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INFLUENCE OF NITROGEN FERTILIZATION ON NICKEL ACCUMULATION AND CHEMICAL COMPOSITION OF COFFEE PLANTS DURING FRUIT DEVELOPMENT

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INFLUENCE OF NITROGEN FERTILIZATION ON NICKEL ACCUMULATION AND CHEMICAL COMPOSITION OF COFFEE PLANTS DURING FRUIT DEVELOPMENT

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□ *Nutritional and physiological significance of micronutrients in coffee plants, especially with regard to nickel (Ni) is still unknown. The dynamics of nitrogen (N), phosphorus (P), potassium (K) and Ni accumulation in coffee fruits, as well as their relationships with total soluble protein, amino acids, reducing sugars, and starch content during coffee fruit development (green, ripe, and dry fruits), were investigated. Coffee trees received three N fertilizer rates (0, 150, and 300 kg of N ha^{-1}) as ammonium sulfate split into three applications per year. Nitrogen fertilization increased reducing sugars and starch concentrations in ripe fruits. In contrast, green fruits showed the highest amino acid and Ni concentrations. Fruit Ni concentration decreased in both green and ripe fruits as N rates increased; thus, indicating the possibility of either a N-associated dilution effect on Ni concentration or that Ni uptake by roots and/or transport to developing fruit was limiting. Plant nutritional status and fruit development stage influenced the coffee grain chemical composition. Furthermore, the variation in reducing sugars and starch content was more closely linked to the stage of fruit development than to N supply. A supposed relationship among the decreased of caffeine, starch, amino acids, and proteins with Ni content during green fruit development suggests a fundamental role for Ni in coffee fruit ripening. The interaction between N and Ni metabolism during fruit ripening might influence the chemical parameters involved in the coffee grain quality. This is the first report documenting changes in Ni concentrations of coffee fruit as a function of N fertilization rates and the development stage, but further research is needed to better understand the significance of N-Ni interaction in developing coffee fruit.*

Keywords: *Coffea arabica*, seed mineral composition, caffeine, amino acids, nitrogen metabolism

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INTRODUCTION

Nitrogen (N) is an essential element required for coffee tree development and their needs are relatively large. Among other things, it is a key component of nucleic acids, amino acids, peptides, proteins, is a constituent of enzymes, including metalloenzymes, and can act as a bridge between enzyme and substrates (Barker and Bryson, 2006). The role of N in agricultural production is closely linked to physiological processes, such as photosynthesis, senescence, and growth of plants (Lawlor et al., 2001; Reis et al., 2009b; Moraes et al., 2009).

Energy is released when a terminal phosphate is split from ADP or ATP. The transfer of phosphate molecules to ATP from energy-transforming processes and from ATP to energy-requiring processes in plants is known as phosphorylation (Sanchez, 2006). Potassium ions that are transported across chloroplasts and mitochondrial membranes are closely related to the energy level of plants. In earlier work, it was shown that potassium (K) has a favorable influence on photoreduction and photophosphorylation (Mengel, 2006).

There are reports indicating that nickel (Ni) is essential for normal seed development (Brown et al., 1990), although the data are restricted to a limited number of plant species. The exact metabolic role of Ni in seed formation is still unknown. Since Ni is an essential component of the urease enzyme (Welch, 1999), the use of stored N in seed compounds require Ni for remobilization of N through urea cycle during seed germination.

Due to the commercial importance of coffee, it has led to a partial analysis of primary chemical components occurring in mature coffee grains (Wöhrmann et al., 1997). Information on the accumulation of organic and inorganic compounds during coffee grain maturation is scarce (Rogers et al., 1999). However, a study has been done on physiology of coffee fruit development in regard to caffeine biosynthesis and transport (Crozier et al., 1997). There have also been attempts to correlate grain maturity to the generation of flavor and aroma components in the final product (Rogers et al., 1999), and to certain enzyme activities during fruit ripening (Golden et al., 1993).

The objective of this study was to develop useful information for create new tools in order to evaluate the N nutritional status of coffee plants cultivated in commercial farms. In the present study, it is hypothesized that N nutrition of coffee trees may influence nutrient concentration [phosphorus (P), K, and Ni], carbohydrate (reducing sugars and starch), and N compounds (amino acids and soluble protein) in developing fruit.

MATERIALS AND METHODS

Plant Material and Experimental Design

The experiment was conducted during 2006–2007 at the experimental farm of the University of Sao Paulo (USP/ESALQ), Piracicaba, Sao Paulo State, Brazil (22°42′ S, 47°38′ W and 580 m above sea level). Six year old coffee trees (*Coffea arabica)*, variety 'Catuaí Vermelho IAC-44', planted in a spacing 1.75 × 0.75 m, with a population of approximately 7,600 plants per hectare were used. According to Köppen and Geiger (1928), the region characterizes a Cwa tropical climate, with annual average temperatures of 21.1°C, average rainfall per year of 1,257 mm and air relative humidity of 740 g kg^{-1}. The wet season is between October and March and the dry season is between June and September. Climate data were daily monitored by the computerized agrometeorological station of USP/ESALQ located at the experimental farm.

The soil at the experimental site was an Eutroferric Red Nitosol, moderate A horizon with a clayey texture. Chemical characteristics of the 0–20 cm profile is as follows: pH (calcium chloride; $CaCl_2$), 5.3; organic matter (OM), 31 g dm^{-3}; phosphorus (P) = 8 mg dm^{-3} (Mehlich 1 extraction and colorimetric determination); potassium (K^+), 4.3 $mmol_c$ dm^{-3} (Mehlich 1); calcium (Ca^{2+}), 29 $mmol_c$ dm^{-3}; magnesium (Mg^{2+}), 20 $mmol_c$ dm^{-3} [titration against ethylenediaminetetraacetic acid (EDTA); potential acidity (H + Al), 30 $mmol_c$ dm^{-3}; base sum (SB), 53.1 $mmol_c$ dm^{-3}; effective cation exchange capacity (CEC; T), 83.1 $mmol_c$ dm^{-3}; and base saturation (V), 64%.

The experimental design was a complete randomized, factorial scheme 3 × 6 with five replications. The treatments consisted of three N rates (0, 150, and 300 kg ha^{-1}) applied as ammonium sulfate, and six different phases of coffee fruit development (January, February, March, April, May, and June 2006). The fertilizer was applied as topdressings on the middle of the area between the central stem and the projection of the plagiotropic branches, on top of dead leaf mulch.

The rate of K 300 kg ha^{-1} was split into two applications, 150 kg ha^{-1} each in November 2006 and January 2007. The levels of fertilizers were based on the yield expectancy and official recommendation for coffee plants cultivated in Sao Paulo State (Raij et al., 1997). Two leaf applications of 1.5% Zn sulfate were made in the months of December and March 2006.

The measured parameters were N, P, K, Ni, caffeine, reducing sugars, starch, free aminoacids and proteins in leaves and fruits, with samples taken at six evaluation period of fruit development. Evaluations were done on January, February, March, April, May and June, covering the stages of development from pinhead drop to dry fruits.

Samples of leaves and fruits were collected for chemical analysis. Compound samples were obtained for each plot, consisting of 50 leaves removed

from the third leaf pairs, starting at the end of the branches in the upper third of the plant according to the procedures described by Malavolta et al. (1997). A third leaf sample was taken from January through June 2006 to analyze the chemical compounds, following the same methodologies previously cited. Sampled fruit were harvested of the branches in the upper third from the same plants.

Statistical analyses were made via ANOVA using the GLM procedure (Snedecor and Cochran, 1989). Depending of the significance levels obtained from F test and Snedecor test for N-rates, a regression was calculated for the first and second degree components, through GLM procedure of Statistical Analysis System (SAS, Cary, NC, USA). A Tukey's mean comparison test ($P < 0.05$) was carried out for the grain chemical composition. The Pearson correlation (or relationship) among N, P, K and Ni content, caffeine, reducing sugars, starch, amino acids, and protein in leaves and fruits of coffee were obtained and tested by the CORR procedure of SAS.

Analytical Methods

For mineral element analysis, plant materials were dried in a forced air oven at 65°C for 48 h, ground and stored in hermetically sealed flasks for later analysis. Total N, P, K and Ni concentrations were determined according to procedures described by Malavolta et al. (1997).

Amino acids were extracted from dried leaf and bean materials (500 mg per 2 mL^{-1}) with methanol:chloroform:water (12:5:3, v/v/v) (Bielesk and Turner, 1966). The methanol, chloroform and water solution was added to the leaf and bean materials and stored in a refrigerator for one week at 4°C. During this time a phase formed at the top was recovered. The phase was dissolved in distilled water, filtered through 0.22 μm filters to remove impurities, and stored at −20°C for analysis. Amino acids concentrations in the samples were quantitatively determined using a leucine standard calibration curve (Cocking and Yemm, 1954).

Total proteins were extracted from dried leaf and bean materials by grinding each sample in a mortar with 0.1 M sodium hydroxide (NaOH; 100 mg of dried material per 5 mL). The concentrations were determined using a ready-to-use reagent from Bio-Rad (Bradford, 1976). Bovine serum albumin was used as standard.

Reducing sugars were determined in 80% methanolic extracts. Extraction (100 mg of dried material per 5 mL 80% methanol) was carried out in a boiling water-bath for 2 hours with occasional agitation. After cooling at room temperature, the extracts were diluted with distilled water to 10% of methanol. Reducing sugars were determined by colorimetry (Nelson, 1944), and glucose was used as standard calibration curve.

Starch was extracted from dried material with chloroform:ether (1:1, v/v), (500 mg per 2 mL), centrifuged at 4°C and the supernatant discarded.

After this process, 2 mL of ether was added to the extracts, centrifuged at 4°C for 15 minutes, after which the supernatant was discarded and the extract was dried. Then, 5 mL of 0.1 M NaOH was added to the dried extract followed by heating the solution at 100°C for 15 minutes. After cooling, the supernatant was stored until analysis. Starch contents were determined using the phenol method (Dubois et al., 1956).

For quantification of caffeine, coffee beans were first dried at 80°C for a week before being finely ground. Afterwards four drops of 1M ammonium hydroxide (NH_4OH) were added to 60 mg of the ground coffee beans, then the mixture was left to rest for one hour before the material was refluxed with 8 mL of chloroform for 30 minutes, and then the cooled extract was then filtered. Next, the extraction flask was washed with 4 mL of chloroform and the solution was again filtered. The volume of the combined extract and the washing solution was measured to be 12 mL, and 0.3 mL aliquots. Then the solution was diluted ten times with chloroform and optical densities at 257, 277, and 297 nm were applied in the equation below to calculate caffeine contents (Lopes, 1971)

$$\text{Caffeine (\% DW)} = \frac{\text{B} - 0.5(\text{A} \pm \text{C}) \times 12\ \text{mL} \times 0.001\% \times 3\ \text{mL}}{\text{b} - 0.5(\text{a} + \text{c})0.3\text{mL}0.06}$$

A, B, and C are extract absorbances at 257, 277 and 297 nm, respectively. a, b, and c are caffeine (0.001%) absorbances at 257, 277 and 297 nm, respectively.

RESULTS

Monthly mean air temperature and rainfall, from September 2005 to June 2006, are shown in Figure 1. Air temperature and rainfall began to increase by mid-January but then slowed down through mid-March, and remained low until June.

Nitrogen supply increased N concentration in coffee beans during green and mature grain growth stages. It is known that during fruit development, the amino acids derived from leaf senescence fill the grains through the formation of N compounds in the endosperm (Malavolta, 2006). The N-deficient coffee plants (control treatment) showed the highest fruit N concentration during the mature stage, which indicated high N mobility in the phloem. However, the highest N rate (300 kg N ha^{-1}) showed the highest fruit N concentration during the green stage (Table 1).

High K concentration occurred during the green stage of growth, and was independent of the N rates, including N-deficient plants from control plants (Table 1). On the other hand, low P concentration in green fruits was

TABLE 1 Nitrogen, phosphorus, potassium, and nickel concentrations in coffee beans during fruit development in relation to nitrogen supply (values are the means of five replicates, ± SEM indicates the standard error of the mean)

Rate of N (kg ha^{-1})	N (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})	Ni (mg kg^{-1})
		Green fruit stage		
0	11.01	0.72	15.23	3.13
150	20.41	0.78	15.73	2.38
300	26.46	0.77	12.66	2.36
±SEM	0.17	0.01	0.17	0.56
R^2	0.98	ns	0.62	0.76
Equation	y = 0.0515x + 11.57	ns	y = -0.0086x + 15.828	y = -0.0026x + 3.0118
		Ripening fruit stage		
0	13.42	0.78	11.89	3.38
150	16.08	0.81	14.35	3.41
300	21.19	0.81	13.07	2.80
±SEM	0.76	0.01	1.36	0.34
R^2	0.97	ns	ns	0.71
Equation	y = 0.0259x + 13.008	ns	ns	y = -0.0019x + 3.4892
		Dry fruit stage		
0	11.99	0.80	12.49	3.69
150	20.65	0.80	14.80	3.59
300	20.65	0.80	14.80	4.15
±SEM	0.40	0.01	0.95	0.15
R^2	0.75	ns	ns	0.60
Equation	y = 0.0288x + 13.436	ns	ns	y = 0.0016x + 3.5769

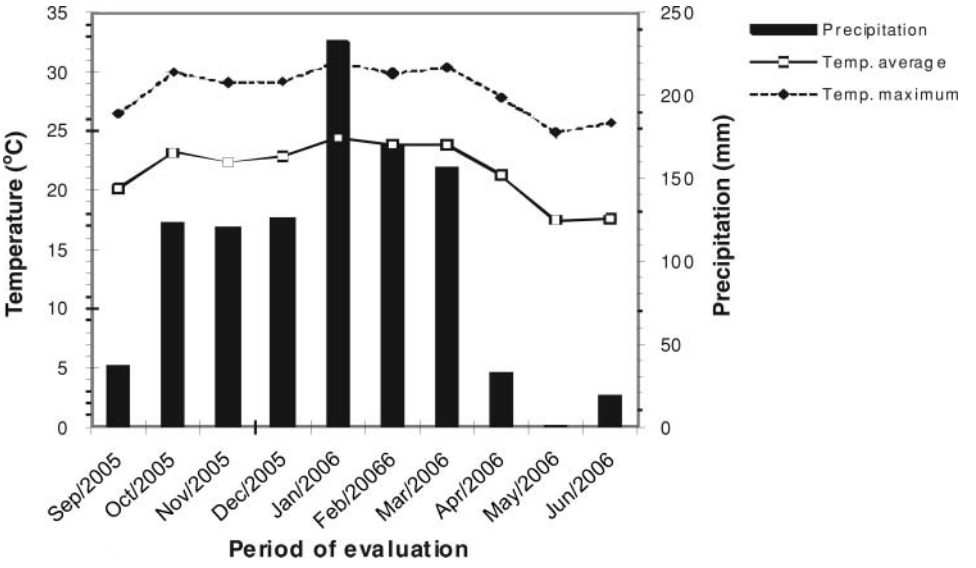

FIGURE 1 Monthly mean air temperature and precipitation from September 2005 through June 2006.

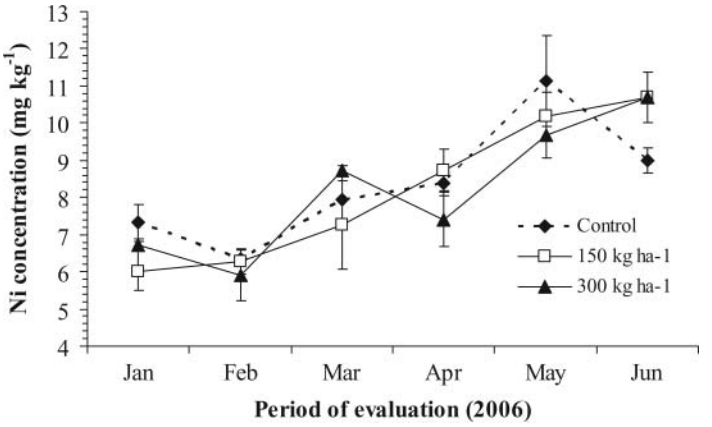

FIGURE 2 Ni concentrations in coffee leaves during fruit development stage. Values represent the mean ± standard error of five composite samples per treatment per sampling time. Where standard error bars are not visible, the error bar is smaller than the symbol.

equivalent to those of the mature fruit. This result of P concentration was lower than that previously reported by Valarini et al. (2005).

Ni concentration in coffee beans increased when fruit matured, regardless of N treatments and it was especially evident at the highest N supply (Table 1). The maximum Ni accumulation in dry fruits was recorded during the May sampling time and coincidentally, a maximum Ni accumulation in coffee leaves was also recorded at the same time (Figure 2). The increase in reducing sugars, starch, amino acids and soluble protein in coffee fruits was associated with the developmental stages in relation to N supply (Table 2). Maximum reducing sugar and starch concentrations were found in mature grains (Table 2). Reducing sugars concentration values found in the present work were similar to the ones reported by (Mazzafera, 1999). Nevertheless, Chagas (1994) observed that high quality coffee beverage is associated with beans containing both high reducing and non-reducing sugar concentrations. Free amino acids and soluble protein levels increased with increasing N supply but dry fruits showed low amino acid concentration than green fruits and the opposite was observed with the soluble protein concentration, which was lower in green than in dry fruits.

DISCUSSION

Sugar metabolism in higher plants has been shown to be essential for grain development (Herbers and Sonnewald, 1998). For example, as fruit mature there is a gradual increase in fruit reducing sugars concentration, reaching a maximum during the ripening stage. Sugar concentration then decreases as the fruit dry because they lose the pulp mucilage which is rich in carbohydrates. This effect can be attributed to an early fruit senescence,

TABLE 2 Caffeine, reducing sugars, starch, free amino acids, and protein content in coffee beans during fruit development in relation to nitrogen supply (values are the means of five replicates, ±SEM indicate the standard error of the mean)

Rate of N (kg ha^{-1})	Caffeine (%)	Reducing sugars (mg g^{-1})	Starch (mg g^{-1})	Free amino acids (mg g^{-1})	Protein (mg g^{-1})
			Green fruit stage		
0	1.16	2.75	7.49	2.83	79.93
150	1.49	1.55	10.42	3.80	120.37
300	1.75	1.58	10.51	5.23	156.79
±SEM	0.08	0.01	0.24	0.11	0.23
R^2	0.99	ns	ns	0.98	0.99
Equation	$y = 0.002x + 1.1683$	ns	ns	$y = 0.008x + 2.753$	$y = 0.2562x + 80.603$
			Ripening fruit stage		
0	0.70	7.35	16.68	0.94	98.80
150	0.84	11.72	20.54	1.99	135.47
300	0.95	10.47	19.32	4.22	166.61
±SEM	0.02	0.15	0.17	0.09	0.28
R^2	0.99	ns	ns	0.95	0.99
Equation	$y = 0.0008x + 0.7072$	ns	ns	$y = 0.0109x + 0.7424$	$y = 0.2261x + 99.716$
			Dry fruit stage		
0	1.07	1.43	12.68	0.56	120.07
150	1.39	2.73	15.47	1.22	152.70
300	1.61	2.06	12.51	3.08	195.50
±SEM	0.11	0.11	0.10	0.04	0.60
R^2	0.99	ns	ns	0.92	0.99
Equation	$y = 0.0018x + 1.0889$	ns	ns	$y = 0.0084x + 0.3554$	$y = 0.2514x + 118.37$

when sugars are processed by anaerobic pathways which might be fermented into alcohols and acids (Pimenta, 1995).

In the present work, the reducing sugar content in each treatment during fruit development was above the range proposed by Tango (1971) and Njoroge (1987), which is between 0 and 5 mg g^{-1} for ripe coffee grains. In addition, the levels of reducing sugars in mature beans (Table 2) were above of the values 5 and 1.8 mg g^{-1} reported by Pimenta (1995) and Leite (1991), respectively, for coffee grains harvested in the same stage of development at Lavras, southeast of Brazil. Climatic variations may have influenced the grain composition because the research was carried out in different regions and seasons. This might indicate that, during grain filling, most of the carbohydrates metabolized are stored as polysaccharides in coffee fruits, such as glucomannans (Wolfrom et al., 1960).

In developing storage organs, such as seeds and fruits, translocated photoassimilates are converted into carbon and N reserves, such as starch, fructans, and proteins. Research has shown that starch is the main carbon reserve found in developing storage organs for many plants (Kavakli et al., 2000). In starch-containing storage organs, N may also be sequestered as a reserve, since plants can accomplish it synthesizing and depositing proteins.

Although the biochemical reactions involved in starch and protein biosynthesis are distinct and unrelated, these processes are closely linked as first noted by Nelson (1982).

This correlation between carbon and N storage indicates that a modification in either of these two components will potentially impact the other. In the following section, some of the results this study on reducing sugars, starch, amino acids, and soluble protein biosynthesis in coffee fruits which have features common to all plants will be presented. Results from these studies have shed new light on the nutritional status associated to the processes responsible for the biosynthesis of coffee grain compounds.

Higher free amino acid concentrations were found in green beans and increased in relation to increasing N supply. The free amino acid concentration decreased from green to dry fruits, showing a negative correlation with soluble protein (Table 3). According to Miflin and Habash (2002), the amino acid metabolism in plants include the primary assimilation of ammonia, the interconversion of the amino group into transport and storage compounds, the carbon skeleton synthesis of the protein amino acids, and its control. Amino acids released from the storage proteins of coffee beans are broken down prior to transport to the developing fruits, as shown in Table 2.

Caffeine concentration decreased during developmental stages, but increased in all treatments in relation to increasing N supply (Table 2). A similar behavior was also reported by Malta et al. (2003) and higher caffeine concentrations in immature fruit endosperm as well as in the whole fruit have also been reported (Suzuki and Waller, 1984; Mazzafera et al., 1991). On the other hand, Clifford et al. (1987) and Clifford and Kazi (1987) reported very small changes in caffeine during the coffee fruit development. Caffeine is the main coffee bean purine alkaloid (i.e. 1,3,7-trimethylxanthine, derived from xanthosine) present in the grain cytoplasm and linked to the cell wall. In addition, the minor caffeine contribution to beverage bitterness led these authors to conclude that this alkaloid is not responsible for any change in beverage quality associated with fruit maturity.

Increases in Ni concentration during fruit development might be attributed to the fact that Ni is a component of the urease enzyme (Dixon et al., 1975), and possibly of other N-associated enzymes, which are likely present in a wide range of plant species (Welch, 1981); thus, Ni plays at least one critical role in metabolism during the reproductive growth phase (Walker et al., 1985). Ni is also highly mobile within the phloem (Malavolta, 2006) and as result it is readily transported to the reproductive organs through the phloem sap and can be accumulated in seeds and grains (Welch, 1995; Brown, 2006). During leaf senescence, up to 70% of the Ni accumulated in the shoots can be remobilized to fruits (Cataldo et al., 1978).

The cause of the decreasing Ni concentrations as N-rates increased in green and mature stage fruits is unknown, but it might be due to a N induced

TABLE 3 Correlation coefficients among rates of nitrogen, grain nitrogen, phosphorus, potassium, nickel content, caffeine (Caf), reducing sugars (RS), starch, amino acids (AA) and protein (Prot) during fruit development

	Rate	N	P	K	Ni	Caf	RS	Starch	AA	Prot
					Green fruit stage					
Rate	1	0.99***	0.74**	−0.62*	−0.69**	0.97***	−0.86**	0.77**	0.99***	0.99***
N		1	0.82**	ns	−0.73**	0.97***	−0.91***	0.82***	0.96***	0.99***
P			1	ns	ns	ns	−0.97***	0.86**	0,67*	ns
K				1	ns	ns	ns	ns	ns	ns
Ni					1	−0.74**	0.79**	−0.75**	−0.61*	−0.71*
Caf						1	−0.89**	0.76*	0.95***	0.98***
RS							1	−0.89**	−0.79**	−0.87***
Starch								1	0.71**	0.78**
AA									1	0.98***
Prot										1
					Ripening fruit stage					
Rate	1	0.97***	0.66*	ns	ns	0.99***	0.68**	0.66**	0.97***	0.99***
N		1	ns	ns	ns	0.96***	ns	ns	0.99***	0.96***
P			1	ns	ns	ns	0.88***	0,85**	ns	ns
K				1	ns	ns	ns	ns	ns	ns
Ni					1	ns	ns	ns	ns	ns
Caf						1	0.72**	0.71**	0.96***	0.99***
RS							1	0.98***	ns	0.72**
Starch								1	ns	0.70**
AA									1	0.96***
Prot										1
					Dry fruit stage					
Rate	1	0.83***	ns	ns	ns	0.96***	ns	ns	0.96***	0.99***
N		1	ns	ns	ns	0.89***	0.84**	ns	0.70**	0.82**
P			1	ns	ns	ns	ns	ns	ns	ns
K				1	ns	ns	ns	ns	ns	ns
Ni					1	ns	ns	ns	0.62*	ns
Caf						1	ns	ns	0.90***	0.95***
RS							1	0.82**	ns	ns
Starch								1	ns	ns
AA									1	0.98***
Prot										1

ns: not significant, ***; ** and *: significant at 0.01; 0.05 and 0.1 levels ($P <$ F 0.0001), respectively by student T test.

dilution effect as a consequence of increased fruit growth. Alternatively might mean that root uptake of Ni did not keep up with the Ni needs of fruit, or that *in planta* Ni did not remobilize rapidly to developing fruit. Due to the insufficient information regarding the optimal Ni concentration in developing coffee fruits, it is tentatively assumed that ≈5–10 mg kg^{-1} (instead of 2–3 mg kg^{-1}) is the adequate Ni concentration range for green and mature stage coffee fruits (Table 1); hence, Ni within fruit might be below the limiting concentration under certain soil situations when high amounts of N fertilizer is used. In the case of dry fruits, the increase in grain Ni concentration as N-rate increased, might be due to the effect of water

loss (drying effect), or else, to a greater need for Ni, since such beans also contain more proteins and free amino acids (Table 2). In other words, it is postulated that the high protein and amino acids in a bean would lead to a potential need for higher Ni concentrations when the bean germinates (i.e., facilitating the conversion of stored nitrogen to enzymes, RNA, DNA, and proteins).

Correlations among N rates and all variables analyzed during fruit development (green, mature, and dry fruit stages) are presented in Table 3. Although grain N concentration was much higher in fertilized than in control plants ($P < 0.00001$), N concentration varied over the stages of fruit development. In all coffee plants, N concentration was correlated to the concentration of caffeine (r = 0.97), starch (r = 0.82), amino acids (r = 0.96), protein (r = 0.99), Ni (r = -0.73), and reducing sugars (r = -0.91) during the green fruit stage. The inverse relationship between Ni concentration and N application rates is an evidence of the Ni-dilution effect within the fruit due to apparent increased growth (dry weight) of the fruit due to N supply. This may mean that coffee fruit require supplemental Ni (as foliar sprays) during fruit development if orchards receive high amounts of N.

Ni nutritional status can disrupt N metabolism in plants but the exactly role of Ni in plant nutrition of higher plants and its physiological significance are poorly understood, especially in woody perennials, which have received little attention (Bai et al., 2006). In this regard, Bai et al. (2007) observed metabolic disruption of N metabolism via ureide catabolism, and the presence of amino acid, and ornithine-cycle intermediates while studying young foliage of Ni-deficient pecan trees. In the available literature, studies demonstrating the relationship between Ni concentration and alteration of N compounds during coffee fruit development could not be found.

As described above, the effects of N on Ni concentrations indicate that a Ni deficiency in orchards trees might potentially influence the conversion efficiency of N plant reserves as well as that of N management of agricultural ecosystems (Bai et al., 2007). Thus, the interaction of N and Ni might be important from the perspectives of cost and coffee grain yield. There is circumstantial evidence on Ni role in the activity of several N associated enzymes in addition to urease. Although ureides have been found in xylem sap of young coffee trees (Mazzafera and Gonçalves, 1999), it cannot be concluded that young coffee trees transport nitrogen partially as ureide-N to fruits. However, according to Vitória and Mazzafera (1999), considerable amounts of ureides (allantoin and allantoic acid) were found in immature and mature fruits of *Coffea arabica* and *C. dewevrei*. The ureide amounts determined by these authors (around 1 μg mg^{-1} of fresh weight), was a little higher than those in soybean seeds (around 0.31 μg mg^{-1} of fresh weight) found by Mosquim and Sodek (1992).

The physiological roles of ureides accumulation in coffee is unknown and has been suggested that it may have some importance in N transport in

the coffee plant (Mazzafera and Gonçalves, 1999), then Ni is likely to play an important role in coffee management. Research is now in progress to evaluate enzyme activities, such as nitrate reductase and glutamine synthetase, as well as total leaf Ni, N, and leaf soluble protein, during the coffee fruit stages (Reis et al. 2009a). This research will hopefully contribute to a better understanding of the physiology of coffee grain yield.

CONCLUSIONS

Chemical composition of coffee fruits was influenced by the plant N nutrition and stage of fruit development. In the green fruit stage there was an intense metabolism in beans than during other stages and it might be suggested that the green fruit stage is a good physiological stage to evaluate the effects of N fertilizer supplies. Additionally, the Ni concentration of coffee leaves increased during fruit development and was negatively correlated to N compounds in coffee grains.

These data also indicate that there is merit in gaining greater understanding of the interaction between Ni concentration in coffee plants and nutritional N physiology as a function of fruit development. We also suggest that monitoring the chemical composition of green coffee fruits (especially with respect to Ni, N, amino acids, and proteins) is potentially an useful tool for assessing the nutritional status of coffee plants in the latter stages of fruit development. There is therefore merit in conducting further research on how monitoring of fruit chemical composition might be used to improve nutrient management of coffee in commercial orchards.

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