced (*p* <0.05, F-test) by the seasons, the treatments, and their interaction (Supplementary material Table 1). It is possible that the greater amount of precipitation during the spring and summer seasons did not cause statistically significant differences between the treatments, showing similar values of water potential. On the other hand, the reduction in precipitation for later seasons led to more negative values of SWP for treatment E (Table 1). The RWC was significantly influenced (*p* <0.05, F-test) by the interaction between the seasons and the treatments (Supplementary material Table 1). The season with the highest values was spring (on average 80.6%), with a decrease in this characteristic during the other seasons (Figure 3). The reduction of water availability in the E treatment may be related to the decrease of almost 8% in relation to the other treatments (Table 1).

The leaf and canopy variations found in coffee trees, especially in the E treatment, could be associated with the partial exclusion of rain in seasons when, due to the natural variation of the climate, there is less water availability, especially during the winter. In those months, the most negative values of soil water potential and the lowest RWC values were presented (Figure 3). Some of these modifications (discussed below) were found in our research because of the imposed treatments (Figures 4 and 5).

3.2 Changes in leaf anatomy

The area of xylem and phloem were significantly influenced (*p* <0.05, F-test) by the interaction between the seasons and the treatments, while the area of midrib was significantly influenced (*p* <0.05, F-test) just for the season (Supplementary material Table 2). In general, for these three variables, there was a decrease throughout the experimental period, with lower values in the dry season (Supplementary material Figure 1). In Sp-15 and Su-16, the mean values of the xylem and phloem area were more uniform among the treatments, whereas in Au-16 and Wi-16, lower values were present, especially in plants from E treatment, under conditions of rain exclusion (Table 2). Considering the ratio between the area of vascular tissues and the midrib area, there was an interaction between the factors (p < 0.05, F test) only for the phloem/midrib ratio. The xylem/midrib ratio did not present statistical differences for any of the factors or their interaction (Supplementary material Table 2).

On the other hand, there were no statistical differences (*p* <0.05, F-test) for the thickness of the mesophyll. In turn, the thickness of the adaxial and abaxial sides epidermis was significantly influenced (*p* <0.05, F-test) by the season, with no effect on the treatment or the interaction between the two factors (Supplementary material Table 2). However, these two variables do not appear to have a season change pattern, with the lowest values of 22.5 and 25.0 μm being observed in the treatments C/ Sp-15 and C/ Su-16, respectively (Table 3). Meanwhile, the cuticle thickness was significantly influenced (*p* <0.05, F-test) by the interaction between season and water treatment, with plants of E treatment showing an increase of almost 50% in the thickness of the cuticle during Wi-16 (Table 2). The trend for these variables in each treatment and season is found in the Supplementary material Figure 2.

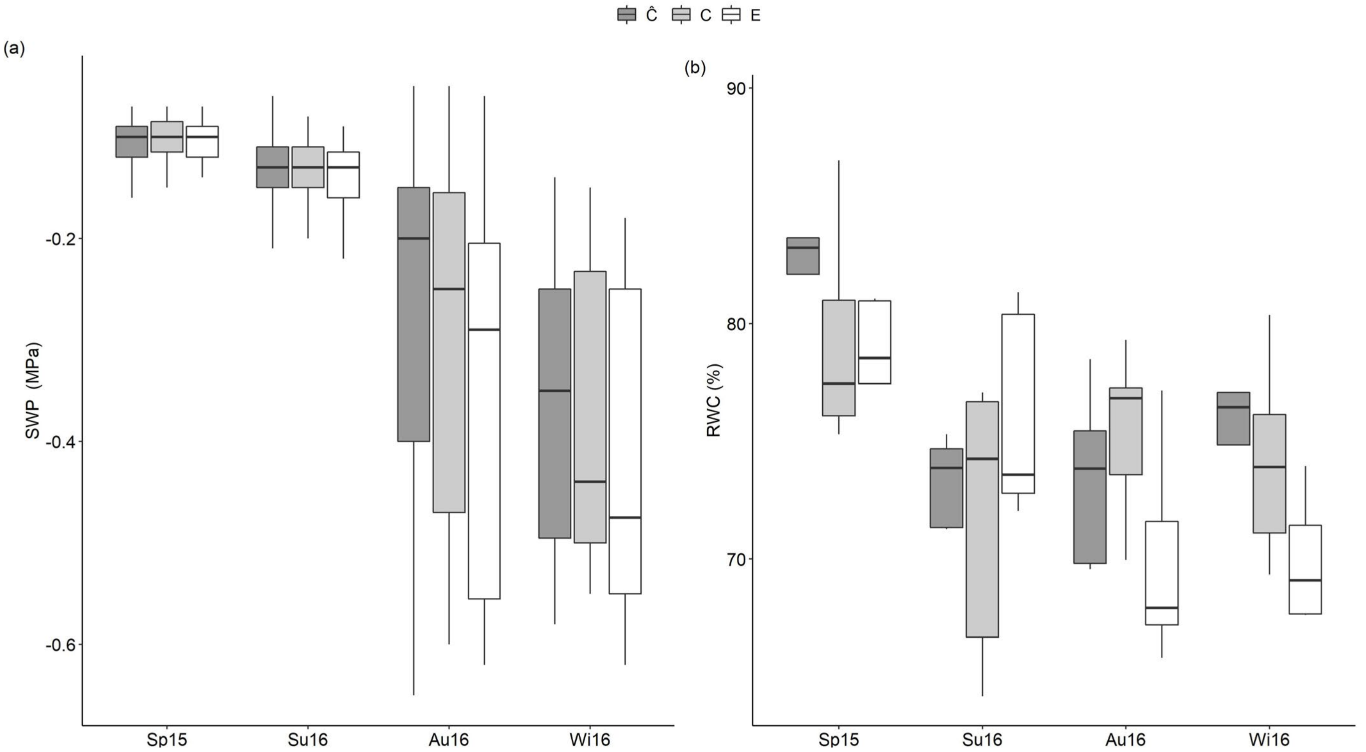


Figure 3: (a) Soil water potential (SWP - MPa) for the three water conditions in each season (b) Relative water content (WRC -%) for the three water conditions in each season.

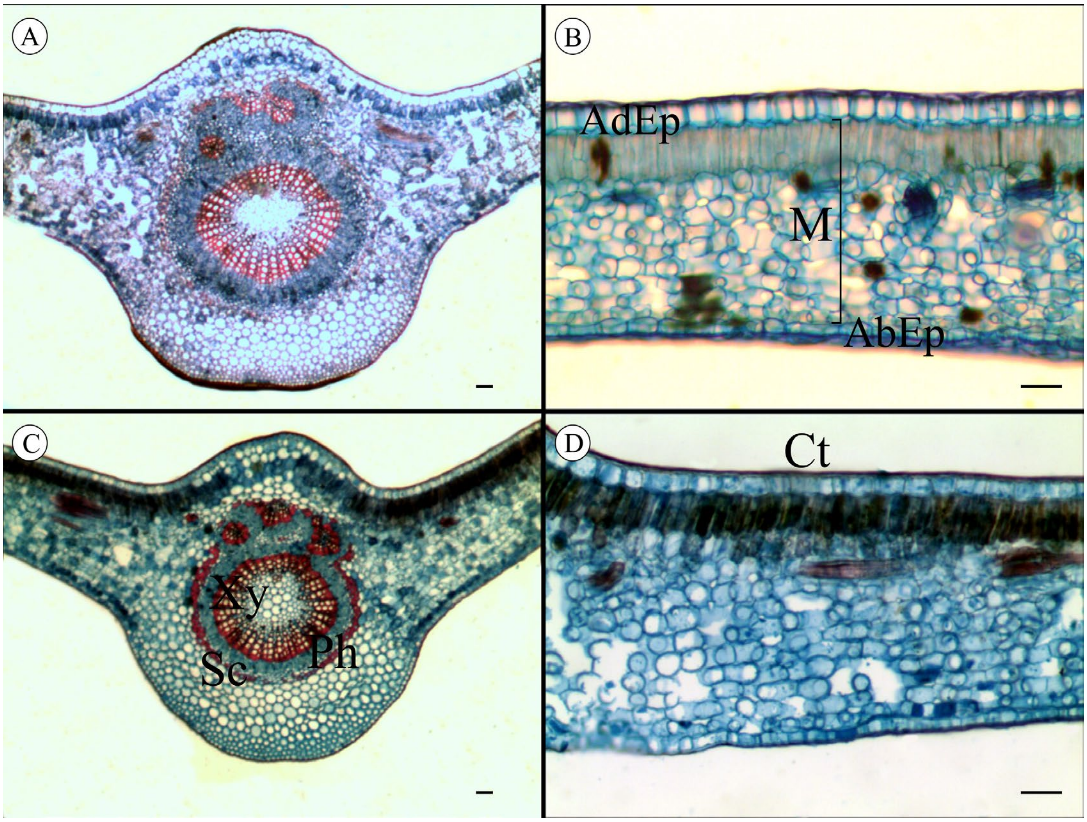


Figure 4: Leaf cross- sections of coffee leaves (*Coffea arabica* L. cv. Mundo Novo) grown during the summer-16 in the C treatment (A, B) and the winter- 2016 in the E treatment (C, D). Xy- xylem; Ph- Phloem; Sc- Sclerenchyma; AdEp- Adaxial side of Epidermis; M- Mesophyll; AbEp- Abaxial side of Epidermis; Ct- Cuticle. Scale Bar= 50 μm.

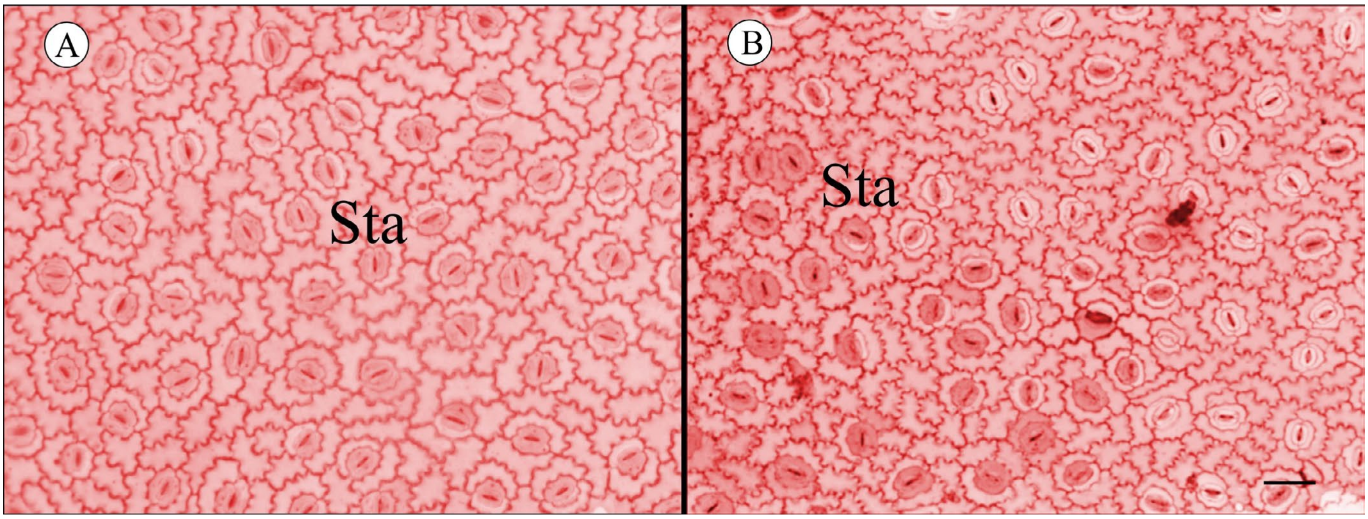


Figure 5: Paradermal sections of coffee leaves (*Coffea arabica* L. cv. Mundo Novo) grown during the summer-16 in the C treatment (A) and the winter- 2016 in the E treatment (B). Sta- Stomata. Scale Bar= 50 µm.

Table 2: Anatomical traits evaluated in coffee leaves (*Coffea arabica* L. cv. Mundo Novo) in three water conditions in different seasonal periods. Variables that presented two-way interaction between the factors.

Factor	XA	PA	PA/RA	CT	PD	TRA	AFE	IAF
	µm^{-2}	µm^{-2}	µm^{-2}	µm	µm	%	cm^{2} g^{-1}	m^{2} m^{-2}
				Season (S) x Treatment (T)				
Sp15-C	113980.63±6409.98	75503.55±4042.07	0.30±0.01	6.80±0.42	25.66±0.33	83.53±1.62	152.15±11.57	3.71±0.11
Sp15-Ĉ	119716.33±4947.82	77846.52±4185.15	0.34±0.02	6.34±0.17	25.70±0.40	79.35±2.13	147.35±6.36	3.45±0.18
Sp15-E	111739.17±7255.07	93416.56±1690.65	0.41±0.03	6.56±0.44	26.45±0.47	77.86±1.78	162.96±2.81	3.52±0.24
Su16-C	122314.24±8968.14	99305.92±6987.03	0.38±0.06	5.86±0.64	25.20±۰,۲۸	73.28±0.84	244.50±16.05	4.30±0.02
Su16-Ĉ	131708.05±9390.49	93030.39±5557.59	0.34±0.03	6.03±0.28	25.43±0.31	71.77±2.67	226.11±11.58	4.32±0.06
Su16-E	136863.16±3964.29	95723.28±7530.61	0.36±0.06	5.90±0.60	25.57±0.40	76.03±1.99	243.60±22.85	4.29±0.07
Au16-C	136483.19±2708.92	114361.04±4388.77	0.40±0.03	6.14±0.72	25.16±0.25	73.43±1.70	209.39±8.67	3.74±0.18
Au16-Ĉ	110677.79±4632.06	98479.35±6242.88	0.40±0.03	4.64±0.36	25.47±0.24	75.39±1.64	221.00±18.7	3.51±0.12
Au16-E	85357.05±4985.80	67554.98±6619.02	0.29±0.02	5.59±0.25	25.48±0.16	69.93±2.05	205.99±8.20	3.33±0.09
Wi16-C	92768.10±10229.56	71286.52±9385.68	0.33±0.02	7.85±1.75	22.04±2.71	75.97±2.26	229.54±12.19	3.57±0.19
Wi16-Ĉ	115443.45±7916.86	67924.52±5022.77	0.30±0.03	7.27±1.02	24.42±0.42	74.17±1.94	235.62±8.11	3.33±0.41
Wi16-E	79092.28±5043.72	47802.23±3517.27	0.26±0.02	11.26±1.42	23.20±0.30	69.95±1.21	190.96±3.52	1.80±0.32
SED	23429.500	20021.589	0.114	2.843	2.927	6.501	42.597	0.685
LSD$_{0.05}$	19231.830	16434.490	0.094	2.333	2.402	5.336	34.964	0.562

Note. Sp-15: spring 2015; Su-16: summer 2016; Au-16: Autumn 2016; Wi-16: Winter 2016. C: control - no gutter system at all; Ĉ: control plus roof - system with no rainfall exclusion; E - exclusion - system with rainfall exclusion. XA: xylem area; PA: phloem area; PA/RA ratio: phloem area/ midrib area; CT: cuticle thickness; PD: polar diameter of stomata; RWC: relative water content; SLA: specific leaf area; LAI: leaf area index. SED, standard error of the difference between two treatment means; LSD$_{0.05}$, least significant difference at p< 0.05; residual degrees of freedom = 48. Values are reported as means ± standard error (n=5 for S x T).

Regarding the characteristics of the stomata, the equatorial diameter was significantly influenced (p <0.05, F-test) just for the season, the polar diameter by the interaction between season and water treatments and the PD/ED ratio showed no differences for any of the factors (Supplementary material Table 2). The polar diameter of the stoma was smaller in all the treatments during the winter in relation to the other seasons (Table 3). The equatorial diameter had the same trend with the lowest values during autumn and winter. This suggests that the availability of water has an influence on the diameter of the stoma. The trend of these variables in each treatment and season is found in the Supplementary Material Figure 3. In our results, we found no differences in stomatal density among treatments and there was no clear trend regarding seasonality (Table 3).

To drill down on all previously found results, the correlations between the RWC values and the anatomical variables are found in Table 4. Significant correlations were found only for treatment E. Positive correlations were found for the area of the vascular tissues (R^2= 0.57, 0.49 for xylem and phloem, respectively) and midribs (R^2=0.45), which agrees with the lowest values found for these variables in the seasons in which there is less water availability (autumn and winter). On the other hand, negative correlations were found for the thickness of the epidermis in adaxial and abaxial sides (R^2= -0.56 and -0.5 respectively), which agrees with the effect of the season found for these variables, so that higher values of thickness are to be expected in seasons with less water availability (although there was no clear pattern for these variables - Table 3). In this context, it is possible the set of anatomical modifications observed in the leaf blade was responsible for preventing excessive water loss, allowing the coffee trees to maintain a relatively constant RWC (above 70%) in the drier seasons, even under rain exclusion condition (Figure 3).

3.3 Changes in canopy characteristics

The leaf area index was significantly influenced (p <0.05, F-test) for the season, the treatments, and the interaction between the factors (Supplementary material Table 1). While the Su-16 presented the highest LAI values, during the Wi-16 there was a notable reduction in this variable. This may be related to the decrease in precipitation and the water potential of the soil during this season; with the rain exclusion in E treatment leading to a reduction of the LAI in coffee trees greater than 50% (Figure 6a). This can be associated with the phenology of the coffee tree, since the lowest LAI values were obtained in the quiescent phase, when the metabolism slowdown due to the environmental conditions of the region, as low precipitation, and temperatures. In our case, it was observed that the condition of rain exclusion increased leaf abscission so the plants of the E treatment showed lower LAI values in winter (2.9 m^2 m^{-2}). Subsequently, with the resumption of growth that accompanies the beginning of the rains in the spring, new branches and leaves emerge, with the consequent increase in LAI until reaching the highest values in the summer (4.3 m^2 m^{-2}) (Figure 6a).

On the other hand, the SLA was significantly influenced (p <0.05, F-test) just by the season individually and by the interaction between season and water treatment (Supplementary material Table 1). For this variable, the lowest values were presented in the spring (154.56 cm^2 g^{-1}) while the highest values were presented in the summer (238.06 cm^2 g^{-1}) (Figure 6b). Additionally, there was a statistically significant negative correlation between SLA and soil water potential (R^2= -0.97) for treatment E (Supplementary material Table 3), as well as between SLA and RWC (R^2= -0.45) for treatment C (Table 4).

Table 3: Anatomical traits evaluated in coffee leaves (*Coffea arabica* L. cv. Mundo Novo) in three water conditions in different seasonal periods. Mean effects of the factors separately (variables without two-way interaction).

Factor	RA	XA/RA	MT	AdET	AbET	SD	ED	PD/ED
	µm^{-2}	µm^{-2}	µm	µm	µm		µm	µm
				Season (S)				
Sp15	238420.10±6181.90	0.49±0.02	247.55±5.00	23.00±0.42	27.32±0.35	167.21±3.43	16.32±0.28	25.94±0.24
Su16	278550.30±14072.31	0.49±0.03	257.61±6.17	25.81±0.64	26.89±0.89	177.73±5.53	16.40±0.22	25.40±0.18
Au16	256248.20±9984.81	0.43±0.02	249.03±5.94	27.79±0.67	33.09±0.68	167.95±3.77	15.38±0.17	25.37±0.12
Wi16	210360.80±9059.65	0.46±0.02	254.22±4.45	25.10±1.03	29.84±0.88	178.67±17.17	15.50±0.28	23.22±0.89
SED	20445.529	--	--	1.449	1.465	18.746	0.486	--
LSD$_{0.05}$	28632.600	--	--	1.914	1.938	26.198	0.640	--
				Treatment (T)				
C	256845.30±10490.12	0.46±0.02	255.85±5.30	24.30±0.63	28.48±1.07	165.04±11.75	16.19±0.20	24.52±0.71
Ĉ	245728.70±6307.84	0.49±0.01	249.72±4.92	25.91±0.74	29.20±0.70	182.69±5.72	15.85±0.21	25.26±0.20
E	235110.50±12927.42	0.45±0.03	250.74±3.79	26.07±0.76	30.18±0.67	170.95±4.40	15.67±0.27	25.17±0.32
SED	--	--	--	--	--	--	--	--
LSD$_{0.05}$	--	--	--	--	--	--	--	--

Note. Sp-15: spring 2015; Su-16: summer 2016; Au-16: Autumn 2016; Wi-16: Winter 2016. C: control - no gutter system at all; Ĉ: control plus roof - system with no rainfall exclusion; E - exclusion - system with rainfall exclusion. RA: leaf midribs area; XA/RA ratio: xylem area/midrib area; MT: mesophyll thickness; AdET: adaxial epidermis thickness; AbET: abaxial epidermis thickness; SD: stomatal density; ED: equatorial diameter of stomata; PD/ED: PD/ED ratio. SED, standard error of the difference between two treatment means; LSD$_{0.05}$, least significant difference at p< 0.05; residual degrees of freedom = 48. Values are reported as means ± standard error (n=15 for season, n=20 for treatment).

Table 4: Pearson's correlation analysis between the RWC and anatomical variables of coffee trees (*Coffea arabica* L. cv. Mundo Novo) in three water conditions in different seasonal periods.

	E Treatment		C Treatment		Ĉ Treatment	
	R^2	*p*	R^2	*p*	R^2	*p*
XA	0.577	0.008	0.222	0.348	-0.391	0.088
PA	0.494	0.027	-0.141	0.554	-0.378	0.101
RA	0.454	0.044	-0.327	0.160	-0.216	0.361
MT	-0.351	0.129	-0.213	0.367	-0.331	0.153
ADET	-0.563	0.010	-0.242	0.304	-0.233	0.323
ABET	-0.504	0.023	-0.181	0.446	-0.191	0.420
CT	-0.331	0.154	-0.232	0.326	0.133	0.600
SD	-0.058	0.809	-0.091	0.702	-0.169	0.475
PD	0.409	0.073	-0.116	0.627	0.125	0.598
ED	0.381	0.097	-0.085	0.721	0.048	0.842
PD/ED	-0.061	0.798	0.019	0.935	0.016	0.946
LAI	0.398	0.082	-0.016	0.946	-0.282	0.229
SLA	-0.153	0.521	-0.448	0.047	-0.588	0.006
XA/RA	-0.291	0.709	0.070	0.930	0.682	0.318
FA/RA	0.301	0.699	0.060	0.940	0.664	0.336

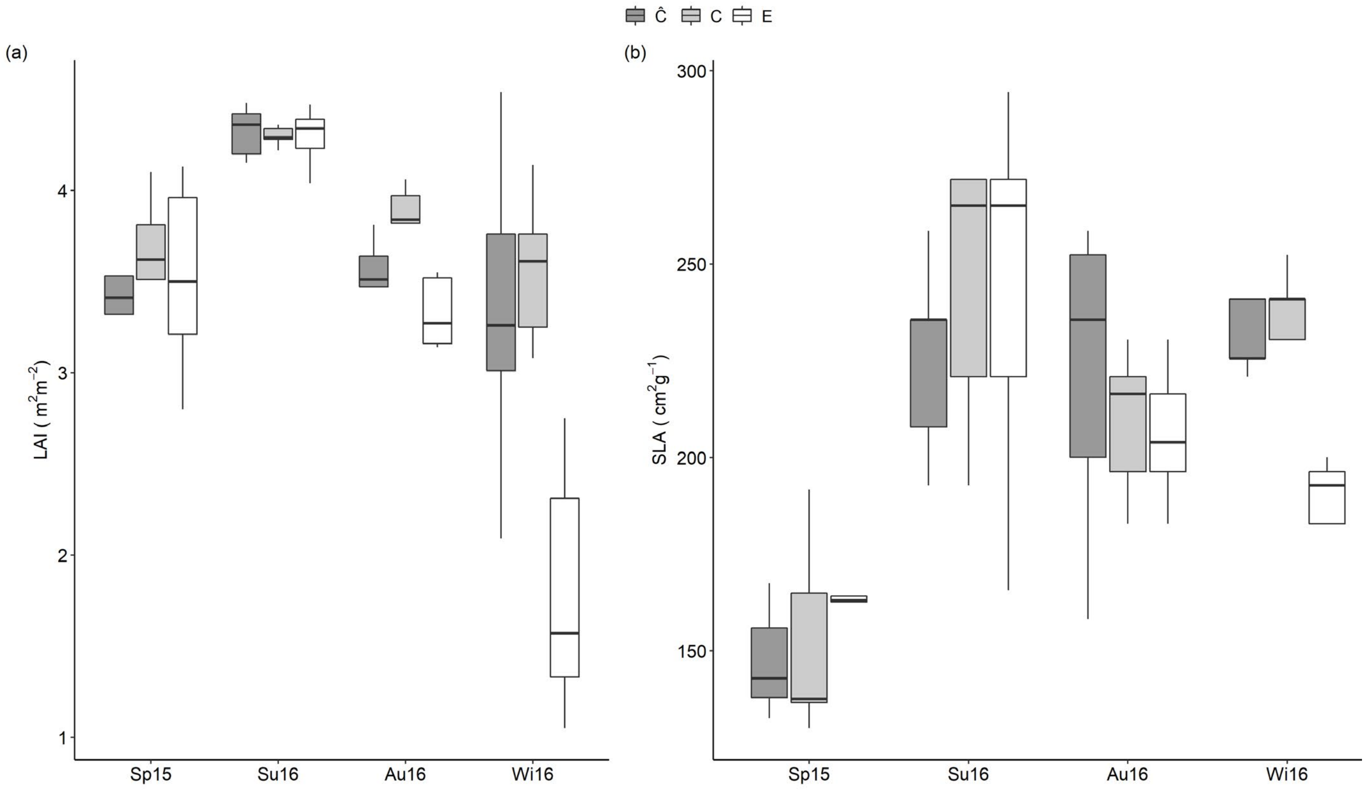


Figure 6: Leaf area index (LAI) and specific leaf area (SLA) of coffee plants (*Coffea arabica* L. cv. Mundo Novo) in three water conditions in different seasonal periods.

3.4 Climatic control of changes in leaf anatomy and canopy characteristics

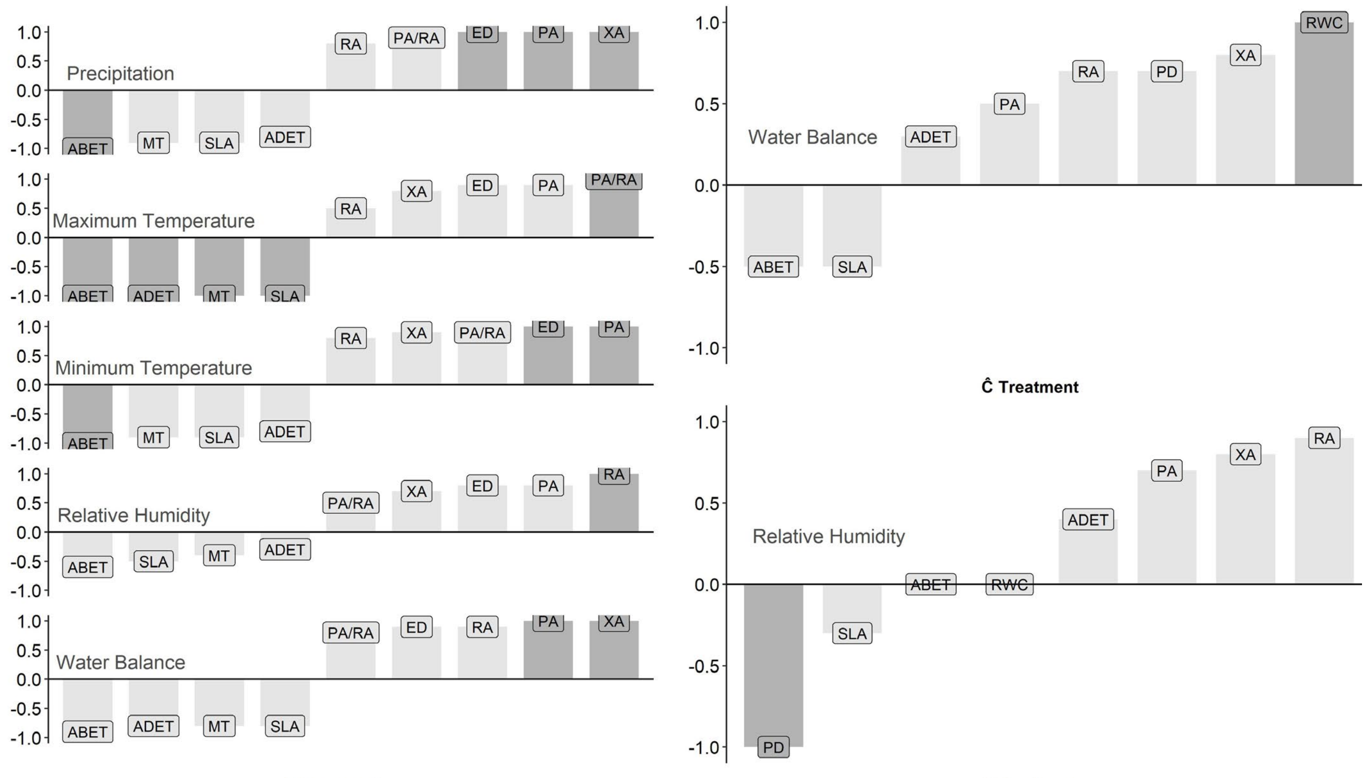
Regarding Pearson's correlation analysis ($p<0.05$), the treatment that showed the most significant correlations between anatomical and climatic variables was the E treatment (Figure 7). For this treatment, it was observed positive correlations between the xylem area and precipitation; between the phloem area and equatorial diameter with precipitation and minimum temperature; and between the midrib area with relative humidity and the water balance. In turn, the negative correlations were between the thickness of the abaxial side epidermis with precipitation and the maximum and minimum temperatures; and between the thickness of the mesophyll, the thickness of the adaxial side epidermis, and the specific leaf area with the minimum temperature. The Ĉ treatment showed only a statistically significant and positive correlation between the water balance and the relative water content. The C treatment also showed only a significant, but negative, correlation between the relative humidity and the polar diameter of the stoma (Figure 7).

The strong correlation between leaf and canopy adjustments of coffee trees and climatic factors, especially water availability, may indicate the positive or negative influence of this condition, depending on the analyzed variable and the phenological phase considered (Figure 7). The thickness of the leaf blade, which comprises the thickness of the abaxial and adaxial sides of the epidermis and the mesophyll, was mainly responsive to the higher temperatures (R^2= -0.98, -0.99 and -0.97 respectively), and in the case of the abaxial side epidermis to precipitation (R^2= -0.97).

4 DISCUSSION

Our results indicate that the plants of *C. arabica* showed adjustments at the **leaf- and canopy-level** in relation to the water conditions evaluated in this experiment. Considering the climatic characterization of the region during the experimental period, the intervals measured for temperature and precipitation fluctuated in the optimum interval for the growth and development of the culture, as reported by DaMatta et al. (2007). This aspect is important since functional leaf traits are largely related to environmental conditions and maximize fitness in variable environments, allowing the maintenance of leaf function during exposure to stress (Grisi et al., 2008; Haffani et al., 2017; Rodrigues et al., 2016).

The leaf and canopy variations found in coffee trees, especially in the E treatment, could be associated with the partial exclusion of rain, which is accentuated in seasons when, due to the natural variation of the climate, there is less water



E Treatment

Ĉ Treatment

C Treatment

Figure 7: Pearson's correlation analysis between climatic and anatomical variables of coffee trees (*Coffea arabica* L. cv. Mundo Novo) in three water conditions in different seasonal periods. Dark gray bars represent statistically significant interactions ($p <0.05$); light gray bars represent statistically non-significant interactions ($p> 0.05$).

availability, especially during the winter. In those months, the most negative values of SWP and the lowest RWC values were presented (Figure 3). Periods of water restriction can induce diverse anatomical modifications; for example, changes in the structure of the leaf mesophyll (Bacelar et al., 2004), thickening of the leaf cuticle (Binks et al., 2016), increase or decrease in the diameter of the xylem vessels (Das et al. 2015; Fernández-de-Uña et al. 2017), and increased stomatal density (Haffani et al., 2017). In turn, these modifications may induce hydraulic changes, influencing the water potential of leaves and/or stems (Marsal et al., 2008) and the vascular flows within the plant (Morandi et al., 2014).

During the winter, the lowest values of xylem and phloem areas were present, especially in the E treatment. It has been reported that the dimensions and the arrangement of the vascular elements can change under water stress to avoid cavitation and transport disorders due to the decline in cell expansion driven by the turgor (Castro et al., 2009; Agustí; Sorek et al., 2021; van Bel, 2003; Woodruff, 2014). Our results agree with the data reported by Fernández-de-Uña et al. (2017), who evaluated the functional adjustments of the xylem and leaf to the drought in *Pinus sylvestris* L. through a rain exclusion experiment, finding that the formation of the xylem was significantly reduced in the treatment of rain exclusion. On the other hand, our results contradict the data reported by Das et al. (2015), who reported an increase in the size of the vascular tissue in response to water deficit conditions, which would indicate greater transport of water and nutrients from the soil and greater discharge of phloem as a strategy to adapt to the decrease in water availability.

In agreement with these results, it has been reported in other coffee cultivars such as Bourbon Amarelo, Catimor and in the IAC H 8421-2 line, that the increase in the thickness of the central rib may be another strategy related to a greater flow of photosynthates and water in the plant, ensuring cell turgidity in the leaves and greater phloem thickening for greater translocation of carbohydrates (Batista et al., 2010; Queiroz-Voltan et al., 2014). These results contradict our data because the midrib area decreased with the lower water availability typical of the autumn and winter seasons (Supplementary material Figure 1).

Regarding the characteristics of the leaf blade, although there was no pattern of variation influenced by seasonality in the adaxial and abaxial sides epidermis, in general there was a greater thickening of these structures in the E and Ĉ treatments in all seasons (Table 3). In turn, the cuticle thickness increased significantly in the dry season (Supplementary material Figure 2). The modifications in the thickness of leaf structures are commonly favorable in conditions of low water availability, since it constitutes a strategy of mechanical resistance of the leaf, which reduces the harmful effects of lack of water (Morais et al., 2004; Haffani et al., 2017). Thus, we observed that the strategy to deal with the low availability of water used by the coffee trees was the preferential modification of the outermost leaf layers, especially the cuticle. A thicker cuticle, due to its lipid nature, can prevent excessive water loss due to leaf transpiration (Dias et al., 2005 Castro et al., 2009; Akram et al., 2016). Certainly, drought-sensitive coffee plants have leaf characteristics contrary to those found in our results (Batista et al., 2010; Queiroz-Voltan et al., 2014). In this context, it is possible the set of anatomical modifications observed in the leaf blade were responsible for preventing excessive water loss, allowing the coffee trees to maintain a relatively constant RWC (above 70%) in the drier seasons (Figure 3).

Other strategies are related to stomata characteristics. The PD/ED ratio is a formula that defines stomatal functionality, and greater functionality can be associated with reduced transpiration, as stomata become more elliptical (Bosabalidis; Kofidis, 2002; Castro et al., 2009; Batista et al., 2010; Zhao et al., 2015). For this elliptical shape, there is usually an increase in the polar diameter of the stoma. Although PD/ED ratio remained unchanged, there were differences in the diameters evaluated separately, with lower values for both in the E treatment in winter (Supplementary material Figure 3). These results may suggest that, although there was no increase in the polar diameter, there was a reduction in the total dimensions of the stomata, which may be associated with a smaller stoma opening area as a strategy to avoid water loss. Some authors report that these changes are usually accompanied by a higher stomatal density to increase water retention and have greater efficiency in gas exchange (Haffani et al., 2017). In contrast, low stomatal density has also been linked to drought tolerance, as it results in less stomatal conductance and, consequently, greater water use efficiency (Franks et al., 2015; Ouyang et al., 2017). In our results, we found no differences in stomatal density among treatments and there was no clear trend regarding seasonality (Table 3).

Considering the canopy-level characteristics of coffee trees, the highest and constant LAI values were related to the rainy season in the summer, while the lowest values were found in the treatment of rain exclusion in the winter, the driest season (Figure 6). It is known that LAI is one of the characteristics that vary seasonally according to environmental conditions, including rainfall patterns (Veneklaas; Poot, 2003; Doughty; Goulden, 2008). Therefore, the reduction in water availability is related to the decrease in LAI, since there is an acceleration of the leaf abscission process (Souza et al., 2016). In addition to seasonality, the changes in LAI are related to the renewal of canopy leaves and, consequently, to abscission and leaf production (Barbosa et al. 2012). This can be associated with the phenology of the coffee tree since the lowest LAI values were obtained in the quiescent phase when the metabolism slowdown due to the environmental conditions of the region, such as low precipitation, and temperatures. In our case, it was observed that the condition of rain exclusion increased leaf abscission so that the plants of the E treatment showed lower LAI values in winter. Subsequently, with the resumption of growth that accompanies the beginning of the rains in the spring, new branches and leaves emerge (DaMatta et al., 2007), with the consequent increase in LAI and SLA, until reaching the highest values in the summer (Figure 6). Plants grown in E condition showed a decrease in SLA in winter, which represents less investment in the area to reduce the transpiratory surface.

The strong correlation between leaf and canopy adjustments of coffee trees and climatic factors, especially water availability, may indicate the positive or negative influence of this condition, depending on the analyzed variable and the phenological phase considered (Figure 7). The thickness of the leaf blade, which comprises the thickness of the abaxial and adaxial sides epidermis and the mesophyll, was mainly responsive to the higher temperatures and in the case of the abaxial epidermis side to precipitation. On the other hand, the area of the vascular tissues was more responsive to water status, with precipitation and water balance having the greatest influence on these variables. It guarantees an adequate transport of water and nutrients as a strategy for conditions of low water availability.

In summary, our results show that the rainfall exclusion condition had a strong influence on the anatomical and canopy adjustments of coffee trees. Thus, the decrease in xylem area, stomata dimensions, and SLA; and the increase in the thickness of the cuticle allowed trees to deal with the reduction of water availability due to the exclusion of rain. Besides that, the canopy characteristic- LAI, followed a seasonal pattern that also accompanies the phenology of coffee trees. Thus, as observed, the decrease in precipitation and increase in temperatures, especially in the dry season, resulted in an increment in leaf thickness as a mechanism of resistance. On the other hand, the area of the vascular tissue was more responsive to water status, with precipitation (R^2= 0.97) and water balance (R^2= 0.96) having the greatest influence on these variables. It guarantees an adequate transport of water and nutrients as a strategy for conditions of low water availability.

5 CONCLUSIONS

The plants of *C. arabica* analyzed in this experiment were able to change the characteristics evaluated, to cope with the restriction of water. A remarkable reduction in LAI was observed, especially in the E treatment during the winter. This response followed a seasonal pattern; however, it represented a canopy-level strategy of reduction of transpiratory area, when the availability of water decreased by around 25%. In the leaf-level, the avoidance of water loss showed a similar response, once the leaves invested in thicker cuticles, reduced xylem and phloem areas, and smaller stomata. Thus, in future scenarios of reduced water availability, we may expect that coffee trees may display some modifications at both levels, to reduce possible damages caused by this abiotic stress in the vegetative stage. Nevertheless, further studies considering the reproduction phase and coffee production are needed.

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7 AUTHOR’S CONTRIBUTION

DVS and VLN performed the experiment, MATH and AMCM conducted all statistical analyses and wrote the manuscript, DAV co-worked the manuscript and SR and JPRADB reviewed and approved the final version of the work.

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