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Clonal cutting production by *Coffea canephora* mother plants under increasing nitrogen doses[1]

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ABSTRACT

This study aimed to establish production curves of cuttings for *Coffea canephora* coffee plants in response to nitrogen doses. A clonal garden of the botanical variety Robusta was used to evaluate the effects of seven nitrogen doses divided into four applications. The subplots corresponded to two evaluation periods: January and June 2019. The following traits were evaluated: number of stems, number of viable cuttings, number of cuttings per stem, cutting fresh and dry mass, and cutting macronutrient contents. The results showed that *C. canephora* produced a higher amount of vegetative mass and propagules during the period from September to January compared to the period from January to June. Nutrient concentrations in the tissues varied with the period of the year due to soil nutrient availability and the amount of accumulated dry mass. Increasing nitrogen doses resulted in higher vegetative and propagules mass associated to higher nitrogen and potassium concentrations in the cuttings; but had no effect on phosphorus, calcium, magnesium, and sulfur contents. Nutrient contents in the cuttings followed the order K>N>Ca>P>Mg>S regardless of the applied nitrogen dose.

Keywords: robusta; conilon; clonal garden; nutrients.

INTRODUCTION

Clonal garden, where mother plants are grown, is an important component of the structure of rural enterprises destined to the seedling production of allogamous woody species. Typically, these areas are composed of mother plants selected according to traits of interest such as productivity and resistance to diseases and water deficit aiming at the production of vegetative propagules throughout the year for seedling production.[1] The vegetative shoot production at the expense of fruits is more important in these fields, as it is the part of commercial interest, in which vegetative propagules are taken for seedling production.

Allogamy and the consequent gametic segregation in *Coffea canephora* give rise to heterogeneous offspring that hinder crop management.[2,3] Thus, vegetative propagation has been essential for productivity gains and the success of cultivations.[4,5] It occurs because crop fields formed from seedlings that were originated through vegetative propagation have characteristics of uniformity and precocity during formation and production.[6]

Cuttings are the main vegetative propagation technique used in the seedling production of *C. canephora* coffee due to rapid, large-scale multiplication[4] ensuring the preservation of traits of interest from the mother plants. However, the sequential removal of vegetative parts for cutting production is responsible for the export of essential nutrients to the plant.[7,8] These nutrients, in addition to being exported, can be and lost in the system and need to be replaced.

The nutritional management of clonal garden plants must be carried out to supply the physiological needs to guarantee the growth and development of mother plants and propagule production. Nitrogen (N) stands out among the essential nutrients for being the most required in quantity by coffee plants at all vegetative stages.[3,9,10] Moreover, the vegetative growth of *C. canephora* in the Amazon is influenced by climate conditions (rainfall and temperature), which potentiate the growth process.[11]

Despite all the benefits provided by N to the vegetative growth of coffee plants, the excess of this nutrient can negatively influence the plants by causing a nutritional imbalance in its relationship with other elements, leading to lower sugar content and excessive growth[12], which can compromise the physiological quality of cuttings.

Thus, considering the importance of information regarding nutritional management for adequate conduction of clonal gardens and the current recommendations of cultural management based on fruit production, this study aimed to establish production curves of clonal cuttings for *C. canephora* coffee plants in response to increasing nitrogen doses.

MATERIAL AND METHODS

The experiment was carried out from August 2018 to June 2019 at EMBRAPA Experimental Field (10°43′55″ S and 62°15′19″ W, with an altitude of 245 m) located in Ouro Preto do Oeste, Rondônia. The predominant climate in the region is Aw (Köppen), i.e., a rainy tropical climate[13] with an average annual temperature of 25 °C and an average rainfall of 2,000 mm year^{-1}. The rainy season is from October-November to April-May.

The average values of air temperature, relative air humidity, and rainfall during the experimental period were obtained through an automatic weather station installed at the Experimental Field (Figure 1A, B, and C). Complementary irrigation was performed using a conventional sprinkler irrigation system to replace the water lost through evapotranspiration during the dry months (Figure 1C).

The experimental area was being cultivated with a coffee crop *C. canephora* of the Robusta botanical variety belonging to the Genetic Breeding Program of Embrapa-RO. The coffee field was planted in November 2016 in reduced spacing, 2 meters between rows and 0.5 meters between plants, and the plants were conducted with only one orthotropic stem, which was not bent to induce sprouting, by bending, as is commonly used. In this garden, the objective is to explore the entire length of the stem in the vertical direction, therefore, the new system, which is in the final stage of development, will be called "Vertical clonal garden" (Figure 2). This new model allows up to three harvests of clonal stakes per year and facilitates the crop cultivation.[4]

The first sprout harvest was carried out in December 2017, 13 months after the area was implemented, for clonal cutting production. The second sprout harvest was performed in April 2018, while the third harvest was carried out on August 20, 2018, before the experiment setup. The average cuttings production reached 11.07, 19.97, and 35.91 in the first, second, and third sprout harvest, respectively. In addition to sprout harvest, 33% of plagiotropic stems were removed from the plants at the harvest time and 60 days later.

The nutritional management considered technical recommendations for crops intended for fruit production.[14] The

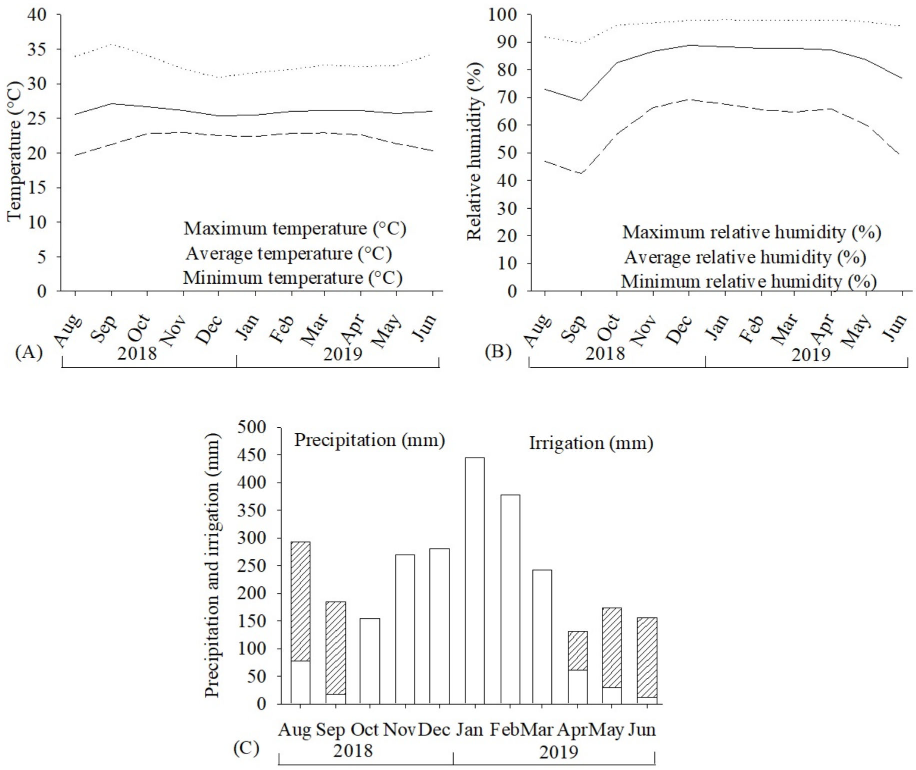

Figure 1: Maximum, average, and minimum air temperatures (A), relative air humidity (B), and accumulated precipitation and complementary irrigation (C) during the experimental period of 2018 and 2019. Ouro Preto do Oeste, Rondônia, Brazil, 2019.

last nitrogen fertilization before the treatments was applied was carried out on July 20, 2018, when 135 kg ha^{-1} N was applied in the form of urea, the last fertilization of the third sprouting cycle.

The soil of the area is a Red-Yellow Argisol[15] and its chemical attributes were determined, in August 2018, before the implementation of treatments, at depths of 0–20, 20–40, and 40–60 cm (Table 1).

A total of 1,000 kg ha^{-1} of dolomitic limestone (90% total neutralizing power) was applied in September 2018. The limestone was applied in a localized way covering an area of 0.5 m within the row and 1.0 m towards the inter-rows, 0.5 for each side of the plant, totaling 0.5 m^2. In this area, 0.5 m^2, 50g of limenstone was applied.

The experiment was carried out in a split-plot scheme in time, consisting of a combination of seven nitrogen doses and two evaluation periods. The main plots were composed of doses of 0, 50, 100, 150, 200, 250, and 300 kg nitrogen ha^{-1} $cycle^{-1}$ applied in the form of urea. The subplots consisted of the two evaluation periods: January (cuttings grown from August to January) and June 2019 (cuttings grown from January to June).

The growth cycles lasted 150 days and comprised the period from August 20, 2018, to January 18, 2019, for the first cycle (January harvest) and January 18, 2019, to June 18, 2019, for the second cycle (June harvest).

The nitrogen doses were divided into four applications carried out every 30 days within each cycle. The application started 30 days after the initial standardization, performed on August 20, 2018, in the first cycle and 30 days after the cutting harvest, carried out on January 18, 2019, in the second cycle. Moreover, 120 kg potassium ha^{-1} $cycle^{-1}$ was applied using potassium chloride (60% K_2O), being split into four applications together with nitrogen. Also, 30 kg of boric acid and 30 kg zinc sulfate were applied per hectare in October 2018 to meet the demands for zinc (Zn) and boron (B).

Production of secondary orthotropic stems and cuttings

All secondary orthotropic stems (sprouts) were collected in each evaluation period (January and June) to determine the vegetative characteristics related to the cutting production. The number of secondary orthotropic stems, number of viable cuttings per plant, and fresh and dry mass of these stems were measured.

Figure 2: Coffee plants grown in a clonal garden system. (A) Before and (B) after harvesting sprouts to produce clonal cuttings. Ouro Preto do Oeste, Rondônia, Brazil, August 2018.

The number of viable cuttings was determined considering those that had a characteristic for seedling formation. Therefore, the basal and apical parts of the stems were disregarded, and cuttings that had a pair of leaves and plagiotropic stems, with 7 cm in length (0.5 cm above the plagiotropic stem insertion and 6.0 cm below the node, that is, leaf insertion) were counted.[7]

The stem fresh mass was determined using an analytical balance (0.001 g of precision) immediately after the stems were harvested. The dry mass was also determined on an analytical balance after drying in a forced-air circulation oven at 65 °C until constant mass. The experimental design at this stage consisted of random blocks with information within the plot with two replications. The experimental plot consisted of seven plants in a sequence within the planting row. However, each plant was considered as a replication for statistical purposes, excluding the borders and the first and last plan of the plot.

Macronutrient content in clonal cuttings

The nutritional analysis was performed in each replication using two cuttings taken from the stems collected in the field with straight cuts, according to the dimensions mentioned above, excluding leaves and plagiotropic stems, totaling 20 cuttings. These cuttings were homogenized aiming to form four replications with five cuttings each, being dried in a forced-air circulation oven at 65 °C until constant mass, ground in a Willey mill, and sent to the Laboratory of Soil and Plant Nutrition of Embrapa Rondônia. The experimental design at this stage was completely randomized, with four replications.

The nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S) contents were determined as follows: N was determined by distillation using the semi-micro Kjeldahl method after sulfuric digestion; P by molecular spectrophotometry; K by flame photometry and Ca, Mg, and S by plasma spectrophotometry, all of them after nitroperchloric digestion.[16] The results were expressed in g kg^{-1} of plant material.

Statistical analysis

The data were subjected to analysis of variance ($p < 0.05$). The Tukey test (< 0.05) was performed when there was a significant effect to compare the means of different periods and regression analysis for the effects of nitrogen doses. Mathematical models were chosen for the regression analysis according to the behavior of the biological phenomenon and the highest values of the coefficients of determination (r^2/R^2) and the significance of the regression coefficients (βi) and the F-test of regression, both up to a 5% probability.

Table 1: Chemical attributes of a Red-Yellow Argisol from the clonal garden of the Experimental Field of Embrapa, Ouro Preto do Oeste, Rondônia, Brazil, 2018

Sample	pH	P	K	Ca	Mg	H+Al	Al	CEC
cm	Water	mg dm^{-3}	cmol$_c$ dm^{-3}					
0–20	4.36	53.13	0.35	1.11	0.76	8.01	0.88	10.24
20–40	4.63	13.75	0.36	1.02	0.45	4.81	0.54	6.64
40–60	4.98	5.50	0.45	1.59	0.53	3.37	0.05	5.94
Sample	**OM**	**m**	**V**	**Cu**	**Fe**	**Mn**	**Zn**	
cm	g kg^{-1}	%		mg dm^{-3}				
0–20	15.20	31.00	21.13	42.61	230.16	119.27	49.98	
20–40	7.70	25.50	28.00	19.38	247.83	120.34	46.24	
40–60	4.95	2.50	43.00	15.75	159.01	119.74	40.98	

pH in water 1:2.5; OM determined by wet digestion; P and K determined by the Mehlich I method; Exchangeable Ca, Mg, and Al extracted with 1 mol L^{-1} KCl.

RESULTS AND DISCUSSION

Production of secondary orthotropic stems

The number of secondary orthotropic stems in January was similar to that in June, regardless of the dose. However, the average stem fresh and dry mass, the average number of viable cuttings, and the number of cuttings per stem in January were higher than in June (Table 2).

Variations in the vegetative growth of *C. canephora* reported in other studies are generally associated with genetic factors,[5,17] nutritional condition of plants,[1,9] and climate conditions,[18,19] mainly the water deficit.[11,20] These variations directly reflect the formation of stems for cutting production, as these plants are grown exclusively for producing vegetative and non-reproductive biomass.

The similar number of secondary orthotropic stems found in the two harvest periods indicates that the capacity to emit new stems under the West Amazon conditions is not influenced by the time of year if there is supplementary irrigation during the dry season (Figure 1) to minimize the negative effects of water stress on the physiology of coffee plants.[4,19,21,22]

On the other hand, the fresh and dry mass of stems of the plants evaluated in January was higher than in June even though no difference was observed regarding the number of orthotropic stems. These differences are possibly related to the effect of seasonal climate variations, such as temperature[23] and relative humidity,[11] on the growth of plagiotropic and orthotropic stems.[7] Higher water availability and air humidity and lower thermal amplitude were observed in the last three months preceding the January evaluation (November, December, and January) than in the months preceding the June evaluation (Figure 1A and B).

Mother plant growth under May and June conditions may have been affected due to the reduction in the nutrient absorption rate, which is compromised by a reduction of plant transpiration when there are high temperatures associated with lower relative humidity and soil water content availability.[24]

In addition to the influence of climate conditions on growth, plants evaluated in January may have been benefited from the residual effect of nitrogen fertilization on July 20, when 135 kg of N were applied in the form of urea. Zinc and boron, applied in October 2018, may have favored plant growth in the first evaluation period, but not during the cycle of plants evaluated in June. In this case, B participates in several processes, such as cell elongation and wall formation, while Zn is part of the synthesis of indoleacetic acid (IAA), which is the hormone responsible for growth.[25]

Also, B and Zn are nutrients required by coffee plants at the vegetative and reproductive growth stages[26-28] The supply of B and Zn increases productivity,[29] and their deficiency reduce growth, the number of plagiotropic stems, leaf area, and dry mass.[30] Thus, the effect of climate conditions and the residual effect of nitrogen and fertilization with B and Zn may have contributed to higher vegetative growth and production of viable cuttings from plants evaluated in January than those evaluated in June (Table 2).

A linear increase as a function of N doses was observed at both periods of evaluation for the number of stems per hectare (Figure 3A) and the number of cuttings per hectare (Figure 3B). The fresh mass of secondary orthotropic stems increased linearly as a function of N doses in January, but presented a quadratic behavior in June, with the maximum point at the dose of 258 kg ha^{-1} (Figure 3C). Dry mass presented a quadratic behavior in both periods with a maximum point at the dose of 198 kg ha^{-1} in January and 272 kg ha^{-1} in June (Figure 3D). Finally, the number of cuttings per stem showed an increasing linear response in January, but a mathematical model could not be adjusted in June and an average of 2.35 cuttings per stem was considered (Figure 3E).

The increase in the vegetative characteristics of plants (Figure 2A, B, and C) is related to the effect of N on important physiological processes.[25] However, although initially N doses provided increments in dry mass, the responses were quadratic with a maximum accumulation point at the dose of 198 kg ha^{-1} in January and 272 kg ha^{-1} in June (Figure 2D). A decrease in the dry mass increment was observed from these doses. It happens because high N doses hinder protein synthesis[12] and induce excessive plant growth, which causes self-shading by reducing solar radiation within the canopy and hence photosynthesis, that is, reduction in photoassimilate production and crop yield.[31]

Still, the growth showed that the productivity varied from 4,000 kg ha^{-1} to more than 6,000 kg ha^{-1} of dry mass of orthotropic stems per cutting cycle. This value is similar to that exported in fruit production, around 3,000 kg ha^{-1} (Ramalho et al.[6]) to 6,000 kg ha^{-1} (Silva et al.[32]), demonstrating the need for replacement of nutrients, especially nitrogen, which is the most accumulated nutrient in the vegetative aerial part for cutting production.[7]

Table 2: Number of stems, number of clonal cuttings, stem fresh and dry mass production, and number of cuttings per stem in *Coffea canephora* coffee plants grown in a clonal garden system at different periods and under different nitrogen doses. Ouro Preto do Oeste, Rondônia, Brazil, 2019

Period	**N doses (kg ha^{-1})**							**Mean**
	0	**50**	**100**	**150**	**200**	**250**	**300**	
Number of secondary orthotropic stems per hectare (CV = 20.52%)								
January	193,500a	200,000a	205,000a	205,000a	214,166a	211,500a	223,750a	207,559a
June	168,750a	185,000a	192,000a	205,250a	213,333a	219250a	216,000a	199,940a
Number of cuttings per hectare (CV = 18.14%)								
January	487,500a	531,000a	546,250a	533,750a	605,000a	601,750a	662,500a	566,821a
June	383,750b	407,500b	467,500b	493,750a	495,000b	503,750b	534,000 b	469,321b
Fresh mass of secondary orthotropic stems (kg ha^{-1}) (CV = 16.90%)								
January	19,665a	21,090a	21,829 a	22,200a	22,050a	24,240a	24,944a	22,288a
June	11,558b	11,917b	13,700b	14,987b	15,212b	14,987b	15,195b	13,936b
Dry mass of secondary orthotropic stems (kg ha^{-1}) (CV = 23.81%)								
January	4,563a	4,763a	5,626a	6,016a	6,215a	5,613a	5,640a	5,491a
June	4,006a	4,315a	4,770b	5,111b	5,558a	5,145a	5,425a	4,904b
Number of cuttings per stem (CV = 20.16%)								
January	2.52a	2.66a	2.66a	2.60a	2.82a	2.85a	2.96a	2.73a
June	2.27a	2.20a	2.43a	2.41a	2.32a	2.30b	2.47a	2.35b

*Means followed by the same letter in the column do not differ from each other by the Tukey test at 5%.

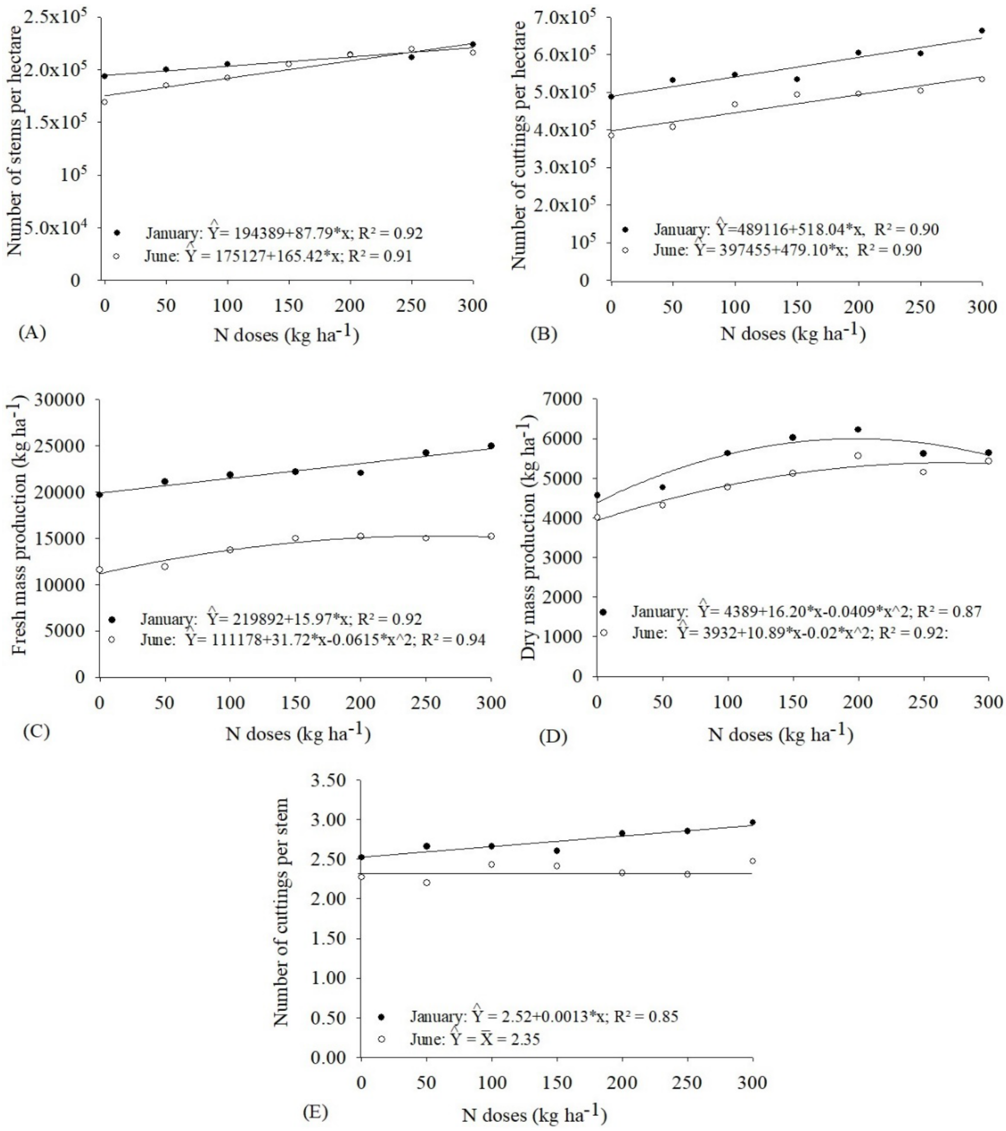

Figure 3: Number of stems, number of clonal cuttings, stem fresh and dry mass production, and number of clonal cuttings per stem of *Coffea canephora* coffee plants grown in a clonal garden system at different periods and under different nitrogen doses. Ouro Preto do Oeste, Rondônia, Brazil, 2019.

Macronutrient content in clonal cuttings

The average N and S contents in the cuttings of January were similar to those of June. The average K values were higher in January, while the average P, Ca, and Mg contents were higher in June. Potassium had the highest content in the cuttings, followed by nitrogen and calcium. (Table 3).

The similarity between periods of evaluation for nitrogen and sulfur may be related to their high mobility in the soil.[33] The highest P, Ca, and Mg contents present in cuttings in the evaluation carried out in June are possibly associated with the higher availability of these nutrients after a reaction of the limestone applied in September 2019. Limestone provides calcium and magnesium and neutralizes aluminum and, consequently, increases phosphorus availability to plants.[34] However, the availability of these nutrients influenced the second period with higher intensity because this corrective reacts slowly in the soil.

The higher potassium concentration in cuttings confirms the importance of this nutrient for coffee plants destined for clonal cutting production.[7] This nutrient can contribute to the humidity maintenance in vegetative propagules during the rooting stage[35] in the nursery. The higher K concentration in cuttings harvested in January may be associated with less competition for cation absorption since low Ca and Mg concentrations were observed in the tissues of cuttings at this period. It occurs because K has preferential absorption among cations, as it is monovalent and has a lower degree of hydration and, therefore, it is absorbed in greater quantity by plants. Also, Ca and Mg concentrations have a trend to reduce when the K contents increase in the plant,[36] which is the result of an antagonistic effect.

Nitrogen doses promoted a linear increase in N contents and a quadratic behavior in K contents (maximum point at 283 kg ha^{-1}) in cuttings harvested in January. On the other hand, a mathematical model could not be adjusted for these nutrients regarding the data obtained in June. Thus, an average of 13.43 g kg^{-1} was accepted for N content and 17.85 g kg^{-1} for K content (Figures 4A and 4C).

The data of the P, Ca, Mg, and S contents in the cuttings did not allow adjusting mathematical models in both periods of evaluation (Figure 4B, D, E, and F). Therefore, the behavior was represented by the average value of each nutrient in each period.

The effects of N doses on the increments in N and K contents in the cuttings in January (Figure 4A and 4C) may be related to the higher movement of ions in the soil as a function of the higher water availability in the period (Figure 1). In this case, the higher movement leads to a higher contact between roots and nutrients, which may increase their absorption. A study carried out on Red-Yellow Latosol under the Amazon conditions showed that the concentration of the NH^{4+} ion decreased exponentially with an increase in irrigation in the first 15 days after urea application.[37] These authors attribute the decrease in ammonium concentration to an increase in the nitrification process and leaching of nitric forms in the soil. The lower soil water availability associated with higher air temperature and lower relative air humidity in the second period may have provided higher nutrient concentration in the application zone, limiting the contact of the roots and, consequently, the absorption.

Similar to nitrogen, increases in potassium contents in January and the lack of effect in June may be related to the movement of this ion in the soil. In this case, potassium was applied together with nitrogen during all applications, and its mobility in tropical soils increases with an increase in soil water availability, as it is a monovalent cation.[38]

The lack of response to an increase in nitrogen doses (Figure 4B, D, E, and F) indicates that P, Ca, Mg, and S contents showed no changes in the cutting tissues, regardless of the higher vegetative growth (Figure 3C and D).

Table 3: Macronutrient contents in clonal cuttings of *Coffea canephora* coffee plants grown in a clonal garden system at different periods and under different nitrogen doses. Ouro Preto do Oeste, Rondônia, Brazil, 2019

Period	N doses (kg ha^{-1})							Mean
	0	50	100	150	200	250	300	
Nitrogen (g kg^{-1}) (CV = 11.90%)								
January	11.18a	12.09b	14.09a	14.82a	14.49 a	15.38a	16.59a	14.09a
June	11.33a	13.92a	15.17a	12.82b	12.13b	14.06a	14.59b	13.43a
Phosphorus (g kg^{-1}) (CV = 7.54%)								
January	1.35b	1.23b	1.47a	1.41b	1.34a	1.32a	1.36a	1.36b
June	1.62a	1.50a	1.62a	1.61a	1.27a	1.44a	1.41a	1.49a
Potassium (g kg^{-1}) (CV = 11.07%)								
January	19.43a	19.73a	24.50a	24.53a	26.00a	25.60a	25.80a	23.65a
June	16.88a	19.50a	19.43b	16.65b	15.38b	18.50b	18.60b	17.85b
Calcium (g kg^{-1}) (CV = 9.09%)								
January	3.36b	3.60a	2.91a	2.96a	3.29a	2.83a	2.89a	3.12b
June	4.50a	4.15a	3.79a	3.51a	3.70a	3.64a	3.91a	3.89a
Magnesium (g kg^{-1}) (CV = 6.14%)								
January	0.69b	0.64b	0.67b	0.77b	0.75b	0.71b	0.76b	0.71b
June	1.09a	1.05a	1.06a	1.06a	0.99a	0.98a	0.93a	1.02a
Sulfur (g kg^{-1}) (CV = 10.23%)								
January	0.48a	0.48a	0.55a	0.52a	0.52a	0.51b	0.55b	0.52a
June	0.50a	0.53a	0.54a	0.57a	0.56a	0.71a	0.65a	0.58a

*Means followed by the same letter in the column do not differ from each other by the Tukey test at 5%.

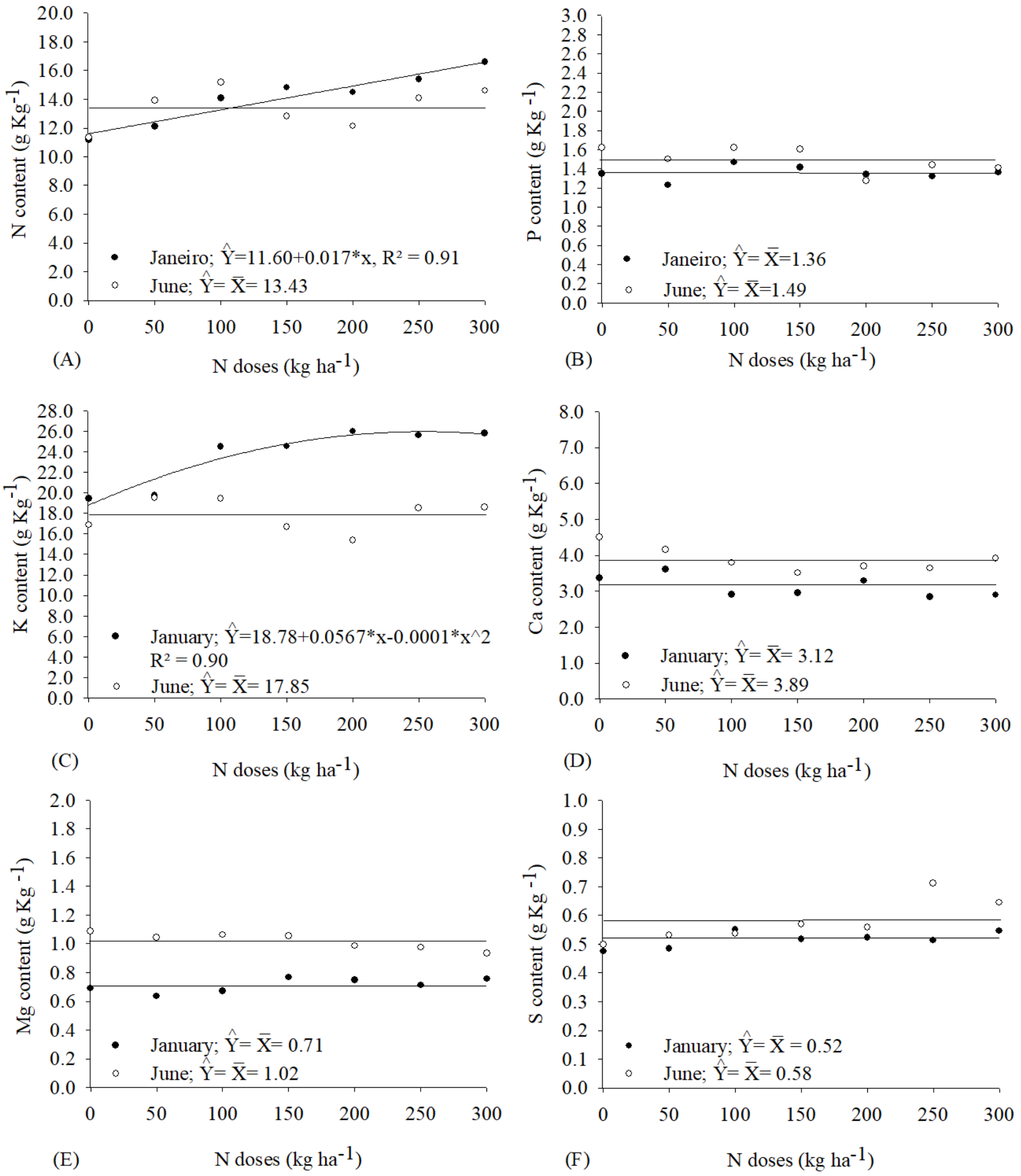

Figure 4: N, P, K, Ca, Mg, and S contents in clonal cuttings of *Coffea canephora* coffee plants grown in a clonal garden system at different periods and under different nitrogen doses. Ouro Preto do Oeste, Rondônia, Brazil, 2019.

It may be related to the availability of these nutrients in soils at a sufficient quantity to meet the plant demand (Table 1) and the Ca and Mg supply by limestone.

The sequence for the nutrient content in the cuttings followed the order K>N>Ca>P> Mg>S, regardless of the applied N dose and evaluation period. It differs from the requirement in the order N, K, and Ca for leaves and fruits[3,9,10,39] and vegetative shoot.[7] In this case, the possibility of having an excess uptake is considered, as the nutrient was highly available in the soil (Table 1). However, K presented the highest content in the cuttings, which reinforces the indication that this nutrient should not be neglected during the nutritional management of clonal garden plants. In this study, the demand for potassium was high for cutting production although this nutrient is usually associated with fruit filling.

CONCLUSIONS

C. canephora coffee plants produced a higher amount of vegetative mass from September to January and, consequently, vegetative propagules, compared to the period from January to June under the South-West Amazon conditions.

Nutrient concentrations in the tissues varied with the period of the year due to the soil nutrient availability and amount of accumulated dry mass.

The increased nitrogen doses resulted in an increase in vegetative mass, propagule mass, and nitrogen and potassium concentrations, not affecting phosphorus, calcium, magnesium, and sulfur contents in the vegetative propagules of *C. canephora*.

Nutrient contents in the cuttings followed the order K>N>Ca>P>Mg>S regardless of the applied N dose.

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