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Nutrient content and cutting anatomy can affect the production of Conilon clonal plantlets

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ABSTRACT

Cutting is the main vegetative propagation method used to produce *Coffea canephora* plantlets. In this method, the nutritional quality of the vegetative propagule (stem cuttings) is one of the determining factors for the rooting speed and the final quality of the plantlets. Thus, the objective in this study was to verify possible variations in nutrient content and anatomical characteristics in cuttings collected at different times of the year and their relationship with the production of *Coffea canephora* clonal plantlets. The study was divided into two phases: 1) Nutritional composition and anatomy of *C. canephora* cuttings grown at different times; 2) Production of *C. canephora* seedlings under greenhouse conditions. The treatments consisted of cuttings collection and plantlets production at different times of the year: January, May and September 2017. We observed that there is seasonal variation for the content of N, P, K and Mg; and anatomical changes in xylem, phloem and vascular cylinder thickness in cuttings harvested at different times of the year. We conclude that although nutritional and anatomical aspects of the vegetative propagule may result in different vegetative growth rates of *C. canephora* clonal plantlets, this result is more dependent on the management of the nursery environment conditions, especially temperature and relative air humidity.

Key words: *Coffea canephora*; cutting nutrition; clonal plantlet; vegetatif growth; physiological quality.

1 INTRODUCTION

C. canephora is a self-incompatible species therefore its crops formed from plantlets are heterogeneous and exhibit high morphological and genetic diversity (Covre et al., 2016; Dubberstein et al., 2020). On the other hand, as asexual reproduction ensures the reproduction of the characteristics of the mother plant (Ramalho et al., 2016), vegetatively propagated crops present greater homogeneity, more vigorous plants, higher productivity, besides promoting earlier production (Fonseca et al., 2017). Therefore, vegetative propagation has been widely used in the commercial multiplication process of coffee trees of this species.

Cutting is the main form of vegetative propagation used in the production of *C. canephora* plantlets (Ferrão et al., 2019). This method uses approximately five centimeters long cuttings, with a pair of leaves (Aquino et al., 2017), taken from secondary orthotropic stems produced by adult coffee plants. These stems are obtained by the induction of shoots in mother plants, usually by bending the stem or by apical pruning of the orthotropic stem (Espindula et al., 2020). However to obtain healthy and vigorous plantlets, it is necessary to know the factors that interfere in this process, as well as the interaction between them.

Vegetative propagation can be influenced by endogenous (genetic and physiological) (Cavalcante et al., 2019; Giuriatto Junior et al., 2020; Guimarães et al., 2019) and environmental factors (Bazoni et al., 2020). In addition, factors such as substrate (Berilli et al., 2020a; Verdin Filho et al., 2018) and fertilization (Berilli et al., 2020b), as well as volume container (Espindula et al., 2018) during the growth phase in the nursery, can alter the final quality of the plantlets.

Nutritional quality of cuttings, for example, is a physiological factor that directly influences rooting (Cavalcante et al., 2019; Guimarães et al., 2019) and occurs because nutrients are involved in metabolic processes associated with de-differentiation and root meristem formation, as well as cell wall formation, lignification and cell elongation, processes necessary for root system growth and development (Cunha et al., 2009).

The anatomy of the cuttings is another factor that influences production of clonal plantlets, since vascular tissues are used to transport water, nutrients and hormones that are involved in their growth. In some cases, the anatomy of the cutting may be a determining factor in the rooting process, since the supporting tissues that constitute it can act as a barrier to the emission of roots, blocking their formation (Bryant; Trueman, 2015; Zottele et al., 2020).

Given the importance of the quality of cuttings to produce clonal plantlets, the objective of this work was to verify possible variations in nutrient content and anatomical characteristics of cuttings collected at different times of the year and their relationship with the production of clonal plantlets of *C. canephora*.

2 MATERIAL AND METHODS

The study was divided into two stages: 1) Evaluation of nutritional and anatomical characterization of cuttings from the clonal garden and, 2) Evaluation of plantlet growth under greenhouse conditions.

2.1 Nutrient content and anatomy of *Coffea canephora* cuttings grown at different times of the year

Four-month-old stem cuttings were obtained from a Conilon - BRS Ouro Preto clonal garden in the Experimental Field of the Brazilian Agricultural Research Corporation - EMBRAPA, in the municipality of Ouro Preto do Oeste, RO, Brazil. (10˚43'55" S; 62˚15'19" W). The place is at an altitude of 300 m, with a predominance of Tropical Rainy - Aw climate (Alvares et al., 2013), with an average annual temperature of 25 °C and average rainfall of 2,000 mm year^{-1}. The rainy season is from October to May.

The average values of temperature, relative minimum, average and maximum humidity, and rainfall during the experimental period were collected at Embrapa's meteorological station in Ouro Preto do Oeste. During the drought period, complementary irrigation was performed to meet the need for water (Figure 1).

The clonal garden was planted in January 2012, with the spacing of 2.5 m between planting rows and 1.5 m between trees in the row. At the beginning of the study the mother trees plants had 4 or 5 stems. The last macro and micronutrient fertilization at this phase was carried out in August 2016, 45 days before the start of the experiment. In this fertilization, 150 g of 20-05-20, 50 g of MIB (B: 1.8%; Cu: 0.8%; Fe: 3.0%; Mn: 2.0%; Mo: 0. 1%), 20 grams of boric acid, 20 grams of magnesium sulfate were applied.

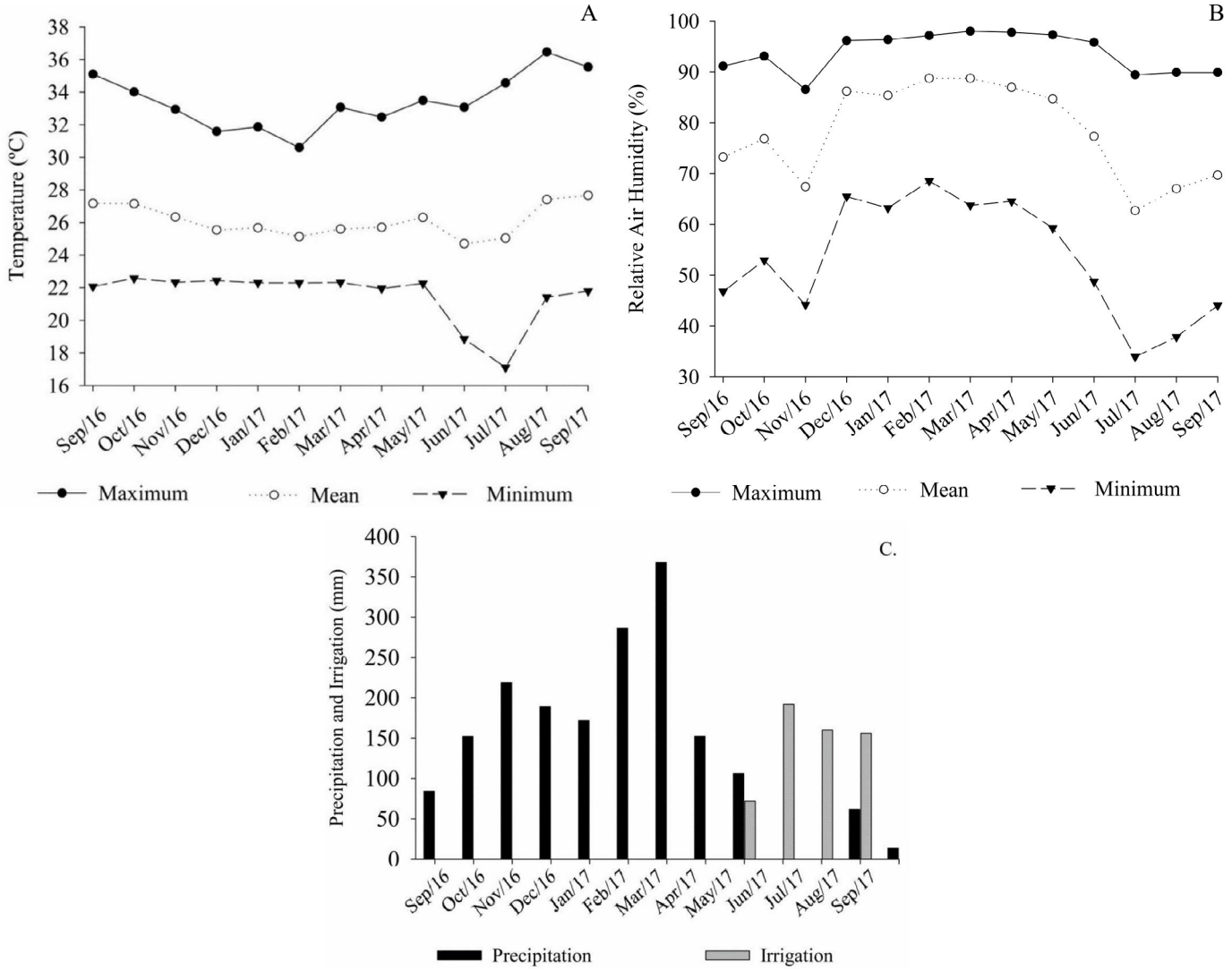

Figure 1: Minimum, average and maximum air temperature (A), minimum, average and maximum relative air humidity (B) and accumulated precipitation and complementary irrigation (C) during the experimental period. Ouro Preto do Oeste, 2017.

Thirty days after the last fertilization of the area, the soil was sampled at a depth of 0-20 cm to determine for chemical analyses. Analysis indicated pH 5.6 in water; P: 46.5 mg dm^{-3}; K: 0.57; Ca: 2.13; Mg: 0.59; H + Al: 4.8; Al: 0.22 and CTC: 8.18 cmolc dm^{-3}, MO: 15.3 g kg^{-1}; Cu: 11; Fe: 95.19; Mn: 196.49 and Zn: 27.6 mg dm^{-3}. During this period, leaves were also collected for tissue analysis and indicated N: 35; P: 1.09; K: 26.9; Ca: 41.4; Mg: 3.31; S: 2.29 g kg^{-1} and Cu: 37.31; Fe: 310.5; Mn: 769.9 and Zn: 54.5 mg kg^{-1}.

To neutralize exchangeable aluminum and to increase Ca and Mg contents, 200 g $plant^{-1}$ of dolomitic limestone (PRNT 100%) was applied on soil surface, 100 grams on each side of the plant, covering approximately one square meter of soil. During each stem growth cycle, 150 grams of the formula 20-05-20 were applied divided into two applications at 20 and 45 days after cuttings removal.

During the installation of the experiment the mother plants were pruned in a way to have four orthotropic stems each and induced to emit secondary shoots. For the induction of shoots, two opposite stems received apical pruning at a height of 1.70 meters from the ground level. All productive branches (plagiotropic branches) that were more than 30 cm long were removed. In November 2016, the plagiotropic branches with cherries were removed.

The corresponding period for the formation of vegetative material (orthotropic stems) in the mother plant was: Season 1, from September 18, 2016 to January 16, 2017; Season 2, from January 16 to May 18, 2017; Season 3, from May 18 to September 19, 2017. Treatments consisted of collecting cuttings at different times of the year: January (Season 1), May (Season 2) and September (Season 3). The experimental design was completely randomized with 24 replications, with one tree per plot.

Nutritional analysis: On January 16 (Season 1), May 18 (Season 2) and September 19 (Season 3) 2017, cuttings, containing a pair of leaves that have been reduced by a third of their length, from the mother plants were collected placed in paper bags to dry in an oven at 65 ° C until constant weight. They were then ground and subjected to analysis of nutrient rate. The nitro-perchloric chemical digestion method was used for nutrients P, K, Ca and Mg followed by analytical determination by plasma spectrometry for Ca and Mg. P was determined by molecular spectrophotometry and K by flame photometry. N was obtained by sulfuric digestion and determined after semi-micro Kjeldahl distillation (Carmo et al., 2000). Nutrient content was calculated using nutrient concentration and cutting dry mass.

Anatomical analysis: A sample of the cuttings collected in January, May and September 2017 were fixed in FAA 70 solution (5 ml of formaldehyde, 5 ml of acetic acid and 90 ml of 70% ethanol) for 24 hours and stored in 70% ethanol in a refrigerator at 5 ° C (Johansen, 1940). Subsequently, the samples were dehydrated in an increasing ethyl series and embedded in paraffin (Kraus; Arduin, 1997). After inclusion, the materials were cross sectioned on a rotary microtome (American Optical) using disposable steel razors. The cuts were 10 µm thick and their structural anatomical characterization was performed with the aid of Safranina and Astra Blue dyes (Bukatsch, 1972).

All blades were prepared using Canada Balm and coverslip and subsequently examined and photographed using a photomicroscope (Leica DM 2500). The images obtained were quantitatively analyzed for xylem thickness (EX), phloem thickness (EF), peridermis thickness (EP) and vascular cylinder thickness (ECV) with the aid of software Image Processing and Analysis in Java (ImagemJ) version 1.8.0.

For each treatment were prepared 54 blades with five cuts each. Three images were obtained per blades, from which, for each tissue analyzed, three measurements were acquired.

Data were subjected to Lilliefors test to assess their normality. Data transformation was used in $\sqrt{x}$, to obtain normal distribution, followed by analysis of variance and F test at 5% probability. The averages were compared by Tukey test ($p \leq 0.05$), with the aid of the GENES® program.

2.2 Production of *C. canephora* plantlets under greenhouse conditions

A sample of cuttings collected in Part I of the study were sent to a greenhouse of Embrapa Experimental Field in Ouro Preto do Oeste, RO, for plantlets production by the conventional cutting method. The cuttings were, five centimeters long and the one pair of leaves was reduced to 1/3 of their length (Aquino et al., 2017), were sowed in 280 cm^3 returnable polyethylene tubes containing commercial Vida Verde Tropstrato HT® substrate with addition of 6 g dm^{-3} of Basacot® Plus 9M fertilizer (16% N, 8% P, 12% K, 2% Mg, 5% S, 0.4% Fe, 0.02% B, 0.02% Zn, 0.05% Cu, 0.06% Mn and 0.015% Mo). The tubes, arranged in trays, were placed in a suspended bench inside the greenhouse, where the minimum and maximum values of temperature and relative humidity were recorded during the experimental period (Figure 2).

The plantlet production was conducted in three seasons: plantlets Season 1 started on January 16, Season 2 on May 18 and Season 3 on September 19, 2017. The management of plantlets in the greenhouse followed technical recommendations for coffee (Fonseca et al., 2017). Intermittent irrigation was performed using nebulizers with an automatic timer programmed to trigger the system for 5 seconds every 10 minutes for the first 40 days, for 10 seconds every 20 minutes from the 40th to the 80th days and for 30 seconds every 30 minutes from the 80th to the 150th days.

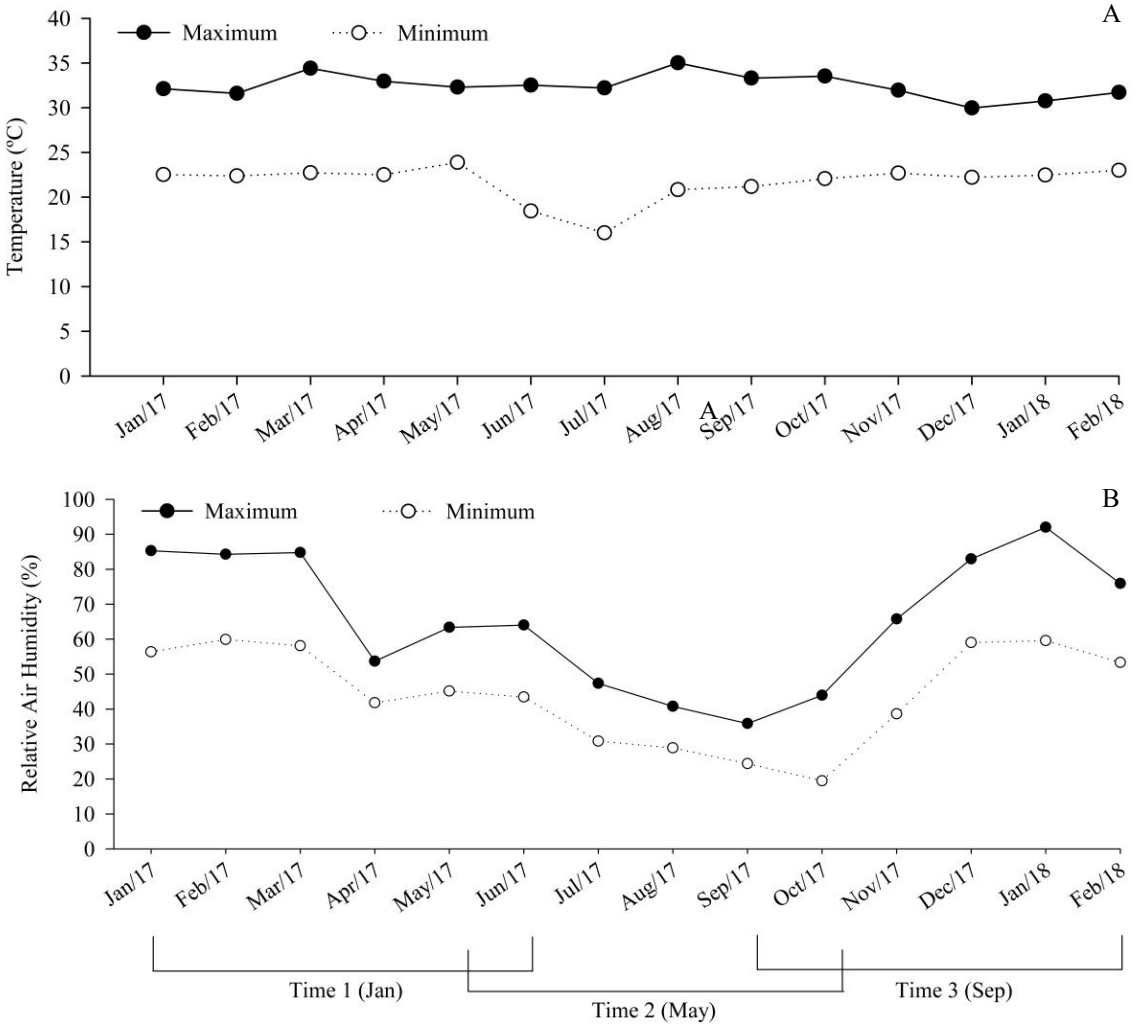

Figure 2: Maximum and minimum air temperature (A), maximum and minimum air relative humidity (B) recorded in the greenhouse during the experiment. Ouro Preto do Oeste, 2017.

Growth analysis: The experiment was conducted in a split plot scheme, 5 × 3, during a combination of five evaluation periods and three plantlets production periods. The evaluation periods were 47, 74, 100, 127 and 152 days after cutting sowing and the production seasons were: January, May and September 2017. It was used a completely randomized design, with 24 replications, and the experimental unit consisted of nine plantlets. During each evaluation period the Dry Mass Accumulation, the Absolute Growth Rate (AGR) and the Relative Growth Rate (RGR) were determined.

To determine the Dry Mass Accumulation, the plantlets, from each period, were washed, packed in paper bags and dried is a oven at 65 °C until a constant mass. The Dry Mass Accumulation was obtained by the difference between the dry mass on the day of evaluation and the initial dry mass of the cutting. The AGR and RGR were calculated based on the dry mass and the number of days between one assessment and another.

The AGR represents the variation or increment of growth, in milligrams per day, between two samples that corresponds to the average growth velocity over the observation period (Benincasa, 2003). The AGR was calculated as follows: $AGR = (W2-W1) / (T2-T1)$ mg day^{-1}; Where, W1 and W2 is the variation of dry mass in two consecutive samples taken at T1 and T2.

RGR expresses the increase in dry mass per unit of mass already existing over a period of time. It was calculated as follows: $RGR = (lnW2 - lnW1) / (T2 - T1)$ = mg g^{-1} day^{-1}, where ln is the neperian logarithm; W1 and W2 represent the dry mass at times T1 and T2.

Data were subjected to analysis of variance and F test at 5% probability with the aid of the GENES program and adjusted by regression with the aid of the Sigma Plot® program.

Physiological quality of plantlets: At 152 days after inserting the cuttings into the tubes, the following biometric components were measured: stem length (CC), determined by direct measurement of the point of insertion of the shoot

in the cut until the apical meristem; stem diameter (DC), determined with an electronic caliper, 2 cm above the point of insertion of the bud in the cutting; leaf area (FA), determined by free software DDA (Digital Area Determinator) (Ferreira et al., 2017) from digital images; root volume (RV), measured using a graduated cylinder, by verifying the difference of displaced volume; root dry mass (MSR), shoot dry mass (MSPA) and total dry mass (MST), determined by analytical balance after drying in a forced air oven at 65 ° C until reaching the state of constant mass; shoot root ratio (R / PA), obtained by dividing between MSR and MSPA and Dickson Quality Index (IQD) using formula IQD = [MST / [(CC / DC) + (MSPA / MSR)].

Data were subjected to Lilliefors test to assess normality. Data was transformed into √x to obtain normal distribution, followed by analysis of variance and F test at 5% probability. The averages were compared by Tukey test (p≤0.05), with the aid of the GENES® program.

3 RESULTS

3.1 Nutritional Composition

N, P and K contents were higher in cuttings collected in May, with an average of 27.48, 1.69 and 32.54 mg cutting^{-1}, respectively. Mg content was higher when cuttings were collected in May and September. For Ca and S content there was no difference between the collection times (Table 1).

3.2 Anatomy

The average thickness of the xylem, phloem and the vascular cylinder of the cuttings collected in January was greater than the cuttings collected in May and September, being 599.2 µm, 133.5 µm and 635.2 µm respectively (Figure 3, Table 2). For periderm thickness, there was no difference between cuttings collection times with an average of 84.84 µm.

Table 1: Nutrient content in vegetative propagule (cutting) for the production of clonal plantlets of *Coffea canephora* 'Conilon' coffee trees grown at different times of the year. Ouro Preto do Oeste, 2017.

Seasons	Nutrient content in the cutting					
	N	P	K	Ca	Mg	S
	---------- mg cutting^{-1} ----------					
Jan	20.63b	1.18b	29.26b	10.28a	1.67b	1.34a
May	27.48a	1.69a	32.54a	11.06a	2.19a	1.29a
Sept	21.66b	1.31ab	30.40ab	10.76a	2.07a	1.34a
Averages	23.25	1.39	30.73	10.7	1.97	1.32
CV (%)	14.22	18.91	13.82	25.86	24.91	23.78

Averages followed by the same letter in the column do not differ from each other by the Tukey test (P≤0.05).

Table 2: Anatomical characteristics of clonal cuttings of *Coffea canephora* 'Conilon' coffee trees grown at different times of the year, in Ouro Preto do Oeste, RO, 2017.

Seasons	XT	PHT	PT	VCT
	----------µm----------			
Jan	599.2a	133.5a	87.3a	635.2a
May	435.6b	109.1b	86.4a	553.6b
Sept	426.2b	104.26b	80.7a	543.4b
Averages	487	115.62	84.84	577.4
CV (%)	12.05	11.89	14.15	11.1

Averages followed by the same letter in the column do not differ from each other under the Tukey test (P≤0.05). EX = Xylem thickness; PHT = phloem thickness; PT = periderm thickness; VCT = vascular cylinder thickness.

3.3 Growth analysis

The plantlets produced in January presented sigmoid growth model for absolute growth rate (AGR) (Figure 4A). At this time, a slow growing phase was observed during the first

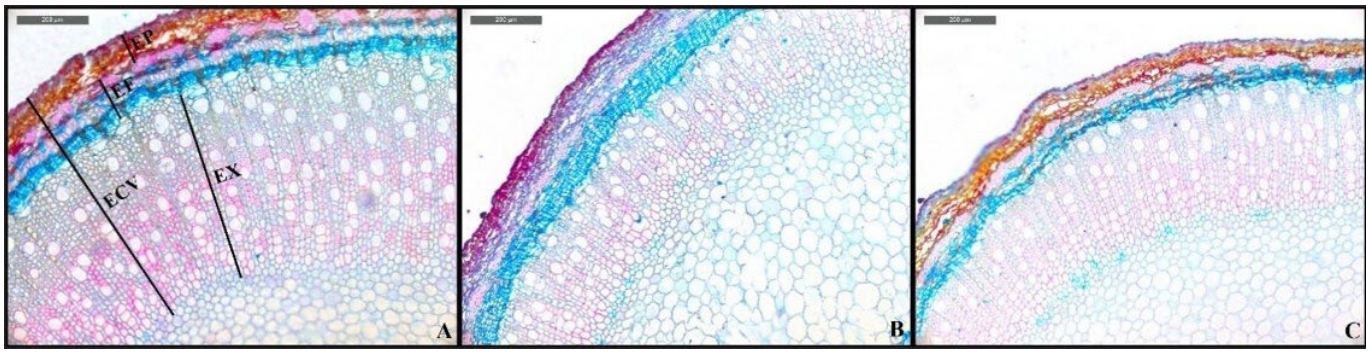

Figure 3: Cross section of *Coffea canephora* 'Conilon' cuttings collected at different times of the year. A - January; B - May; C September. Read: EX for xylem thickness; EF for phloem thickness; EP for peridermis thickness and ECV for vascular cylinder thickness. Ouro Preto do Oeste, 2017.

50 days, followed by a fast-growing phase, up to approximately 110 days followed by a stabilization phase. Differently from January, the plantlets produced in September presented a cubic model with positive and negative AGR peaks, while for the plantlets produced in May, it was not possible to adjust the growth model to the data obtained having established an average of 5.47 mg per day.

The relative growth rate (RGR) of the January plantlets followed a quadratic model, indicating increasing behavior with a maximum point at 100 days and a subsequent decrease at 152 days after staking (Figure 4B). For plantlets produced in September, the growth model was cubic with positive and negative RGR peaks, while for plantlets produced in May, it was not possible to adjust growth model for the obtained data, having established an average of 3.27 mg g^{-1} day^{-1}.

For plantlets dry mass accumulation, exponential models were adjusted in the three study periods. However, the plantlets produced in January showed higher dry mass accumulation when compared to other seasons, and this behavior was observed at 47 days after inserting the cuttings into the substrate. In relation to the other seasons, for the seedlings produced in May, despite the exponential behavior, the accumulation was constant throughout the growth phase, while the plantlets produced in September showed slow growth at 120 days with rapid increase of dry mass at the end of the cycle (Figure 4C).

3.4 Physiological quality of plantlets

The plantlets produced in January presented the highest values of, SL, SD, LA, RV, RDM, SDM, TDM, RDM/SDM and DQI when compared to the plantlets produced in May and September (Table 3).

4 DISCUSSION

4.1 Nutrient content of the cutting

Differences in the nutrient content of *C. canephora* cuttings, collected at different times, are related to the effect of climatic conditions on the development of the mother

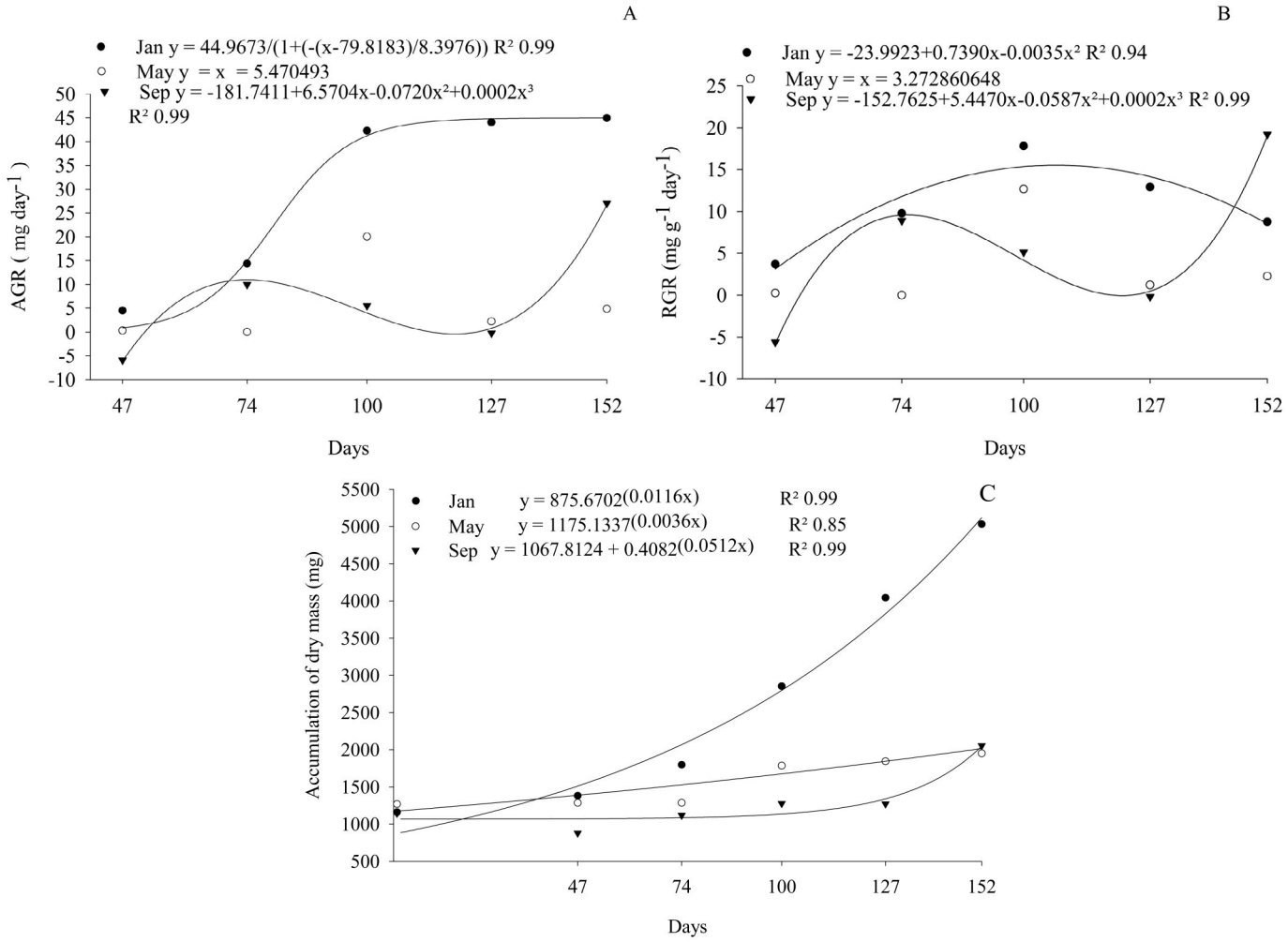

Figure 4: Absolute Growth Rate Curve (AGR) (A), Relative Growth Rate Curve (RGR) (B) and Dry Mass Accumulation Curve (C) of *Coffea canephora* clonal plantlets produced at different times of the year, Ouro Preto do Oeste, 2017.

plants that gave rise to cuttings. Furthermore, the mother plants were at a different stage of development, older, and there may also have been an effect of nutritional management prior to the implementation of the experiment. This is because different environmental conditions during the year promote seasonality of vegetative growth (Dubberstein et al., 2017) and, consequently, impact the accumulation of nutrients by plants.

Higher N, P and K contents observed during May can are associated with higher light (Secretaria de Desenvolvimento Ambiental- SEDAM, 2017) availability compared to January stakes and higher water availability compared to September stakes (Figure 1). Moreover, the presence of reproductive structures during the first and third seasons may also have contributed to the lower content of these nutrients in the cuttings of these periods.

Regarding cuttings grown at the beginning of the rainy season, from September to January, the lower luminosity of the period (SEDAM, 2017) may have resulted in greater stem etiolation, resulting in nutrient dilution in the tissues, compared to cuttings from the end of the rain, from January to May. In addition, the presence of fruits in the plagiotropic branches remaining after pruning in the months of September to November may have contributed to the lower nutrient concentration in the cuttings, since the fruits are priority drains and, by this time, the plants already present nutrient accumulation in fruits (Oliosi et al., 2020).

When comparing nutrient accumulation at the end of the rainy season, from January to May, and during the dry season, from May to September, the higher nutrient content in May cuttings may be associated with higher nutrient uptake due to greater mobility of these nutrients in the soil (Covre et al., 2018), resulting in higher nutrient concentration during this period. Also, the flowering of the plants observed in July and August may have contributed to the lower nutrient accumulation in the cuttings, since these reproductive structures act as the main nutrient drain (Dubberstein et al., 2019).

As for the Ca and S contents in cuttings collected at different times, the similarity suggests that if nutrients are available in the soil solution, stem accumulation is not affected by the presence of reproductive structures, as already observed in Conilon coffee trees grown in the state of Espírito Santo (Bragança et al., 2008). These authors attributed this fact to the reduced mobility of the nutrients, since after being absorbed by the roots most Ca is transported in the xylem, although a small part can be transported through the phloem under specific conditions.

4.2 Cutting Anatomy

Greater thickness of vascular tissues observed in cuttings harvested in January indicates changes in the stem structure of the orthotropic stems of the mother plants that gave rise to the cuttings. These changes in anatomy are associated with the presence of fruits in the remaining plagiotropic branches after pruning. As fruits become the plant's priority nutrient drains (Oliosi et al., 2020), the xylem and phloem conduction tissues undergo changes in their anatomy so that adequate water, nutrient and photoassimilate translocation occurs at this stage of higher nutritional demand.

On the other hand, the mother plants that gave rise to cuttings harvested in September (development period from May to September) usually undergo a period of thermal stress (Ramalho et al., 2018; Rodrigues et al., 2016) due to high temperatures and relative humidity reduction (Figure 1 A and B), while cuttings harvested in May (development period January to May) came from mother plants that developed in a period of lower solar radiation in the region (Instituto Nacional de Pesquisas Espaciais - INPE, 2018). Both situations may have affected the photosynthetic metabolism of these plants and, consequently, the growth of orthotropic stems and their anatomy.

Importantly, the anatomy of the stems that give rise to the cuttings can contribute positively or negatively to rooting. This is because the thickening of vascular tissues can influence the carbohydrate reserve, as well as influence the translocation of water, nutrients, hormones that are involved in the plantlet development process. In addition, the presence of continuous perivascular sclerenchyma sheath may act as an anatomical barrier to the emergence of the roots in coffee plants (Arantes et al., 2019).

In the present study, cuttings harvested in January developed thicker vascular tissues and sclerenchymatous tissue cells occurring in non-continuous bands around vascular tissues, thus not representing an anatomical barrier to rooting (Pimentel et al., 2020; Stevens; Pijut, 2017). Such changes in anatomical characteristics may have influenced the physiological quality of the plantlets, leading to higher means for biometric characteristics, Dickson Quality Index, as well as biomass accumulation by the plantlets (Table 3).

4.3 Growth analysis

The sigmoidal behavior of the absolute growth rate (AGR) of the plantlets observed in January was marked by slow initial growth during the first 50 days after due to the absence of absorbent roots. After this period the roots and leaves appear, and rapid growth begins. At this stage, the plant begins to absorb water and nutrients from the substrate and initiates photosynthesis-dependent anabolic processes as well as the production of auxins essential for root growth (Taiz et al., 2017). Finally, in the final phase of the plantlets cycle, the stabilization of the AGR and reduction of the RGR was observed, possibly due to the fact that the substrate volume (Espindula et al., 2018) or nutrient availability started to limit plant growth (Maekawa et al., 2020).

It was not possible to adjust a representative curve for absolute and relative growth rate data for the plantlets produced in May, possibly because the low relative humidity of the nursery (Figure 2) had a negative influence on plantlet development. At relative air humidity to levels below 50%, as recorded in this study, any minimal change in temperature causes changes in the plantlet's water content and increases transpiration, resulting in higher photoassimilate waste, thus impairing plantlet the development (Pereira et al., 2019b).

The lower accumulation of dry mass during the initial development stage of the plantlets produced in September may be related to environmental stress caused by problems in the nursery's nebulization system water. In this period, the relative humidity of the air was below 50% (Figure 2), which led to the dehydration of the cuttings. As in this initial stage the plantlets do not have the means to absorb water and nutrients from the substrate, the plantlets probably spent their reserves to compensate for the increased transpiration and this, consequently, compromised the rooting process (Pereira et al., 2019a) and later plantlets development. In fact, to preserve the turgidity of the propagules, it is recommended to keep the air humidity above 80%, as they do not have the means to absorb water and nutrients (Dias et al., 2015).

4.4 Physiological quality of plantlets

The plantlets produced in January presented higher physiological quality than the plantlets produced in May and September, a fact evidenced by the higher values of the seedling biometric characteristics (Table 3). However, although plantlets quality is associated with plantlets physiological quality, the results in this study indicate that the lower physiological quality of plantlets produced in May and September are probably related to the low relative humidity of the greenhouse in the most critical period before rooting. For this period, the relative humidity considered ideal for the clonal propagation of *C. canephora* is close to 100% (Fonseca et al., 2017).

Humidity is one of the determining factors for the process of rooting, since a high water potential is needed for cell division and mainly for cell expansion (Taiz et al., 2017). Thus, high air humidity favors the reduction of transpiration and, consequently, the loss of water from the cuttings. In this sense, it is possible to admit that the relative humidity in the greenhouse, below 70% before the cuttings have roots, had a negative influence on the quality of *C. canephora* plantlets. This is because low humidity may have delayed the beginning of rooting and, therefore, delayed the growth of the plantlets. Studies show that the control of the environmental conditions within the nursery is essential for maintaining the survival of these vegetative propagules (Dias et al., 2015; Giuriatto Júnior et al., 2020).

The plantlets produced in January showed a Dickson quality index (IQD) higher to those reported for culture (Berilli et al., 2020a; Berilli et al., 2020b), while the plantlets produced in May and September showed IQD lower than the reference. However, the lowest IQD found in this study was higher than the highest IQD reported for *C. canephora* clonal plantlets produced in different tube volumes, even for treatment using a 280 cm³ tube (Espindula et al., 2018). Thus, the greater development of the plantlets produced in January reveals that the management of the greenhouse was the determining factor for the quality of the seedlings and that the nutritional and anatomical factors of the cuttings, although important to the quality of the cuttings, should not be analyzed in isolation.

5 CONCLUSIONS

The levels of N, P, K, Ca and Mg content of *C. canephora* cuttings vary according the times of the year that they are collected.

The thickness of the xylem and the vascular cylinder vary according to the time of the year they are produced.

Nutrient content and anatomical aspects xylem and vascular cylinder of the vegetative propagule may result in different vegetative performances of *C. canephora* clonal plantlets. However, this result seems to be more dependent on the management of the environmental conditions of the greenhouse, especially temperature and relative air humidity.

6 AUTHORS' CONTRIBUTION

Conceptual Idea: Bazoni, P.A.; Espindula, M.C.; Methodology design: Espindula, M.C.; Vasconcelos, J.M.; Data collection: Bazoni, P.A.; Ton Giuriatto Júnior, J.J.; Data analysis and interpretation: Espindula, M.C.; Aráujo, L.F.B.; Campanharo, M.; and Writing and editing: Bazoni, P.A.; Espindula, M.C.; Aráujo, L.F.B.; Campanharo, M.

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