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Concentration and accumulation of micronutrients in robust coffee

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ABSTRACT. Knowledge on the dynamics of micronutrients in coffee tree assists nutritional diagnosis and fertilization management. Therefore, the objective of this study was to evaluate the concentration and accumulation of micronutrients in the leaves and fruits of *Coffea canephora*. The experiment was managed in crops propagated for 2.5 years in a split-plot-in-time scheme in which the main plots consisted of fertilized and not-fertilized plants, and the subplots stipulated the evaluation periods. The berries and leaves were collected every 28 days, starting from the first flowering to full maturity. The micronutrient content was determined by laboratory chemical analysis, and the accumulation of nutrients in the fruit (mg) was calculated by multiplying the dry matter of the berry (g) × nutrient concentration (mg kg^{-1}). The results showed that fertilization infers the zinc concentration in the fruits and the concentration for manganese, iron and zinc in the leaves in the determined periods. Micronutrient accumulation curves followed the simple sigmoidal model, with accumulation increasing considerably as a function of fruit growth. Thus, fertilization based on micronutrients must be divided over stages with higher accumulation rates, considering the specific requirement of each nutrient.

Keywords: *Coffea canephora*; nutrition; parcelling; reproduction.

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Introduction

Coffee is currently a fundamental factor for the economy of more than 50 countries and stands out as the product with the second greatest commercial value, surpassed only by oil, generating millions of jobs directly and indirectly. In Brazil plays an important social and economic role as the largest producer and exporter of coffee. Approximately 99% of world's production is obtained from two main species, *Coffea arabica* L. and *Coffea canephora* Pierre ex Froehner, which produce ~2/3 and 1/3 of the crop yield, respectively, and differ greatly in origin, cultivation conditions, handling techniques, beverage properties and flavour (DaMatta & Ramalho, 2006; Davis, Tosh, Ruch, & Fay, 2011; Martins et al., 2016; Dubberstein et al., 2018).

The coffee plant is considered to have high nutritional demands due to the large quantities of nutrients that are lost when the berries are harvested. Thus, the nutritional status of the plant itself is characterized as a factor that substantially influences both plant development and reproduction (Quintela, Silva, Bomfim-Silva, Silva, & Bebé, 2011; Partelli, Espíndula, Marré, & Vieira, 2014; Assis, Guimarães, Colombo, Scalco, & Dominghetti, 2015; Covre et al., 2016).

Micronutrients are as important as other nutrients for the growth, development, and productivity of coffee plants, and their required quantities vary, mainly according to the age and productivity of each plant (Tomaz et al., 2011; Carmo, Nannetti, Lacerda, Nannetti, & Espírito Santo, 2012; Covre, Partelli, Bonomo, & Gontijo, 2018). Iron (Fe) is the most accumulated micronutrient in the conilon coffee plant, followed by manganese (Mn), boron (B), zinc (Zn), and copper (Cu) (Bragança et al., 2007). The results of Covre et al. (2016) report the order Fe>B>Cu>Mn>Zn accumulation in coffee fruits. In Arabica coffee berries, the order of accumulation is as follows: Fe>Mn>B>Cu>Zn (Laviola et al., 2007a). Among their countless functions, it should be emphasized that Cu, Mn, Zn, and B operate specifically during the reproductive growth stage and therefore in the determination of the productivity and quality of the plant. Fe, in turn, operates in the synthesis of chlorophyll and in the electron transport network of both photosystems (Taiz & Zeiger, 2010).

Coffee is normally planted in soils with a very low nutrient availability, and for most soils, fertilization with micronutrients is ignored, which leads to the emergence of different deficiency symptoms in the plants. Thus, the need arises for knowledge on the requirements for these nutrients during the reproductive stage of the coffee plant because the plant shows certain peculiarities during its various stages of development involving variation in the concentration and content of accumulated elements. According to Almeida, Nóbrega, Corrêa, Pinheiro, and Araújo (2014), changes are already occurring in this customary fertilization scenario due to the need to achieve high levels of productivity, and there is growing concern regarding the use of micronutrients for fertilization.

During the reproductive period, coffee berries preferentially drain carbohydrates and mineral nutrients from the plant; however, this phase coincides with the largest vegetative growth phase (generally occurring between September and May), which also demands large quantities of nutrients (Partelli, Vieira, Silva, & Ramalho, 2010; Partelli, Marré, Falqueto, Vieira, & Cavatti, 2013; Partelli et al., 2014; Dubberstein, Partelli, Dias, & Espindola, 2016; Covre et al., 2018). Thus, the supply of mineral nutrients to the coffee plant must be sufficient to meet the demands of both the berries and the vegetative organs (Laviola et al., 2008), addressing peaks in the demand for each nutrient in particular (Partelli et al., 2014). Knowledge regarding the accumulation curves and the total nutrients amassed by the organs of the conilon coffee plant would assist in the formulation and adjustment of recommendations for the crop fertilization programme (Bragança et al., 2007; Covre et al., 2016; Dubberstein et al., 2016).

Given the aforementioned difficulties, the present study assessed temporal changes in the concentration and accumulation of micronutrients in coffee leaves and berries under different fertilization management conditions in the southwestern Amazon region.

Material and methods

The experiment was conducted in the municipality of Rolim de Moura, located in the Zona da Mata region of the state of Rondonia, Brazil, at latitude 11°49’43”, longitude 61°48’24”W, and an average elevation of 277 m. The predominant climate in the region, according to the Köppen climate classification scheme, is tropical monsoon (Am), with an average annual temperature of 24-26°C and average precipitation of 1,900-2,200 mm per year. The rainy season lasts from October-November until April-May. The first quarter of the year has the greatest amount of rainfall. The warmest period is between August and October (Alvares, Stape, Sentelhas, & Gonçalves, 2014).

The study was conducted in a coffee crop propagated by 2.5-year-old cuttings, with a spacing of 4 m between rows and 1 m between plants (2,500 plants per ha). The local terrain is flat, and the soil is classified as eutrophic dark-red latosol with a clayey texture. Fertilization was performed according to the recommendation for the function of expected output in culture, divided into four instalments during the fruiting period. During the dry period, irrigation was performed by conventional spraying.

The experiment comprised plots subdivided by time, with the main plots consisting of two types of fertilization management (fertilized and non-fertilized) and the subplots divided according to the period during which the leaves and berries were collected. The experimental design utilized randomized blocks with three repetitions to determine the nutrient concentrations in the leaves and berries. Each experimental plot consisted of 11 plants; on each plant, two productive plagiotropic branches containing 10 to 12 rosettes and positioned at the median portion of the crown at the north and south cardinal points were marked.

The collections were performed at intervals of 28 days, beginning during the pinhead phenological phase of the berry (July 2013) and continuing until its complete maturation (April 2014). For each evaluation time and treatment, five branches were chosen randomly within the repetitions, completely removed from the trunk, and then packed in paper bags. At the same time, 22 leaves were collected in each block from the middle third of the plant, starting from the third pair of leaves; the evaluations extended until two months after harvest (June 2014) so that the behaviour of the foliar concentrations could be ascertained once the berries were removed.

The collected berries and leaves were taken to a forced-air circulation oven and dried at 65°C to constant weight. Subsequently, the berries were separated, counted, and then weighed on highprecision scales. The branches collected in July included flowers, which were grouped together with the pinhead stage berries. The material was ground in a stainless-steel Wiley mill for the performance of chemical analyses. The analyses were performed using the methodology described by Silva et al. (2009).

The nutrient accumulation per berry (mg per berry) was determined by the following formula: accumulation = dry matter of the berry (g) × nutrient concentration (mg kg^{-1}) (Partelli et al., 2014; Marré et al., 2015; Covre et al., 2016; Covre, et al., 2018; Dubberstein et al., 2016). The averages for nutritional concentration in the leaves and berries, as well as for the accumulation of nutrients in the berries, were subjected to variance analysis. A regression analysis of nutrient accumulation in the berries was performed with the aid of statistical software.

Results and discussion

The applied fertilization management influenced the micronutrient concentrations in the berries and leaves during certain evaluation periods, with significant interaction between the treatments applied (fertilization management and time of evaluation) (Table 1).

Table 1. Analysis of variance (ANOVA) for the micronutrient concentration in berries and leaves from post-flowering until maturation and post-harvest of the berries, respectively.

Source of variation	Cu	Mn	Fe	Zn
	F-value			
	Concentration in berries			
Fertilization management (A)	1.18^{ns}	0.87^{ns}	2.47^{ns}	0.70^{ns}
Period evaluated (B)	8.14**	15.43**	16.20**	19.57**
A x B interaction	1.63^{ns}	1.09^{ns}	1.42^{ns}	3.25**
	Foliar concentration			
Fertilization management (A)	1.06^{ns}	5.09^{ns}	3.93^{ns}	5.89^{ns}
Period evaluated (B)	2.76**	12.31**	22.11**	63.82**
A x B interaction	1.73^{ns}	5.39**	5.36**	2.69**

Values followed by "ns" and "**" are "not significant" and "significant" at the 1% level, respectively.

For the micronutrient concentrations in berries, only Zn showed a significant interaction. Compared with the berries of non-fertilized plants, those of fertilized plants contained a higher Zn concentration in late January and April (62.40 and 82.20 mg kg^{-1}, respectively) and a lower Zn concentration in early November (44.47 mg kg^{-1}). The Zn concentration did not differ during the other evaluation periods (Figure 1).

For the leaves, a distinct behaviour was observed: a significant interaction occurred between fertilization management and the timing of the Mn, Fe, and Zn micronutrient evaluations. When averaged over all the evaluation periods and compared between fertilization treatments, the foliar concentration averages were statistically similar for each of the micronutrients, including Cu (Figure 1).

In general, the concentrations of Mn, Fe, and Zn fluctuated during the evaluation periods, making it difficult to observe the benefits of fertilization, specifically for these nutrients. For Mn in fertilized plants, the highest foliar concentrations were observed from early November to early January and during May (128.45, 139.50, 118.07, and 187.14 mg kg^{-1}). For fertilized plants, Fe attained the highest concentrations at the end of January and June (127.24 and 228.26 mg kg^{-1}), conversely showing lower concentrations in early January (89.29 mg kg^{-1}). For Zn, lower concentrations were found in July and November of 2013 (49.71 and 59.40 mg kg^{-1}) (Figure 1).

When the concentrations of Cu, Mn, Fe, and Zn were analysed during the evaluation period (July 2013 to June 2014), variations were diagnosed for all the micronutrients in the leaves and berries of conilon coffee plants. The highest concentrations occurred in the berry during its initial developmental stage, which is known as the pinhead stage (Figure 1). This behaviour results from a low increase in dry matter in the berry during the pinhead phase, which is characterized by intense cell division and high respiratory rates (Laviola, Martinez, Souza, & Alvarez, 2007b). In other words, the nutrients become more concentrated because of the lower biomass of the berry.

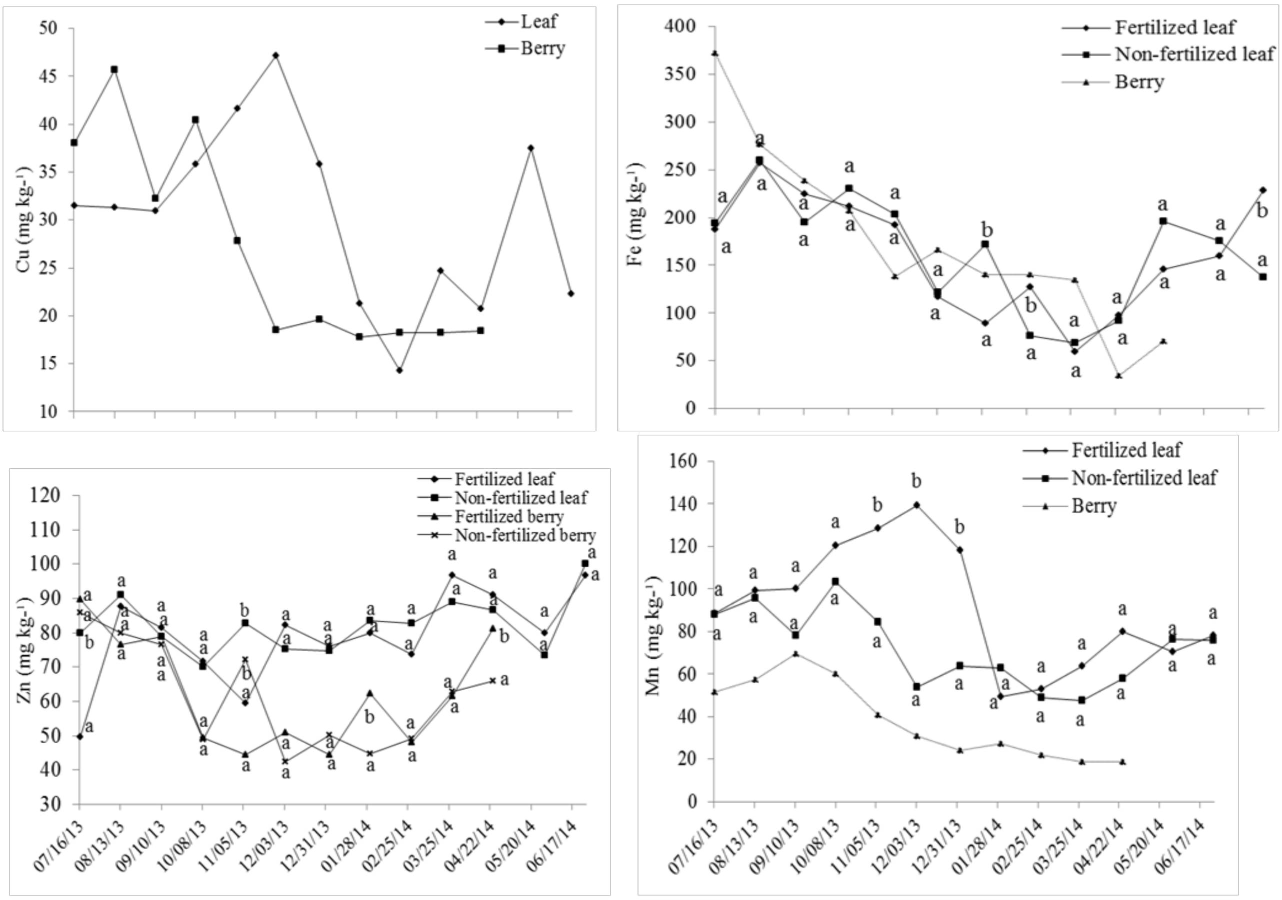

Figure 1. Concentration of Cu, Fe, Mn, and Zn in the berries and leaves of the conilon coffee plant from the post-flowering to post-harvest periods. Different letters represent significant interactions between fertilization management and collection time.

With the growth of the berry and its increased dry matter content during the later stages of expansion and grain formation, the concentrations varied but decreased and reached their minimum values during the last month of evaluation (maturation stage) for Cu, Mn, and Fe. Only Zn behaved differently—an increase in the concentration of this nutrient was observable during March and April. These effects accompanied the dilution of nutrients due to the increased biomass content of the berry (Prezotti & Bragança, 2013).

The foliar concentrations also varied during the evaluation period (Figure 1). Cu, Fe, and Mn exhibited minimum concentrations from the grain formation to maturation phases of the berries. This same behaviour was observed for Cu and Mn by Laviola et al. (2007a); however, the same authors reported distinctly lower Fe concentrations during the berry expansion phase. Despite little variation in concentration, Zn showed a tendency for lower levels during the berry expansion phase, as reported by Laviola et al. (2007a), who found less significant concentrations during the early stage of berry development.

After the berry harvest (May and June), there was a trend towards an increase in the foliar concentration of the micronutrients. This may occur because of the removal of preferential drains (berries), which leaves more nutrients for the plant to invest in vegetative growth. However, Zabini, Martinez, Finger, and Silva (2007) and Pedrosa et al. (2013) reported that Zn shows low mobility in the phloem due to the lower level and chlorosis found in new leaves compared with older leaves.

According to Partelli et al. (2013), plagiotropic branches with no berries exhibit more vegetative growth compared with that exhibited by productive branches and this behaviour can be attributed to the demand of the photoassimilates that are necessary for berry formation during the reproductive phase.

Although the graphs do not clearly indicate competition for micronutrients between the berries and leaves, an upward trend in the foliar concentration could be observed as the berry concentration decreased after the initial development phase. The study by Laviola et al. (2007a), which compared the foliar concentrations in the expansion and grain formation phases with those in the initial phase (flowering stage) to verify the existence of competition between the berries and leaves for nutrients, found that the presence of berries causes strong competition for the allocation of micronutrients, regardless of the state of berry formation. This phenomenon is characterized by nutrient translocation, which involves the transfer of an element from an absorptive organ to other organs to meet their temporary demands (Ferreira et al., 2013).

The management of fertilization did not affect the accumulation of micronutrients in fruits. However, they have different growth rates depending on the fruit collection times, finding significant results for the evaluation periods (Figure 2). This behaviour can be explained in terms of micronutrient concentration values observed in the organs of plants because virtually all the nutrients are present at appropriate or even higher levels.

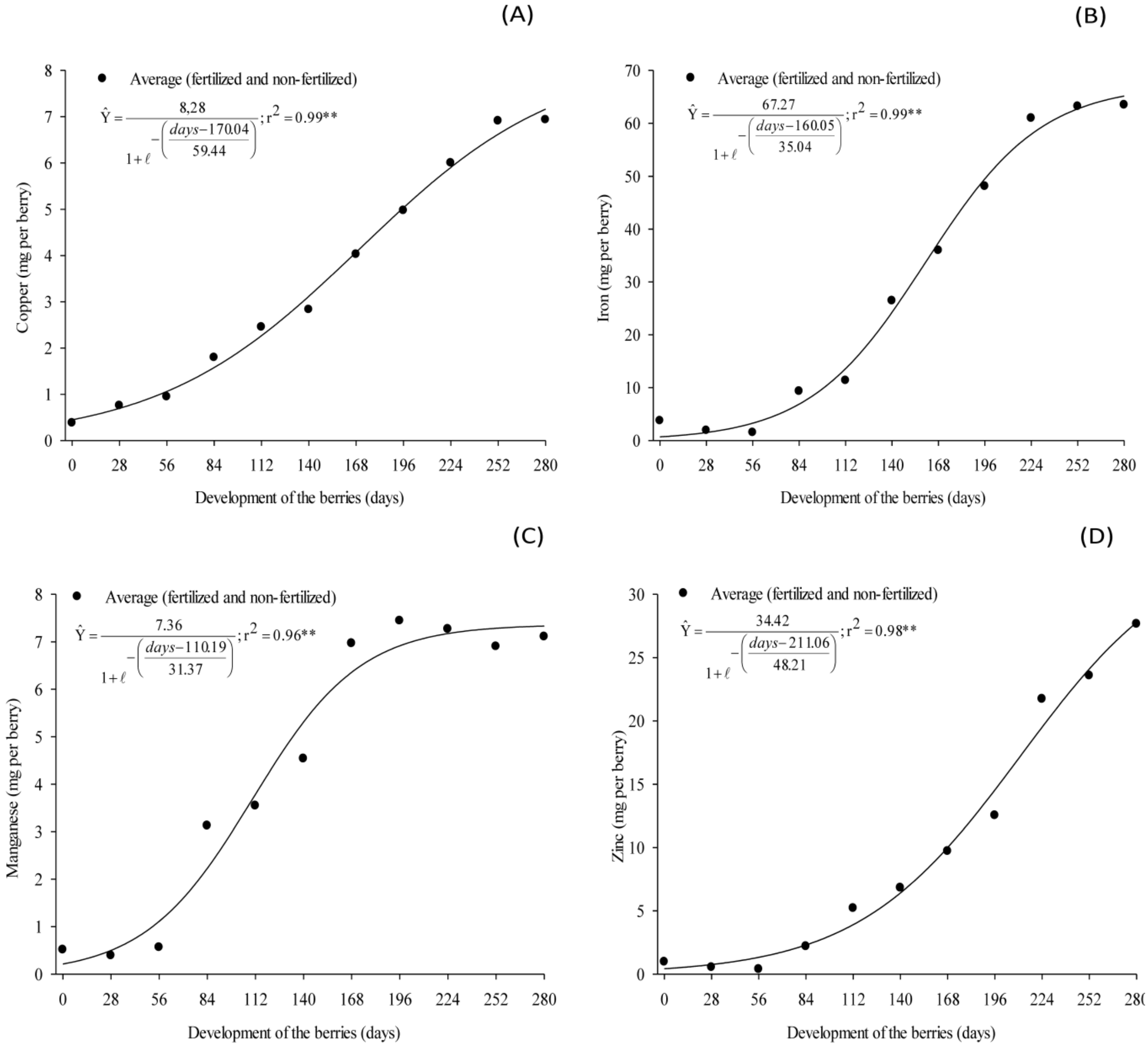

Figure 2. Copper, iron, manganese and zinc accumulation in conilon coffee berries, collected from the pinhead stage of the berry through maturation.

The accumulation curves for the micronutrients followed simple sigmoidal behaviour, overall initially showing (in the pinhead berry stage) low accumulation rates and further gradual increase after this stage. However, each micronutrient presented some peculiarities in the accumulation behaviour. Similar results were reported for the accumulation of macronutrients in fruits of *C. canephora* cultivated in the Brazilian Amazon region (Dubberstein et al., 2016) and macronutrients in fruits of *C. canephora* cultivated south of Bahia (Covre et al., 2018).

Beginning from the first evaluation (July 16, 2013), Cu showed little increase in accumulation rate, exhibiting almost linear behaviour until the penultimate evaluation (March 25, 2014) and stability in the last collection (Figure 2A). This increasing Cu demand could be related to its essential role in photosynthesis, respiration, carbohydrate distribution, N metabolism, proteins, and cell walls, as well as its role in the control of DNA and RNA production and plant reproduction (Mattos Júnior, Ramos, Quaggio, & Furlani, 2010).

Fe behaved differently from the other micronutrients during the initial phase—showing an evident increase in accumulation only after September 2013 and an almost imperceptible change during the first three months of evaluation. However, the accumulation increased significantly from November through March 2014 and was stable by the last month of collection (April 2014) (Figure 2B). Similarly, Covre et al. (2018) reported more spring accumulation rates and iron summer.

Mn displayed a characteristic micronutrient pattern, with more varying accumulation rates compared with those of the other nutrients—first increasing somewhat beginning from the pinhead berry stage (phenological phase) and then significantly between September and October before reaching the highest accumulation peak in January, followed by a small decline during the subsequent months (Figure 2C). Manganese ions are responsible for the activation of many enzymes in plant cells that have photosynthetic reactions as a more definite function where oxygen is produced from the water molecule, called hydrolysis (Taiz & Zaiger, 2010).

There was no significant Zn accumulation during the initial phase, and less pronounced rates were present until the end of January 2014, with greater accumulation during the last three months of evaluation (February, March, and April) (Figure 2D). In other words, the requirement of the berries for this nutrient tended to be more evident during the last three months of their formation. A deficiency of this nutrient immediately and directly affects the production of the plant (Pedrosa et al., 2013) because this nutrient is a precursor of indole acetic acid, which is the main plant growth hormone (Zabini et al., 2007).

In early-, medium-, and late-ripening conilon coffee genotypes, Marré et al. (2015) verified the accumulation of micronutrients during the initial phase of berry maturation, linking such accumulation to the short amount of time available to the plant to completely form berries, given that a super-late clone behaves differently from other genotypes. The berry formation period increased by as much as four, three, and two months for the super-late clone compared with the early-, medium-, and late-ripening genotypes, respectively.

Similarly, for the pinhead berry stage, Laviola et al. (2007a) found a proportionately higher accumulation of B and Zn, attributing this result to the important functions of these nutrients in cell division processes and the stabilization of membranes in newly formed cells (Taiz & Zaiger, 2010). The pinhead berry stage is characterized by cell multiplication and high respiratory rates; thus, during this phase, the accumulation of dry matter does not occur, and berries show low rates of nutrient accumulation.

Cell elongation occurs during the rapid expansion stage, when approximately 80% of the total weight of the berry is reached. Endosperm filling and the deposition of dry matter occur during the grain formation stage, and an increase in sugar content occurs during the maturation stage. Thus, the higher accumulation rates determined during these stages can be explained by the nutrients required for these processes (Laviola et al., 2007a and b; Laviola, Martinez, Souza, & Alvarez, 2007c; Partelli et al., 2014).

Mineral fertilization with micronutrients is not normally prioritized in coffee crops; thus, the presence of plant deficiency symptoms may be common. However, when well managed, such fertilization can provide numerous benefits to coffee plants, which respond efficiently, primarily with increased production (Bragança et al., 2007; Gontijo, Guimarães, & Carvalho, 2008; Tomaz et al., 2011). Proper conservation of mineral content and balance within the plant is crucial for the expression mechanisms of tolerance to abiotic stresses (Ramalho, Fortunato, Goulao, & Lidon, 2013).

In view of these results, it is suggested that the application of micronutrients to coffee plants, when necessary, should be performed before the start of the rapid expansion stage because of the accumulation rates during this stage. The same timing is recommended by Laviola et al. (2007a) and Marré et al. (2015) because of the accumulation results observed in their work. More attention should also be focused on Cu and Mn, in particular, considering the significant increase in the accumulation rate of these nutrients during the initial phase and the tendency with this increase for a greater demand during this phase.

When fertilizing with micronutrients via the soil, the first application should be performed soon after flowering because of the slow release of the micronutrient sources. However, when supplying micronutrients via the foliar route, the first application may be performed approximately 30 days after anthesis because absorption by the leaves and the later distribution of the micronutrients in the plants are faster processes (Laviola et al., 2007a).

Conclusion

Mineral fertilization has an influence on the concentrations and accumulation of micronutrients in the plant organs evaluated. The micronutrient concentrations in the berries and leaves of the coffee plant vary during the crop cycle. In the berries, the concentrations are greater during the initial phase and decrease in the later phases; however, the foliar concentrations are greater after the berries are harvested. The accumulation of micronutrients in the berries exhibits sigmoidal behaviour, with lower accumulation rates during the initial (pinhead) phase and then increased rates during the expansion, grain formation, and maturation phases.

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