Research, Society and Development, v. 11, n. 6, e1311628591, 2022
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v11i6.28591

Diagnosis of the nutritional status of coffee tree according to fruit phenology

Diagnóstico do estado nutricional do cafeeiro em função da fenologia de frutos

Diagnóstico del estado nutricional del café según fenología del fruto

Received: 03/28/2022 | Reviewed: 04/07/2022 | Accept: 04/13/2022 | Published: 04/18/2022

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Abstract
The appropriate time for leaf collection to evaluate the nutritional status of the coffee tree should be carried out when the nutrient is in higher levels in the leaves, which may vary according to the phenological stage of the fruits. Macro and micronutrients do not show the same pattern regarding the period of highest leaf content. Therefore, it is coherent to define different times of leaf collection for each nutrient. The objective of this study was to verify the most suitable times for collecting leaves to evaluate the nutritional status of coffee plants based on the phenological development of coffee fruits. The experiment was carried out in a commercial field, in which six coffee cultivars were used (Acauã Novo, Bourbon Amarelo, Catuaí Vermelho IAC 144, Catucaí 20/15, IAC 125 RN and IPR 100). The collections were carried out in four phenological phases of coffee fruit development (floral bud maturation, chumbinho, fruit expansion, and fruit granulation). The periods with the highest concentrations varied based on the phenological stages of the fruits, and differences in concentrations were also observed between the cultivars examined. The highest zinc concentration in the leaves was observed in the maturation phase of the floral bud. Foliar collection for the diagnosis of the nutritional state of the coffee plant should be done for each nutrient based on the phenology of the fruits.
Keywords: Leaf diagnosis; Nutritional status; Phenological stage; *Coffea arabica*.

Resumo
A época adequada para coleta de folha para avaliação do estado nutricional do cafeeiro deve ser realizada no período em que o nutriente esteja em maiores teores nas folhas, o que pode variar de acordo com a fase fenológica de frutos. Os macro e micronutrientes não apresentam o mesmo padrão quanto ao período de maior teor foliar, portanto, é coerente definir épocas diferentes de coleta de folhas para cada nutriente. Objetivou-se com este estudo verificar épocas mais adequadas para coleta de folhas para avaliação do estado nutricional do cafeeiro com base no desenvolvimento fenológico de frutos de café. O experimento foi instalado em lavoura comercial, no qual foram empregadas seis cultivares de café (Acauã Novo, Bourbon Amarelo, Catuaí Vermelho IAC 144, Catucaí 20/15, IAC 125 RN e IPR 100). As coletas foram realizadas em quatro fases fenológicas de desenvolvimento de frutos do cafeeiro (maturação das gemas

florais, chumbinho, expansão dos frutos e granação dos frutos). Os períodos com as maiores concentrações variaram com base nos estádios fenológicos dos frutos, e também foram observadas diferenças nas concentrações entre as cultivares examinadas. A maior concentração de zinco nas folhas foi observada na fase de maturação da gema floral. A coleta de folhas para o diagnóstico do estado nutricional do cafeeiro deve ser realizada para cada nutriente com base na fenologia dos frutos.
Palavras-chave: Diagnose foliar; Estado nutricional; Estádio fenológico; *Coffea arábica*.

Resumen
La época ideal en recolectar hojas para evaluar el estado nutricional del café debe realizarse en el período en que el nutriente se encuentra en niveles más elevados en las hojas, lo que puede variar según el estado fenológico de los frutos. Los macro y micronutrientes no presentan el mismo patrón en cuanto al período de mayor contenido foliar, por lo que es coherente definir diferentes épocas de recolección de hojas para cada nutriente. El objetivo de este estudio fue verificar las épocas más adecuadas de recolección de hojas para evaluar el estado nutricional de la planta de café en base al desarrollo fenológico de los frutos. El experimento se realizó en un campo comercial, en el que se utilizaron seis cultivares de café (Acauã Novo, Bourbon Amarelo, Catuaí Vermelho IAC 144, Catucaí 20/15, IAC 125 RN e IPR 100). Las colectas se realizaron en cuatro fases fenológicas del desarrollo del fruto del café (maduración del botón floral, chumbinho, expansión del fruto y granulación del fruto). Los períodos con las concentraciones más altas variaron con base en los estados fenológicos de los frutos, y también se observaron diferencias en las concentraciones entre los cultivares examinados. La mayor concentración de zinc en las hojas se observó en la fase de maduración del botón floral. La recolección de hojas para el diagnóstico del estado nutricional del café se debe realizar para cada nutriente en base a la fenología de los frutos.
Palabras clave: Diagnóstico foliar; Estado nutricional; Estadio fenológico; *Coffea arabica*.

1. Introduction

The collection of leaves for the diagnosis of the nutritional status of the coffee tree is carried out between the phenological stages of flowering and lead; however, the nutrient content in plant tissues may vary between cultivars, phenological stage of the fruits, plant age, and in different organs of the same plant (Bragança et al., 2007; Martinez et al., 2003). Short cycle genotypes accumulate dry matter with greater intensity and may dilute nutrients in tissues. This process occurs when the accumulation of dry matter exceeds the relative absorption rate of nutrients, another factor that contributes to changing the nutrient content in plants is the translocation of nutrients via phloem from older leaves to developing organs (Maia et al., 2005).

In addition to genotypic characteristics, climatic conditions can also change nutrient uptake dynamics. A condition of low water availability in the soil combined with high temperatures reduces nutrient absorption and promotes biochemical changes such as an excessive increase in reactive oxygen species (ROS) and activation of antioxidant enzymes (Gill & Tuteja, 2010; Kim et al., 2017; Kunrath et al., 2018). On the other hand, high air temperature associated with high transpiration can promote more excellent absorption and translocation of nutrients(Wielgolaski, 2001). Thus, seasons with higher temperatures and precipitation can induce a more excellent absorption of nutrients, notably in the fruits' phenological phases of expansion and granulation.

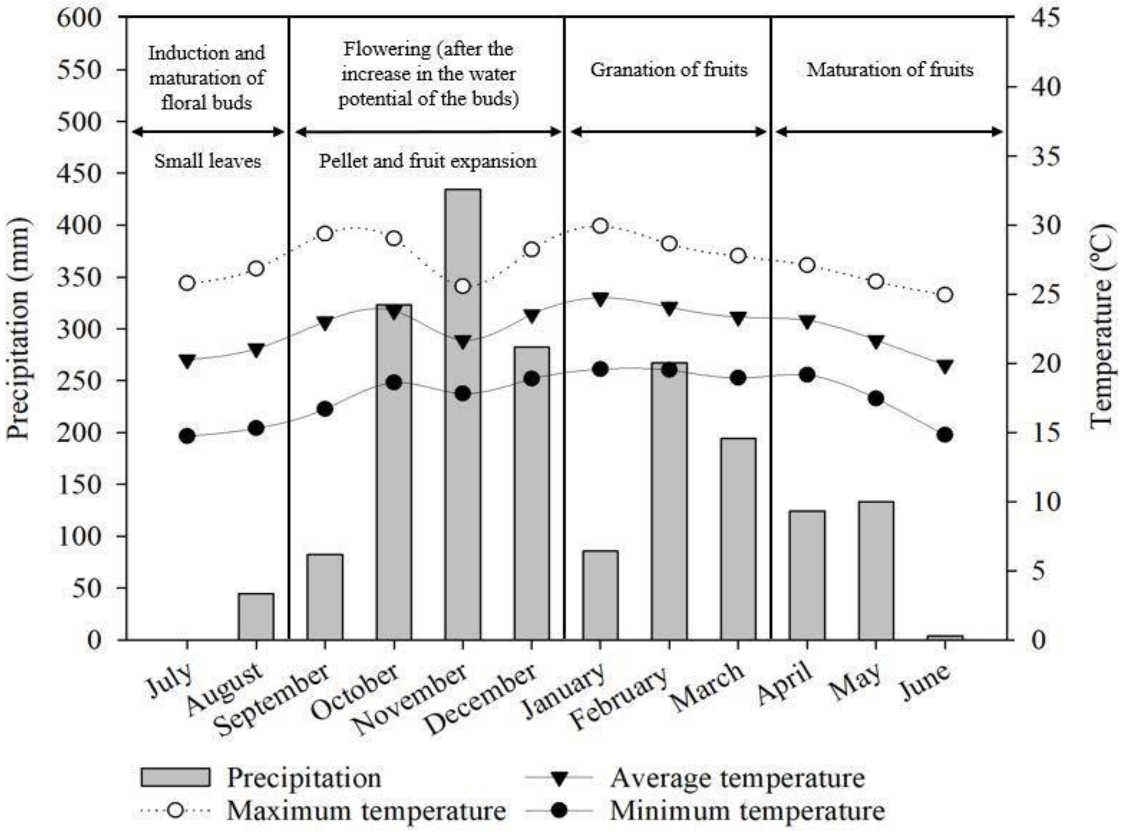
In general, plants subjected to low water availability conditions in the soil tend to close their stomata to prevent water loss to the atmosphere and consequently reduce nutrient absorption (Taiz et al., 2017). However, plants resistant to soil water deficit can maintain stomatal opening, allowing the influx of CO_2 and ensuring physiological and biochemical processes and nutrient absorption by the roots (Martinelli et al., 2009).

As mentioned, cultivar characteristics and ecophysiological interactions can alter nutrient absorption over time and thus alter leaf nutrient content (Amaral et al., 2011). Therefore, the collection of leaves to evaluate the nutritional status of the coffee plant based on the phenology of the fruits would be more coherent, although few studies contemplate this methodology. Given the above, an exploratory study was carried out with six coffee cultivars to verify in which phenological stage of the fruit the plant's nutritional status is best represented.

2. Material and Methods

The experiment was installed in a commercial farm located in the municipality of Rio Paranaíba – MG, at latitude 19°14'13.1"S and longitude 46°16'59.4"W, with an average altitude of 930 m. The region's climate is classified as Cwa (Alvares et al., 2013). The crop was established in December 2016 at a spacing of 3.70 × 0.63 m. The climatic information for the period under study and the phenological phases of the fruits can be found below (Figure 1).

Figure 1. Maximum, average and minimum temperatures and monthly rainfall between July 2018 to June 2019.



Source: Authors.

Crops were fertilized with 350 kg ha^{-1} of nitrogen divided into four applications (November, December, February, and March) and 300 kg ha^{-1} of K_2O divided into three applications (December, February, and March). To determine the chemical characteristics of the soil in the areas, soil samples were collected at depths of 0.0 – 0.20 m and 0.20 – 0.40 m (Table 1).

Table. Soil sample collected in the skirt projection of each cultivar before leaf collection.

Cultivate	Depth	pH	P[1]	K[1]	S	B	Fe[1]	Mn[1]	Cu[1]	Zn[1]	Ca_2^+	Mg_2^+	P[2]
		(H_2O)	-------------------------mg dm^{-3}-------------------------								$cmol_c dm^{-3}$		mg L^{-1}
New Acauã	0-20	5.4	26.9	76	31	0.4	62	31.2	2.2	2.2	2.9	0.6	20.1
	20-40	5.4	7.4	55	49	0.4	61	22.2	2.0	1.9	2.6	0.7	20.8
Yellow Bourbon	0-20	5.3	12.1	187	38	0.4	57	26.5	2.4	1.7	2.7	0.5	18.6
	20-40	5.1	5.3	85	55	0.4	61	20.0	2.2	1.1	2.0	0.5	19.7
Red Catuaí IAC 144	0-20	5.4	57.2	130	25	0.3	61	36.9	2.5	3.4	3.4	0.6	20.2
	20-40	5.3	16.9	8	40	0.4	69	29.4	2.6	2.9	2.5	0.6	19.9
Catucaí 20/15	0-20	4.9	12.2	87	47	0.4	68	23.8	2.2	1.5	1.9	0.5	19.4
	20-40	4.9	10.4	64	50	0.40	67	18.4	2.0	1.5	1.9	0.5	23.8
IAC 125 RN	0-20	5.3	23.8	224	22	0.4	88	30.1	2.2	2.0	3.2	0.8	21.6
	20-40	5.0	7.7	140	40	0.3	77	19.0	2.2	1.3	2.2	0.6	22.1
IPR 100	0-20	5.6	14.7	112	36	0.3	69	32.7	2.6	3.3	3.2	0.9	20.1
	20-40	5.6	7.7	68	49	0.3	59	21.3	2.0	2.0	2.9	0.9	20.7

[1] Extractor: Mehlich-1; [2] Phosphorus remaining. Source: Authors.

To carry out the comparative study, six coffee cultivars were used: Acauã Novo, Bourbon Amarelo, Catuaí Vermelho IAC 144, Catucaí 20/15, IAC 125 RN and IPR 100. Leaf collections were carried out between July 2018 and March 2019. A collection of leaves was carried out at each phenological stage of the coffee plant (floral bud maturation, lead, fruit expansion, and fruit granulation). Four sampling points were taken in each cultivar, and 30 leaves were collected at each point. The leaves of the third pair of the plagiotropic branch, located in the middle third of the plant, were collected (Malavolta et al. 2006).

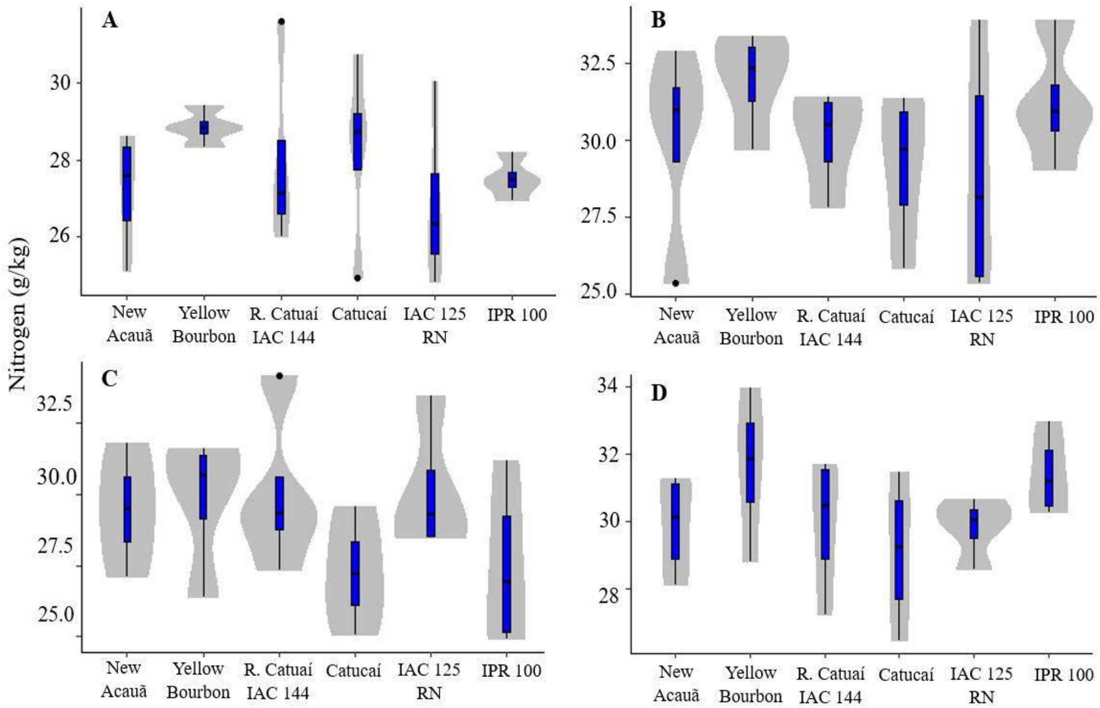
After the collection, the leaves were cleaned to remove any impurities, and they were placed to dry in a forced air circulation oven at a temperature of 70 ºC until reaching constant mass. Then, the leaf samples were ground in a Wiley mill equipped with a 1 mm sieve, and the macro and micronutrients N, P, K, Ca, Mg, S, Cu, Mn, and Zn (Miyazawa et al. 2009).

Leaf content data were submitted to exploratory analysis in the R software using the "ViolinPlot" graph, which indicates the maximum, minimum, first and third quartile and median values of the evaluated data.

3. Results

The cultivars Acauã Novo, Bourbon Amarelo, Catuaí Vermelho IAC 144, Catucaí 20/15 showed increased nitrogen (N) content in the lead phase and remained during the periods of expansion and granulation of the fruits (Figure 2). The cultivar IAC 125 RN showed a gradual increase in N during the four phases under analysis. In the cultivar IPR 100, there was an increase in the N content in the maturation phase of the floral buds for the lead phase, and then there was a reduction in the N content in the fruit expansion phase. The N content increased again in the fruit granulation phase (Figure 2).

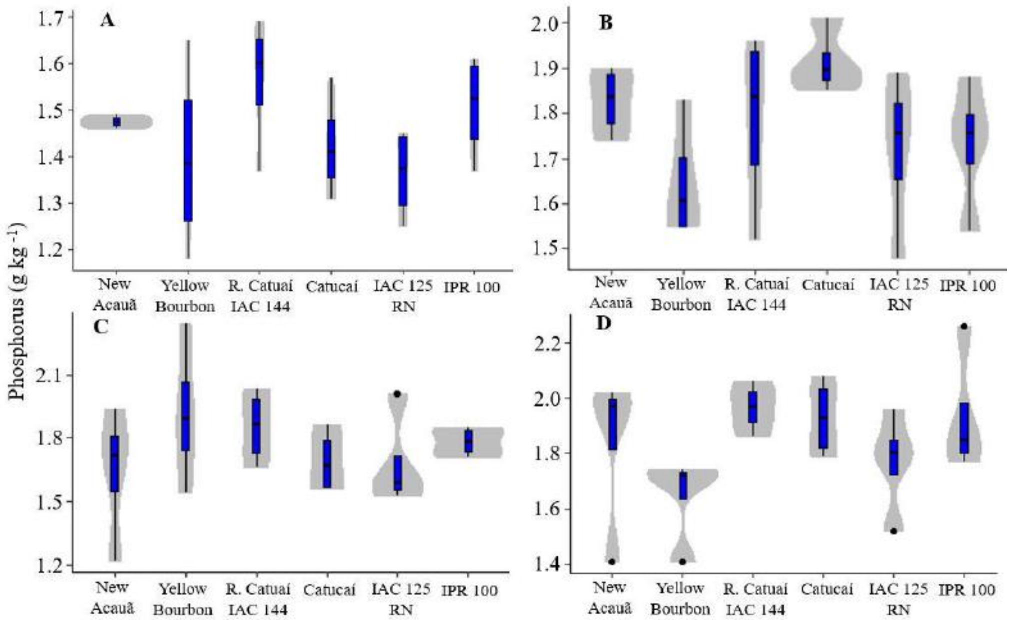
Figure 2. Leaf nitrogen (N) levels in coffee cultivars as a function of leaf collection time. (A) Maturation of floral buds; (B) pellet; (C) Expansion of fruits; (D) Granation of fruits.



Source: Authors.

The cultivars Acauã Novo, Catucaí 20/15, IAC 125 RN, and IPR 100 showed the highest phosphorus (P) content in lead, fruit expansion, and fruit granulation stages. The cultivar Catucaí 20/15 showed higher foliar P content in the lead and fruit granulation phases (Figure 3). The cultivar Bourbon Amarelo had the highest foliar P content in the fruit granulation phase.

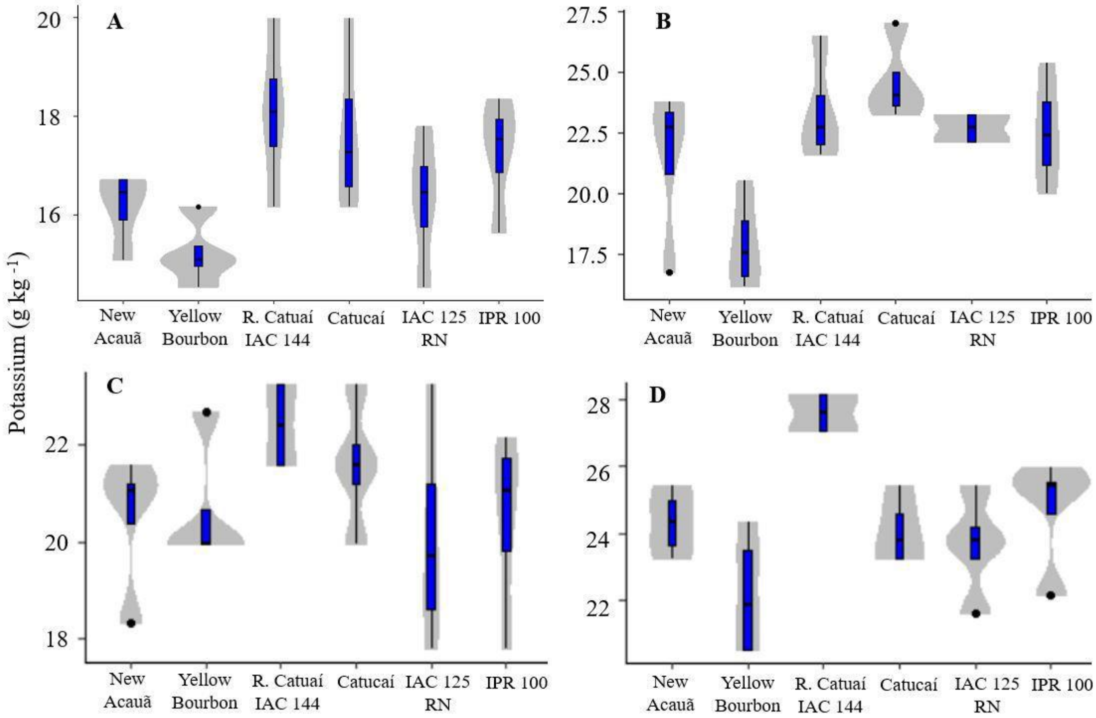
Figure 3. Leaf phosphorus (P) levels in coffee cultivars as a function of the time of collection of leaves for analysis. (A) Maturation of floral buds; (B) pellet; (C) Expansion of fruits; (D) Granation of fruits.



Source: Authors.

In the fruit granulation phase, the fruit granulation phase, the highest potassium (K) content in the Acauã Novo, Catuaí Vermelho IAC 144, and IPR 100 cultivars were observed (Figure 4). The cultivars IAC 125 RN and Catucaí 20/15 reduced the foliar K content in the fruit expansion phase. The cultivar Bourbon Amarelo presented a different result from the other cultivars with a gradual increase in K content until the phases of expansion and granulation of the fruits. Still, the K content in the fruit granulation phase is the lowest of the other cultivars for this cultivar.

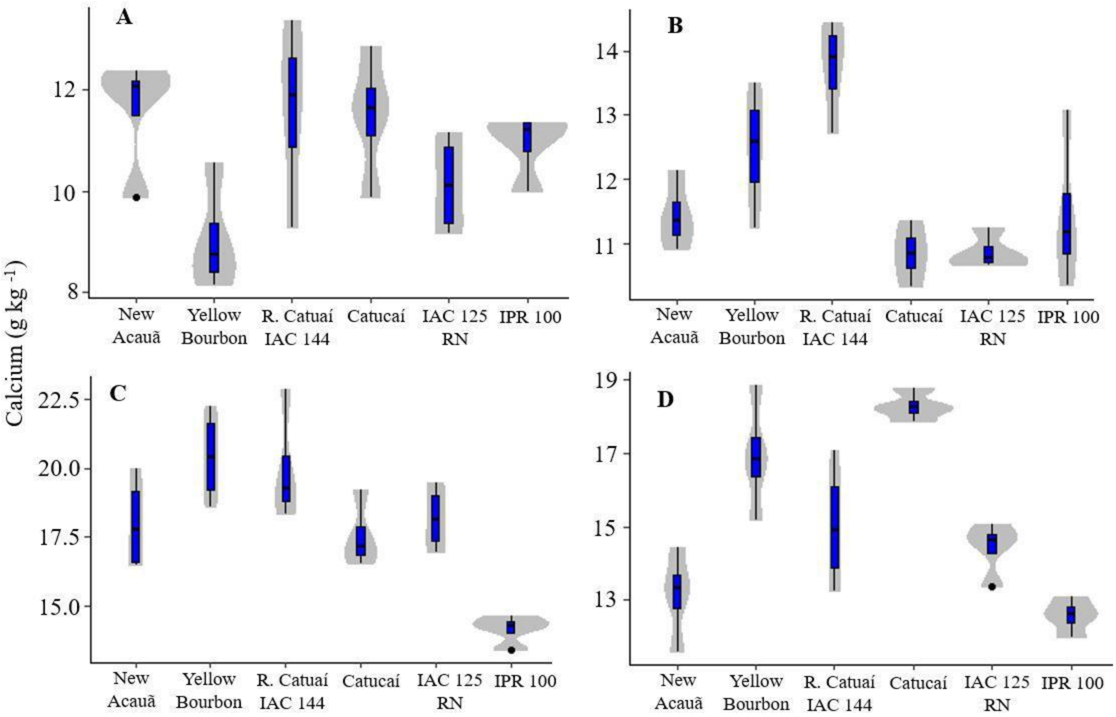
Figure 4. Leaf potassium (K) levels in coffee cultivars as a function of leaf collection time for analysis. (A) Maturation of floral buds; (B) pellet; (C) Expansion of fruits; (D) Granation of fruits.



Source: Authors.

For the cultivars, Acauã Novo, Bourbon Amarelo, Catuaí Vermelho IAC 144, IAC 125 RN, and IPR 100, the highest foliar calcium (Ca) content occurs in the fruit expansion phase. For cultivar Catucaí 20/15, the highest foliar Ca content period occurs during the fruit expansion and granulation phases (Figure 5).

Figure 5. Leaf calcium (Ca) levels in coffee cultivars as a function of leaf collection time for analysis. (A) Maturation of floral buds; (B) pellet; (C) Expansion of fruits; (D) Granation of fruits.

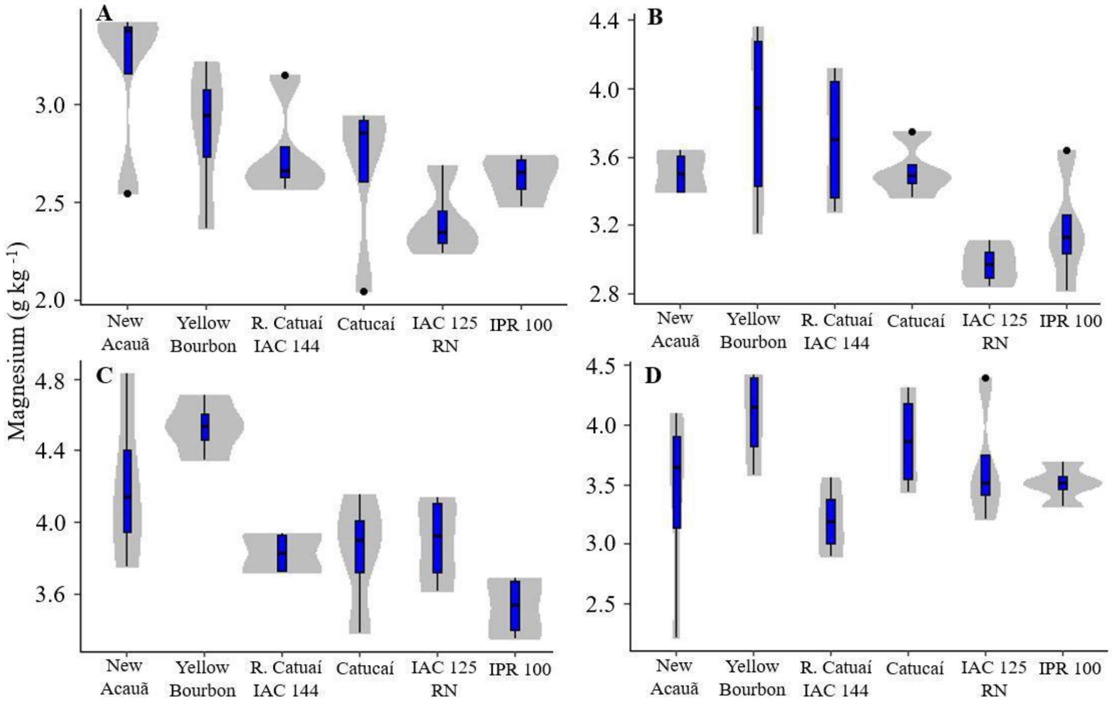


Source: Authors.

The cultivars Acauã Novo, Bourbon Amarelo, and IAC 125 RN showed higher foliar levels of magnesium (Mg) in the fruit expansion phase and reduction in the fruit granulation phase (Figure 6). For Catucaí 20/15 and IPR 100, higher foliar Mg content was obtained in the expansion and granulation phases of the fruits. In Catuaí Vermelho IAC 144, the highest Mg content occurs concomitantly with the lead and fruit expansion phases.

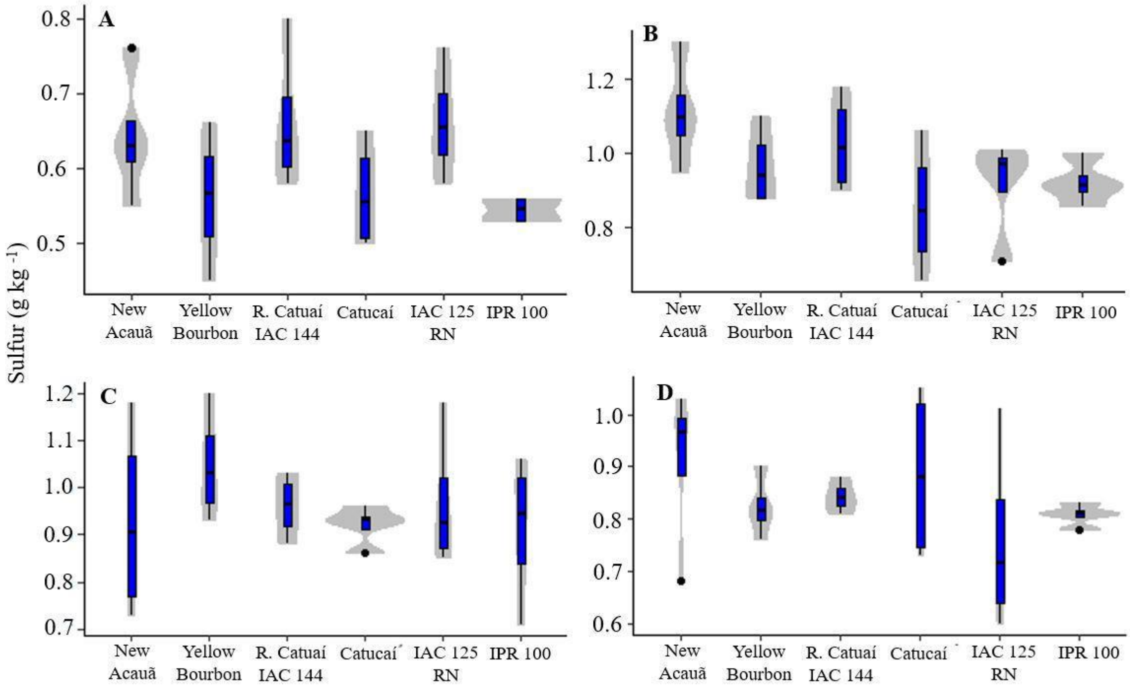
The highest foliar sulfur (S) contents in the cultivars Bourbon Amarelo, Catuaí Vermelho IAC 144, and IPR 100 were obtained in lead and fruit expansion (Figure 7). A similar result was found for Catucaí 20/15 and IAC 125 RN, presenting higher levels in the fruit granulation phase. Acauã Novo showed higher foliar S content only in the lead phase, followed by the expansion and fruit granulation phases.

Figure 6. Magnesium (Mg) foliar contents in coffee cultivars as a function of leaf collection time for analysis. (A) Maturation of floral buds; (B) pellet; (C) Expansion of fruits; (D) Granation of fruits



Source: Authors.

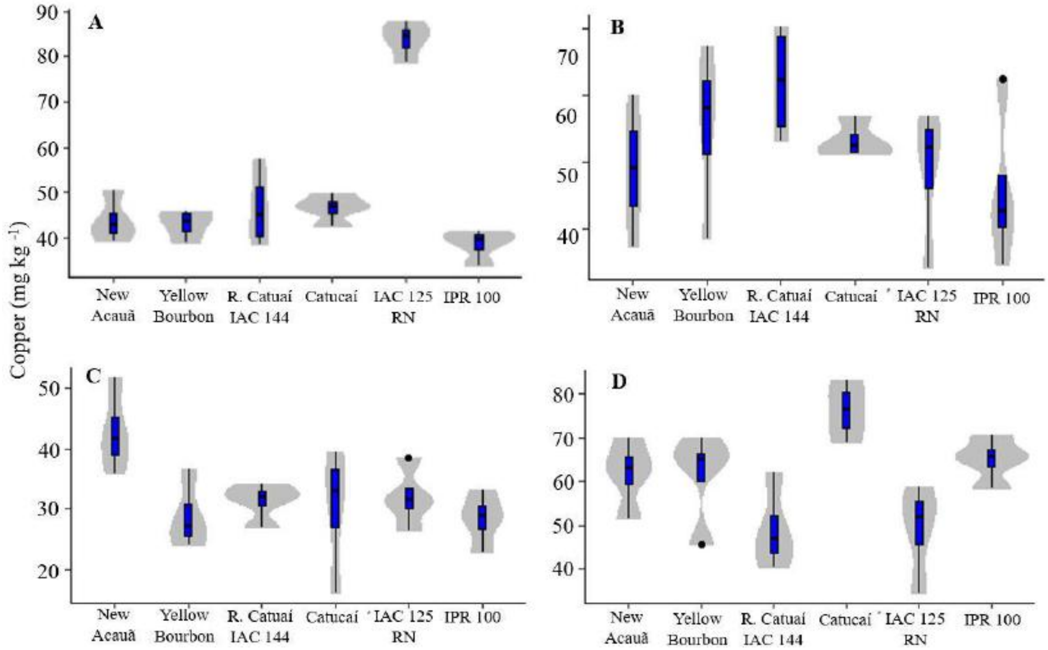
Figure 7. Leaf sulfur (S) levels in coffee cultivars as a function of leaf collection time for analysis. (A) Maturation of floral buds; (B) pellet; (C) Expansion of fruits; (D) Granation of fruits.



Source: Authors.

The periods with the highest foliar copper (Cu) content alternate between the lead and fruit granulation phases (Figure 8). There was a marked reduction in leaf copper content in the fruit expansion phase, except for the cultivar Acauã Novo (Figure 8).

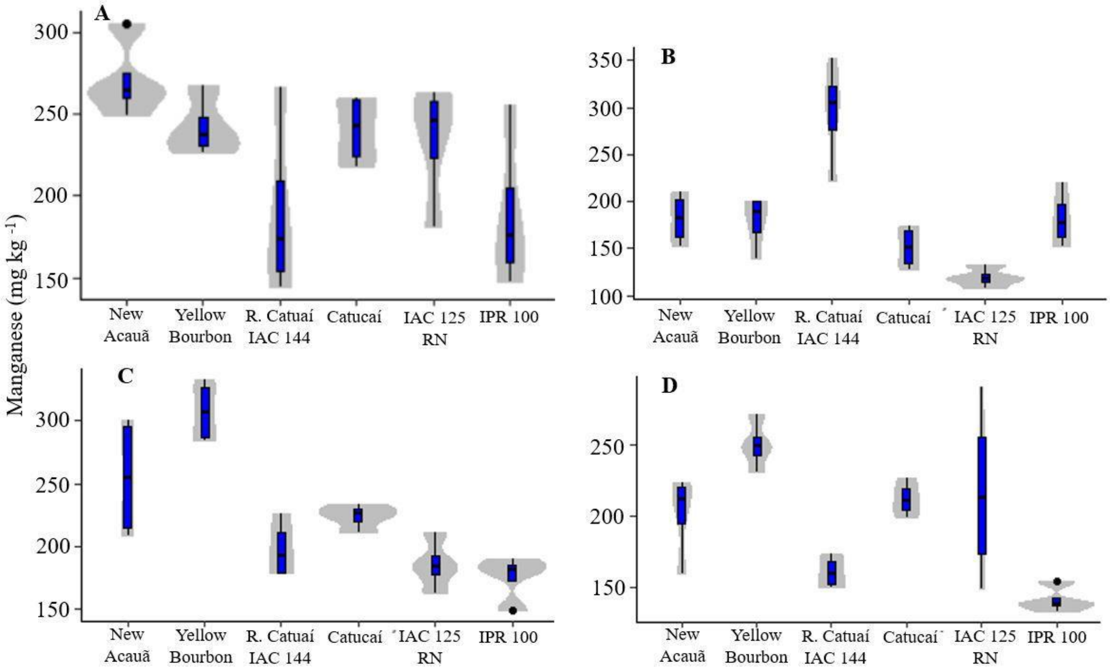
Figure 8. Leaf copper (Cu) levels in coffee cultivars as a function of the time of collection of leaves for analysis. (A) Maturation of floral buds; (B) pellet; (C) Expansion of fruits; (D) Granation of fruits.



Source: Authors.

The cultivar Acauã Novo had the highest manganese (Mn) content in the phase of floral bud maturation and fruit expansion (Figure 9). The highest Mn content in the cultivar Bourbon Amarelo was in the fruit expansion phase, while the cultivar Catuaí Vermelho IAC 144 had the highest Mn content in the lead phase. The cultivars Catucaí 20/15 and IAC 125 RN showed higher Mn levels in the maturation phase of the floral buds. The cultivar IPR 100 had the highest Mn content during flower bud maturation, lead, and fruit expansion.

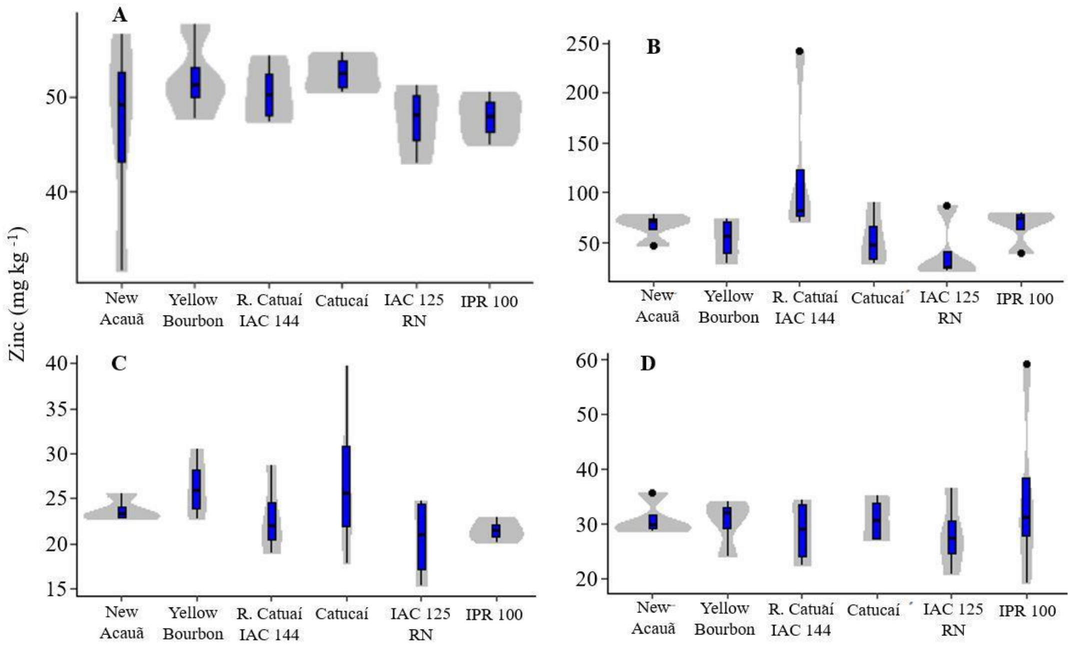
Figure 9. Leaf levels of manganese (Mn) in coffee cultivars as a function of the time of collection of leaves for analysis. (A) Maturation of floral buds; (B) pellet; (C) Expansion of fruits; (D) Granation of fruits.



Source: Authors.

The cultivars in the experiment showed high variability in leaf zinc (Zn) levels in the lead stage (Figure 10). The cultivars Acauã Novo and IPR 100 showed higher foliar Zn contents during the fruit development period in the lead stage. The highest foliar Zn contents occurred in the lead and fruit expansion phases in the other cultivars. The period with the lowest foliar content of Zn occurred in the fruit expansion phase.

Figure 10. Leaf zinc (Zn) contents in coffee cultivars as a function of leaf collection time for analysis. (A) Maturation of floral buds; (B) pellet; (C) Expansion of fruits; (D) Granation of fruits



Source: Authors.

4. Discussion

The N content increases from the lead phase, which coincides with the increase in precipitation. However, a reduction in leaf N content is observed in the cultivars Bourbon Amarelo and IPR 100 in the fruit expansion phase. This reduction is because there is remobilization of sugars and amino acids from the leaves in this period to meet the demand of the fruits (Da Matta et al., 1999). In addition, when in unsatisfactory levels (N), there is a reduction in the chlorophyll content, which impairs the process of light energy dissipation. Therefore, reactive oxygen species may be formed. In this case, there may be damage to cellular components and impairment of photosynthetic activity (Reis et al., 2015).

The increase in P content occurs in the lead phase, a period in which there is an increase in precipitation (Figure 1). In the cultivars Acauã Novo and Catucaí 20/15, there was a reduction in leaf content in the phenological stage of fruit expansion, which may be related to the translocation of P via the phloem to the fruits. The cultivars Catuaí Vermelho IAC 144 and IPR 100 showed the highest P levels in the maturation phase of the floral buds. These cultivars have a well-developed root system and resistance to water deficit in the soil, contributing to greater soil exploitation, reflecting more superior absorption of nutrients transported by diffusive flow, such as phosphorus (Ronchi et al., 2015).

The cultivars Acauã Novo, Catuaí Vermelho IAC 144, IAC 125 RN, and IPR 100 showed the highest levels of K in the fruit granulation phase. On the other hand, the cultivar Bourbon Amarelo had the highest K content in the fruit expansion and graining phase. For the cultivar Catucaí 20/15, the highest levels occurred in lead and fruit granulation phenological stages. The cultivar Bourbon Amarelo had a lower content of K in the granulation phase of the fruits. This reduction may be related to the dilution of potassium in the plant due to the translocation of potassium to the fruits. Potassium plays an essential role in yielding soluble solids, pH, and phenolic compounds. Therefore, there is a high translocation of potassium from leaves to fruits (Karimi, 2017).

The cultivar Catuaí Vermelho IAC 144 presented the highest Ca content in the fruit granulation phase. The high content of Ca in the leaves indicates a stabilization of the membranes, influenced by the water relations and changes in the properties of the cell wall. Ca is not remobilized via the phloem, making developing tissues depends on the available Ca supply in the soil; thus, calcium uptake during the early stages of fruit development is mainly involved in cell division and cell-cell junction (Hocking et al., 2016).

The cultivars Acauã Novo, Bourbon Amarelo, and IAC 125 RN showed the highest levels of Mg in the fruit expansion phase. In the other cultivars, the highest levels occurred in the expansion and maturation phases of the fruits and the lowest levels in the lead stage. The higher Mg requirement in the pellet stage is related to the high ATPase activity since the fruits at this phenological stage have a high respiratory rate (Marschner, 2012).

The highest foliar S levels occurred during the lead and fruit filling phases. Later there was a reduction in the foliar S levels, which may be related to the translocation for fruit growth and accumulation in the amino acid levels in the fruits. The most significant demand for sulfur occurs from green to ripe fruits (Valarini et al., 2005).

A reduction in foliar Cu content was observed in the grain filling phase, as Cu has low mobility via the phloem. This reduction is due to the increase in foliar dry matter, which generated the dilution of Cu in the leaves. The cultivars Acauã Novo, Catucaí 20/15, IAC 125 RN, and IPR 100 showed the highest copper tors in the fruit granulation phase, whereas the Yellow Bourbon presented the highest content in the lead and fruit granulation phases, the Catuaí Vermelho IAC 144 the highest Cu content occurred in the pellet stage.

Only the Catucaí 20/15 and IAC 125 RN cultivars showed higher Mn levels during the floral bud maturation. The IPR 100 cultivar had the highest Mn content during the stages of floral bud maturation, lead and fruit expansion, and the lowest content in the graining phase, corroborating the results of (Laviola et al., 2007), who found content of 110 mg kg-1. The Catuaí

Vermelho IAC 144 cultivar had the highest Mn content in the lead stage. A similar study carried out in Costa Rica with the caturra cultivar verified that the highest foliar Mn content occurs during the lead stage (Ramírez et al., 2002). The cultivars Acauã Novo and IPR 100 showed an increase in the foliar content of Zn in the lead phase, period of high cell division. Zn acts in cell division processes and membrane stabilization of newly formed cells (Marenco & Lopes, 2005). For the cultivars Bourbon Amarelo, Catuaí Vermelho IAC 144, Catucaí 20/15, and IAC 125 RN, the foliar Zn contents remained similar in the maturation stages of the floral buds and lead, even though there was a fluctuation in the Zn content in the lead stage. Variation is not enough to conclude that this phase has outstanding absorption. These cultivars show a Zn storage capacity in periods with low precipitation and subsequent retranslocation to organs such as fruits. Zn is classified as a low mobility micronutrient. However, when in high concentration, it can be translocated via phloem and redistributed to meet the initial demand of organs in early development (Silber et al., 2018).

The present work verified that the foliar contents of macro and micronutrients vary between the phenological phases and between the cultivars studied. Some research has already reported differences in the nutritional requirements of macronutrients in coffee plants. These differences may occur due to the year, days after anthesis, and cultivar characteristics. In addition, there may be a difference between sufficiency ranges from one region to another(Martinez et al., 2003; Partelli et al., 2014). Based on the above, the collection of leaves to evaluate the nutritional state of the coffee plant should be performed at different times. However, we suggest further research be conducted to evaluate coffee cultivars in different regions.

5. Conclusions

Levels of macro and micronutrients vary according to phenological stages of the fruits and among the cultivars evaluated at the same time.

The collection of leaves for the diagnosis of the nutritional status of the coffee tree must be carried out based on the phenology of the coffee fruits.

Acknowledgments

"This work was carried out with the support of the Coordination for the Improvement of Higher Education Personnel – Brazil (CAPES) – Financing Code 001". Santinato Cafés for making the areas available for research development.

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