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Copper can influences growth, disease control and production in arabica coffee trees

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Abstract

The supply of copper to coffee trees is of great importance, but few studies have examined the benefits of copper to plant nutrition, vegetative growth, production and rust management. The objective of this study was to evaluate the influence of a supply of copper on the development, nutrition, and productivity of coffee trees and on the incidence and severity of rust in arabica coffee trees. The experiment was carried out in the Centro de Pesquisas Cafeeiras Eloy Carlos Heringer (CEPEC) located in the municipality of Martins Soares, MG, Brazil. Treatments were arranged in a randomised block design with a split plot in time, with eight replicates. The supply of copper was evaluated in the plots, and the control trees in each subplot were used to evaluate the influence of the year on the experimental results (year 1 and year 2). In this experimental setup, the following variables were studied: the number of nodes per plagiotropic branch, the number of leaves per plagiotropic branch, the copper content in green leaf tissues, the incidence of rust and the yield of coffee beans. To evaluate rust severity and the quality of the coffee fruit (mature, immature and dry; the average percentage of fruit that float; and the quantity of beans that are retained using size 17, 16, and 14 sieves and at the bottom), a randomised complete block design was used, with four replications and eight treatments. The results indicate that Arabica coffee trees supplied with Cu accumulated more Cu in leaf tissues (increase of 81%), experienced a lower intensity of rust (decrease of 23%), were more productive and produced fruit with a larger grain size. The bienniality of coffee plant production caused different levels of vegetative growth, intensities of rust and productivity between the years evaluated.

Keywords: Development, nutrition, coffee rust, productivity.
Abbreviations: AUDPC_area under the disease progress curve; AUDPCI_area under the disease progress curve for incidence; AUDPCS_area under the disease progress curve for severity; Cu_Copper; DF_mass of dry coffee fruits; IMF_mass of immature coffee fruits; MF_mass of mature coffee fruits; NLP_number of leaves per plagiotropic branch; NNP_number of nodes per plagiotropic branch.

Introduction

Coffee trees are often cultivated in regions with low micronutrient availability; thus, it is common to observe nutrient deficiencies in photosynthetic plant tissues (Martinez et al., 2003). Micronutrients (e.g., B, Cu, Fe, Mn and Zn), which are required in small quantities, are of great importance for the growth, development and production of coffee (Laviola et al., 2007). Copper (Cu) has received special attention because of its nutritional effects, which trigger a series of benefits, and because it offers the possibility of disease management. Cu also has a tonic effect; it influences the vigor of plants and leaf retention, as well (Cunha, Mendes and Chalfoun, 2004), thus resulting in higher productivity and greater development. These benefits of Cu occur because Cu ions are cofactors for numerous enzymes (e.g., Cu/Zn superoxide-dismutase (SOD), cytochrome c oxidase, amino oxidases, laccases, plastocyanin oxidase and polyphenol oxidase) and play essential roles at the cellular level. Cu ions are involved in cell signalling, transcription, protein trafficking, oxidative phosphorylation and the mobilisation of iron (Yruela, 2005). Cu is also active in the formation of pollen and in fertilisation (Taiz and Zeiger, 2013), both of which affect fruit production. Cu deficiency affects the photosynthetic apparatus indirectly, via changes in the degree of saturation of lipids of the thylakoid membrane and changes in chloroplast structure (Maksymiec, 1997). In response to Cu deficiency, dark green leaves develop chlorotic spots, become twisted or malformed, and even fall prematurely (Taiz and Zeiger, 2013). Cu deficiency also affects leaf anatomy; it impairs the cell-wall lignification in leaves and xylem vessels (Marschner, 2012), leaving the plant more vulnerable to pathogens and abiotic stresses. The supply of Cu is of fundamental importance in the management of coffee rust *(Hemileia vastatrix* Berk. & Br.) (Zambolim, Vale; and Zambolim, 2005; Pozza, 2008). Thus, special attention is given to copper fungicides in the management of rust; they have a broad spectrum of activity, they carry a low risk of selecting for resistant strains, and they pose a lower risk to the environment (Chalfoun, 1999). When applied on the leaf surface, copper fungicides prevent rust control by forming a barrier capable of preventing the germination and penetration of leaf tissues by urediniospores (Souza et al., 2011). Despite the importance of Cu to plant health, insufficient data on the function of Cu in coffee exists. Because the Cu supply available to plants has effects on vegetative growth, mineral nutrition, disease control and yield performance, there is a great need for more thorough and conclusive studies on the importance of this element to the development and production of coffee. We have examined the influence of the supply of copper on the growth, nutrition, disease control and production of Arabica coffee trees under field conditions during two consecutive years.

Results

Vegetative growth and copper content of leaf tissues

In both years, the supply of Cu did not influence the vegetative development of coffee plants. No difference in the means of either the number of nodes per plagiotropic branch (NNP) or the number of leaves per plagiotropic branch (NLP) was found between plants given a Cu supply and control plants; in the first year, a greater number of shoots were retained on branches (Table 1). Supplying plants with copper increased Cu concentrations in leaf tissues, with no difference between the years evaluated (Table 2).

Coffee bean yields and incidence and severity of coffee leaf rust

Coffee trees without Cu supplies had higher AUDPCI and AUDPCS and consequently higher intensities of the disease. We found a higher incidence of the disease during the second year (Table 3). In the first year, the productivity of coffee trees supplied with Cu did not differ from that of controls (Table 4). In the second year, the supply of Cu increased the grain yield by 10.47% compared to control plants; the productivity of both treated and control trees was higher in the second year than in the first year (Table 4). In Table 5, we observe that the plants treated with copper produce a greater mass of fruit per plagiotropic branch. Analysing the average percentage grain retained in sieves (Table 5), we also verified that a supply of Cu increased the production of larger grains, with a higher percentage of beans retained in the size 17 and 14 sieves and at the bottom.

Discussion

Importance of Cu in the cultivation of coffee trees

The supply of Cu increased the Cu content in the leaf tissues of Arabica coffee trees. At baseline, the leaf copper content was at an appropriate level (Table 8), the Cu content in the soil was low, and the organic matter content was high (Table 7); in this situation, Cu may be retained in soil, and its availability to plants may consequently be limited (Parat et al., 2002). During the two years of cultivation, there was extraction of Cu (vegetative growth and two samples) by control plants, without replacement, implying that the Cu deficit increased over this period (Table 2). Cu deprivation causes a number of metabolic disorders in plants; Cu deprivation alters the photosynthetic apparatus indirectly, by changing the degree of saturation of membrane lipids of the thylakoid and by changing chloroplast structure (Maksymiec, 1997). Coffee trees without a supply of Cu have a greater intensity of rust; this finding is linked to the fact that Cu deprivation most likely affects the lignification of cell walls, shoots and xylem vessels (Marschner, 2012), leaving the plant with increased vulnerability to pathogens and other forms of biotic stress. Overall, in leaves damaged by rust, there is a reduction in photosynthetic activity caused by a loss of photosynthetic area, which reduces radiation interception and radiation use efficiency. A consequence of this fact was a reduction in the production of assimilates, which reduced the level of their supply to reproductive and vegetative organs and thereby resulted in reduced growth and coffee production. Supplying Cu to plants resulted in greater production in year 2 compared to control plants (600 kg/ha), a greater mass of mature fruits and larger grain sizes. This greater productivity occurred because plants supplied with Cu exhibited higher foliar Cu concentrations and lower intensities of rust and thus had fewer injured leaves; a greater number of non-injured leaves allows the plant to have higher photosynthetic activity to meet the demand of fruits, given the available assimilates, thereby enabling its full development. Another factor contributing to increased productivity and the higher quality of grains produced by plants supplied with Cu was the fact that this nutrient also supports in the formation of pollen and in fertilisation (Taiz and Zeiger, 2013), thereby facilitating greater fruit set and the production of a greater number of fruits.

The supply of copper and coffee bienniality

The increased vegetative growth and reduction in disease that was observed in the first year allowed the coffee plants to increase production in the second year, resulting in increased productivity and higher grain quality. This fact can be linked to the physiological nature of Arabica coffee plants, which are characterised by a bienniality phenomenon, in which most plants alternate between vegetative growth one year and increased production in the following year (DaMatta et al., 2007). This phenomenon is related to the way in which coffee plants allocate the use of photosynthates. In a year of high production, photosynthates are directed to fruit production; in the following year, photosynthates are directed to form new vegetative buds (Mendonça et al., 2011). Thus, vegetative growth occurs in two-year cycles (Barbosa et al., 2012), and this fact accounts for the large number of leaves that grow in the first year (Table 1).

In years of high fruit production, an increased incidence of rust is usually found. This increased incidence is likely due to a change in the plant’s resistance to rust that may be caused by nutritional imbalances and the high rate of removal of assimilates from leaves required for the production of fruit (Zambolim et al., 2005; DaMatta et al, 2007). Thus, the deficit levels of Cu observed in control

Table 1. Mean values of the number of nodes per plagiotropic branch (NNP) and the number of leaves per plagiotropic branch (NLP) of arabica coffee trees in years 1 and 2, in the presence and absence of a supply of copper. CEPEC, Martins Soares, State of Minas Gerais, Brazil.

	NNP		NLP	
	Year 1	Year 2	Year 1	Year 2
Supply of copper	10.71aA	07.14 aA	25.28 aA	08.40 aB
Control	10.00 aA	07.00 aA	22.00 aA	05.00 aB
CV (%)	14.11	11.94	31.13	34.92

Means followed by the same lower case letter in the column (Supply of copper and Control) and capitalised in the same row (Year 1 and Year 2) did not differ, as determined by Tukey's test ($p \leq 0.05$).

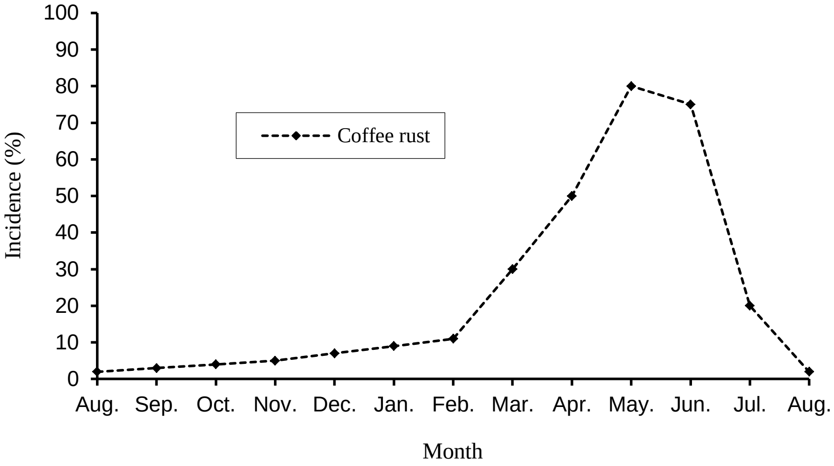

Fig 1. Historical average of rust incidence (%) observed in the CEPEC for the years 1994 to 2013. CEPEC, Martins Soares, MG.

Table 2. Mean values of mineral copper concentrations in leaf tissues (mg/kg) of arabica coffee trees in years 1 and 2, in the presence and absence of a supply of copper. CEPEC, Martins Soares, State of Minas Gerais, Brazil.

Cu	Year 1	Year 2
Supply of copper	37.21aA	32.63aA
Control	06.00 bA	07.00 bA
CV (%)	25.08	25.75

Means followed by the same lower case letter in the column (Supply of copper and Control) and capitalised in the same row (Year 1 and Year 2) did not differ, as determined by Tukey's test ($p \leq 0.05$).

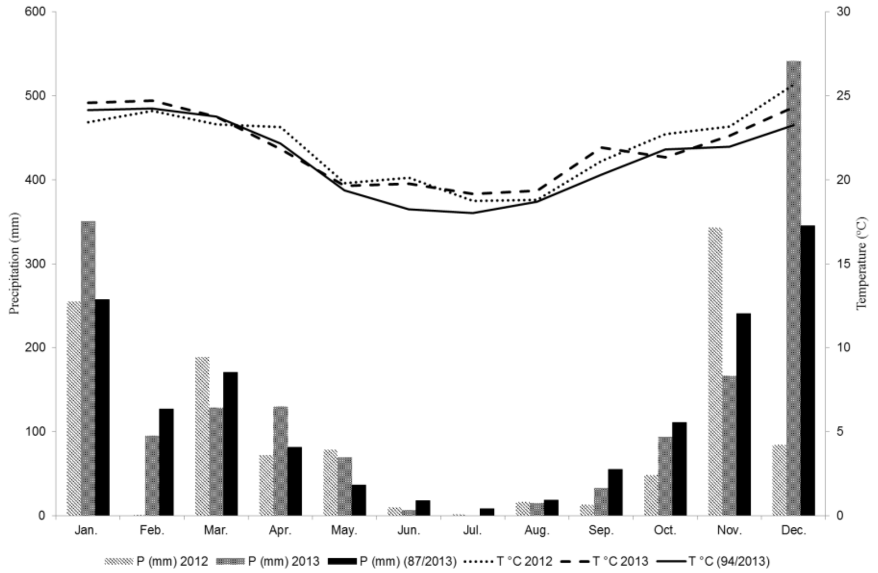

Fig 2. Monthly values of rainfall (mm) and mean air temperature (°C) in the years 2012 and 2013 and the local historical averages. CEPEC, Martins Soares, State of Minas Gerais, Brazil.

Table 3. The area under the disease progress curve for the incidence (AUDPCI) and severity of rust (AUDPCS) in arabica coffee trees in years 1 and 2, in the presence and absence of a supply of copper. CEPEC, Martins Soares, State of Minas Gerais, Brazil.

	AUDPCI		AUDPCS
	Year 1	Year 2	Year 2
Supply of copper	192.70 bB	13679.11 bA	587.42 b
Control	868.20 aB	17175.31 aA	1356.45 a
CV (%)	5.04	5.56	4.19

Means followed by the same lower case letter in the column (Supply of copper and Control) and capitalised in the same row (Year 1 and Year 2) did not differ, as determined by Tukey's test ($p \leq 0.05$).

Table 4. Mean values of productivity (60 kg/ha) and grain yield (%) of arabica coffee trees in years 1 and 2, in the presence and absence of a supply of copper. CEPEC, Martins Soares, State of Minas Gerais, Brazil.

	Productivity (60 kg/ha)	
	Year 1	Year 2
Supply of copper	08.24 aB	100.10 aA
Control	07.04 aB	89.67 bA
CV (%)	05.21	05.14

Means followed by the same lower case letter in the column (Supply of copper and Control) and capitalised in the same row (Year 1 and Year 2) did not differ, as determined by Tukey's test ($p \leq 0.05$).

Table 5. Mean values of the masses (g) of immature coffee fruits (IMF), dry coffee fruits (DF), mature coffee fruits (MF) and grain retained in sieves (sizes 17, 16, and 14 and at the bottom) of arabica coffee trees in year 2, in the presence and absence of a supply of copper. CEPEC, Martins Soares, State of Minas Gerais, Brazil.

	Supply of copper	Control	CV (%)
IMF	14.41 a	09.50 b	04.37
DF	06.04 a	04.58 b	03.65
MF	98.40 a	62.75 b	00.89
Boia	08.71 a	09.00 a	06.30
Sieve 17	41.28 a	35.67 b	05.20
Sieve 16	30.38 a	32.33 a	07.29
Sieve 14	19.19 b	24.33 a	05.32
bottom	10.09 b	15.00 a	12.54

Means followed by the same letter did not differ, as determined by Tukey's test ($p \leq 0.05$).

Table 7. Chemical characteristics of the soil used in the experiment. CEPEC, Martins Soares, State of Minas Gerais, Brazil.

Attributes	Values
pH (H_2O)[1]	4.99
P (mg/dm^3) [1]	22.60
K (mg/dm^3) [1]	48.00
Ca (cmol$_c$/dm^3) [1]	2.24
Mg (cmol$_c$ /dm^3) [1]	0.55
Al (cmol$_c$ /dm^3) [1]	0.24
H + Al (cmol$_c$ /dm^3) [1]	9.80
SB (cmol$_c$ /dm^3) [1]	2.93
CTC (t) (cmol/dm^3) [1]	3.17
CTC (T) (cmol/dm^3) [1]	12.73
V (%)[1]	23.00
m (%)[1]	7.60
MO (dag/kg) [1]	3.70
P-rem (mg/L) [1]	19.61
Zn (mg/dm^3) [1]	5.80
Fe (mg/dm^3) [1]	28.60
Mn (mg/dm^3) [1]	5.50
Cu (mg/dm^3) [1]	0.20
B (mg/dm^3) [1]	0.19
S (mg/dm^3) [1]	28.18

[1](EMBRAPA, 1997).

Table 8. Nutritional content of the leaf tissues of the coffee plants used in December 2011. CEPEC, Martins Soares, State of Minas Gerais, Brazil.

N	P	K	Ca	Mg	S	Zn	Fe	Mn	Cu	B
29.1	16	24.5	8.3	2.1	2.3	7.6	91.41	32.70	14.75	33.00

plants in year 2, contributed to the occurrence of higher rust intensity and greater AUDPCI in these plants. Overall, observed that independent of the supply of Cu, in years with smaller hanging loads, coffee trees have greater vegetative growth and a lower intensity of rust, and in years with greater hanging loads, coffee trees have reduced vegetative growth and a greater intensity of rust.

Materials and Methods

Description of the study and plant materials

The experiment was carried out in the Centro de Pesquisas Cafeeiras Eloy Carlos Heringer (CEPEC) [Eloy Carlos Heringer Coffee Research Center], located in the municipality of Martins Soares, MG, Brazil (latitude 20º14'45"S, and longitude 41º50'47"W) at an altitude of 736 m. The climate of the region, according to the Köppen classification, is mesothermal, with rainy summers (Cwa).
The chemical characteristics of the soil are shown in Table 7. The soil in the area of Latossolo Vermelho-Amarelo is dystrophic (oxisol) (Embrapa, 2007); thus, before the experiment, liming and fertilisation of the soil was performed as recommended (Guimarães et al., 1999).
The variety of coffee tree investigated in this study was Catucaí Vermelho 44. The trees, which were 15 years old, were planted at a spacing of 2.5 × 0.8 to achieve a population density of 5.000 plants/ha. They were pruned in September 2010, and before implantation was performed, the nutritional content of the leaf tissue of the plants was analysed (Table 8). The experiment was conducted from February 2012 through September 2013.
Climatological data was collected from the local weather station, which is 100 m from the test site. Monthly averages for temperature and precipitation were obtained during the conduction of the experiment (Figure 2).

Experimental design and conduct of the study

Treatments were arranged in a randomised block design with a split plot in time, with eight replicates. The supply of copper was evaluated in the plots, and the control trees in each subplot were used to evaluate the influence of the testing year (year 1 and year 2). In this experimental setup, the following variables were studied: the number of nodes per plagiotropic branch, the number of leaves per plagiotropic branch, the copper content in green leaf tissues, the rust incidence and the yield of coffee beans.
To evaluate the severity of rust and the quality of the coffee fruit (mature, immature and dry; the average percentage of fruit that float; and the quantity of fruit that is retained in sieves (sizes 17, 16, and 14) and at the bottom), a randomised complete block design was used, with four replications and eight treatments. The experimental plots consisted of four rows of 10 plants; considering the two central rows to be most useful, with six plants in each row, a total of 12 plants were examined in each plot.

Copper used in the study

Treatment groups consisted of control trees, which had access to very little Cu, and trees given a supply of copper. Recommended copper doses of 0.6 kg of Cu/ha were applied (Guimarães et al., 1999). To minimise the effects of specific copper sources, seven different sources were used: carbonate of copper (48% Cu), copper hydroxide powder (35% Cu), copper hydroxide liquid (35% Cu), oxychloride of copper (50% Cu), copper sulfate (25% Cu), copper sulfate and lime (25% Cu) and cuprous oxide (50% Cu). Thus, the supply of copper consisted of seven applications of isolated copper sources.
The treatments were divided into three applications, spaced 30 days apart, from February to April. These applications were grounded in the local history of an early occurrence of rust, obtained through monthly monitoring over the years 1994 to 2013, in experimental crops grown at the CEPEC without fungicide application (Figure 1).
The treatments were performed by the same user using the same spray and were always administered in the morning. To calculate the amount of slurry required, a "blank test" was performed. The sprayer (PJH, Jacto) had a 20-liter plastic tank with metal internal components, a brass piston and a brass pressure chamber. A cone-shaped spray nozzle was used (JD-10, DSCO 1.0 mm, Jet) with an empty cone, stainless steel disc, small drops, an angle of 80 degrees and 60 psi.

Evaluation of the study

Number of vegetative nodes and leaves of plagiotropic branches

The number of vegetative nodes on the plagiotropic branches (NNP) and leaves (NLP) was monitored monthly during the period from February 2012 to September 2013. Two plagiotropic branches were evaluated per plant, in the middle third, on opposite sides, previously marked in February 2012.

Leaf cooper content

Before the first spraying and 30 days after each spraying, leaves were collected; they were packaged in paper bags and dried in an oven with forced air circulation at 60 °C until reaching constant mass. Subsequently, the leaves were ground in a Wiley mill and passed through a 20-mesh sieve (0.841 mm). A portion of the samples was then subjected to nitric-perchloric digestion for determination of the copper concentrations by flame atomic absorption spectrophotometry (Malavolta et al., 1997) in the Laboratory of Plant Mineral Nutrition of the Centre for Agricultural Sciences, UFES.

Incidence and severity of coffee leaf rust

Evaluations of rust incidence on the leaves of coffee plants were performed at intervals of 30 days, from February 2012 to September 2013, referring to the two harvests of the agricultural years 2011/2012 (year 1) and 2012/2013 (year 2). A non-destructive method of evaluation was chosen, based on evaluating leaves from the middle third, between the 3rd and 4th pair of leaves from two plagiotropic branches per plant, previously marked in February 2012 with a ribbon tied to the first vegetative internode. The incidence of disease was determined according to the following equation 1:

$$\text{Incidence} = \frac{number\ of\ leaves\ with\ rust\ (unit)}{total\ number\ of\ leaves\ (unit)} . 100\ (\%) \qquad Equation\ 1$$

Rust severity assessments were performed monthly, from February 2013 to September 2013, referring to harvest of the agricultural year of 2012/2013. Leaves were sampled in the middle third, between the 3rd and 4th pair of leaves from the plagiotropic branches, ranging between the four quadrants of 12 plants per plot, totalling 24 leaves per plot. Rust severity was assessed using the scale proposal by Kushalappa and Chaves (1978). This scale consists of three diagrams of coffee leaves with 30, 50, and 70% of the areas marked indicating severity, where in each leaf a known quantity of the area (1, 3, 5, 7, and 10%) is occupied by individual pustules that coalesced. The data of rust incidence and severity in coffee leaves observed during the evaluation period were transformed into area under the disease progress curve (AUDPC) according to the equation 2 proposed by Shaner and Finney (1977):

$$\text{AUDPC} = \frac{\sum_{i=1}^{n-1} Yi + Yi + 1}{2}(\text{Ti} - 1 - \text{Ti}) \qquad \textit{Equation } 2$$

where:

AUDPC= Area under the disease progress curve;

Yi = Proportion of the disease in the i^{th} repetition;

Ti = Time in days at the i^{th} observation;

n = Total number of observations.

Productivity

Harvest was performed in the month of June (for both years) when, on average, 90% of the fruits were ripe, in the "cherry" stage. Fruits were harvested from all plants in the plot after measuring the harvested volume to determine the productivity of each plot. In the 2013 harvest, coffee grains were removed from four plagiotropic branches per plot and then washed and separated into floating, dry, unripe and ripe grains, quantifying the weight of each group. The fruits were then dried in an oven with forced circulation at a temperature of 60 ºC until the grains reached 12% moisture. After drying, the samples were weighed, and the grains were separated from the parchments and hulls. The grains were weighed to determine the yield of dry processed grains from each plot. Samples of flat grains were classified using number 14, 16, and 17 sieves, where the percentage of grains retained on each individual sieve was evaluated, including smaller grains that passed through all the sieves and were deposited on the bottom.

Statistical analysis

Data were subjected to analysis of variance, and in the presence of significant differences, the treatments were differentiated and studied with appropriate statistical techniques, using the statistical analysis program SISVAR (Ferreira, 2011). Tukey's test ($p \leq 0.05$) was used to verify differentiation between treatments and the control.

Conclusions

We observe that arabica coffee trees supplied with Cu have higher levels of this nutrient in the leaves, lower intensities of rust, increased productivity and larger grain sizes.

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