JUNIA MARIA CLEMENTE

BORON, COPPER AND ZINC EFFECTS ON PHOTOSYNTHESIS, ENZYMATIC ACTIVITY, NUTRITIONAL STATUS, PRODUCTION, CHEMICAL COMPOSITION AND CUP QUALITY OF COFFEE

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Fitotecnia, para obtenção do título de *Doctor Scientiae.*

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Júlio César Lima Neves

Ricardo Henrique Silva Santos

Adriana Farah de Miranda Pereira

John Lonfover Jifon

Herminia Emilia Prieto Martinez
(Orientadora)

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SUMMARY

RESUMO

CLEMENTE, Junia Maria, D.Sc., Universidade Federal de Viçosa, julho de 2014. **Efeito do boro, cobre e zinco na fotossíntese, atividade enzimática, estado nutricional, produção, composição química e qualidade da bebida do café.** Orientadora: Hermínia Emilia Prieto Martinez. Co-orientadores: Fernando Luiz Finger, Yonara Poltronieri Neves e Paulo Roberto Cecon.

A ordem de exigência de micronutrientes pelo cafeeiro é Fe>Mn>B>Zn>Cu>Mo, o que permite inferir que os maiores problemas estão relacionados ao B, Cu e Zn, uma vez que os solos brasileiros apresentam teores elevados de Fe e Mn. As formas usuais de fornecimento do B, Cu e Zn a cafeeiros são via solo ou via pulverização foliar, porém, ambas as formas possuem desvantagens, além disso, a literatura é bastante rica quanto às melhores fontes e doses para que sejam obtidas boas produtividade, no entanto, pouco ainda é conhecido sobre seus efeitos na qualidade da bebida. Assim, objetivou-se com esse trabalho verificar a viabilidade da suplementação do B, Cu e Zn via inserção de comprimidos na haste ortotrópica do *Coffea arabica* L., bem como, avaliar a resposta do cafeeiro em termos de produtividade e qualidade dos grãos. Foram executados três experimentos em campo sob delineamento em blocos casualizados com 5 repetições. Para avaliar o efeito do B foram aplicados os tratamentos sem o fornecimento de B, pulverização foliar contendo ácido bórico (0,4%) e inserção de comprimidos contendo sais de B, B+Cu, B+Zn e B+Cu+Zn; para avaliar o efeito do Cu foram aplicados os tratamentos sem o fornecimento de Cu, pulverização foliar contendo sulfato de cobre (0,4%) e inserção de comprimidos contendo sais de Cu, B+Cu, Cu+Zn e B+Cu+Zn e para avaliar o efeito do Zn foram aplicados os tratamentos sem o fornecimento de Zn, pulverização foliar contendo sulfato de zinco (0,4%) e inserção de comprimidos contendo sais de Zn, B+Zn, Cu+Zn e B+Cu+Zn. Adicionalmente, foi executado um experimento em casa de vegetação com objetivo de determinar as concentrações de B, Cu e Zn que se relacionam com a máxima eficiência fotossintética e relacioná-las também às atividades da polifenoloxidase e superóxido dismutase. O experimento foi executado usando-se vasos de 50 L contendo areia lavada e plantas de café cultivadas nessa condição durante três anos, em sistema hidropônico, sob delineamento inteiramente casualizado com três repetições. As doses de 0.4, 0.8, 1.6 e 3.2 µmol/L de Cu; de 1.0, 2.0, 3.0 e 4.0 µmol/L de Zn e 10, 20, 30 e 40 µmol/L de B constituíram os fatores em estudo. A inserção de comprimidos no tronco, contendo B, Cu e Zn embora necessite de ajustes

quanto a composição e doses é eficaz no fornecimento dos nutrientes às plantas e é mais regular que a pulverização foliar. O B, Cu e Zn fornecidos via pulverização foliar ou inserção de comprimidos na base do tronco influenciaram a qualidade dos grãos de café, caracterizada pelos teores de cafeína, trigonelina, sacarose, glicose, arabinose, manose, ácido 3-cafeoilquínico, ácido 5- cafeoilquínico, atividade da polifenoloxidase e compostos fenólicos totais. Apenas o Cu influenciou a qualidade da bebida avaliada pela prova de xícara. O Cu e Zn fornecidos via inserção de comprimidos foi equivalente à pulverização foliar tanto em produção quanto em qualidade. Com base nos resultados do experimento adicional pode-se dizer que a atividade da polifenoloxidase pode ser um indicador do estado nutricional das plantas em B e Cu. A atividade da superóxido dismutase é proporcional aos teores foliares de Cu e Zn e a atividade fotossintética é ótima quando os teores foliares de B e Cu encontram-se dentro da faixa de suficiência previamente estabelecida, resultando também em máxima atividade da polifenoloxidase.

ABSTRACT

CLEMENTE, Junia Maria, D.Sc., Universidade Federal de Viçosa, july 2014. **Boron, copper and zinc effects on photosynthesis, enzymatic activity, nutritional status, production, chemical composition and cup quality of coffee.** Adviser: Herminia Emilia Prieto Martinez. Co-adviser: Fernando Luiz Finger, Yonara Poltronieri Neves and Paulo Roberto Cecon.

The amounts of micronutrients required for the coffee trees follows the order Fe> Mn> B> Zn> Cu> Mo, which allows us to infer that the biggest problems are related to B, Cu and Zn, since the Brazilian oxisoils have high contents of Fe and Mn and coffee trees do not need high amounts of Mo. Soil fertilization or foliar sprays are the usual ways of supplying such nutrients to coffee plants, but in some conditions both forms are ineffective. The literature has many references about sources and doses of micronutrients for providing good productivity, however, little is known about its effects on the quality of the beans and beverage. Thus, the objective of this study was to verify the feasibility of B, Cu and Zn supplementation by injections of tablets containing salts of these micronutrients in the orthotropic branch of *Coffea arabica* L., and evaluate the response of coffee in terms of nutritional status, yield and quality. Three experiments were performed in field conditions under randomized block design with 5 replications. In order to assess the effect of B it was applied the following treatments: without B, foliar sprays containing boric acid at 0.4% and injections of tablets containing B, B+Cu, B+Zn and B+Cu+Zn in the trunk. Similar procedure was done to assess the effects of Cu and Zn, being the treatments as follow: without Cu, foliar sprays containing copper sulphate at 0.4% and injections of tablets containing Cu, Cu+B, Cu+Zn and B+Cu+Zn in the trunk; without Zn, foliar sprays containing zinc sulphate at 0.4% and injections of tablets containing Zn, Zn+B, Zn+Cu and B+Cu+Zn in the trunk. A fourth experiment was performed in a greenhouse in order to determine B, Cu and Zn nutritional status of the coffee-plants related to the maximum photosynthetic efficiency and activities of polyphenol oxidase and dismutase superoxide. The experiment was performed using pots of 50 L containing washed sand and coffee-plants cultivated in such way for three years, in a hydroponic system, under completely randomized design with three replications. The treatments were constituted of doses of 0.4, 0.8, 1.6 and 3.2 µmol/l of Cu; 1.0, 2.0, 3.0 and 4.0 µmol/l of Zn and 10, 20, 30 and 40 µmol/l of B. The results obtained permit conclude that tablets containing B, Cu and Zn injected into the trunk, although needing

adjustments in the composition and doses, are effective in providing these micronutrients to the coffee-plants. Boron, copper and zinc supplied by foliar sprays or tablet injections at the base of the trunk influenced the quality of the coffee beans, characterized by the contents of caffeine, trigonelline, sucrose, glucose, arabinose, mannose, 3-caffeoyilquinic acid, 5-caffeoyilquinic acid, PPO activity and total phenolic compounds. Only Cu nutrition influenced the cupping quality. For Cu and Zn, coffee production and beans quality were equivalents with either forms of supply. PPO activity is a good indicator of the B and Cu nutritional status of coffee trees. SOD activity is proportional to the foliar contents of Cu and Zn and the photosynthetic activity is high when the contents of B and Cu, are within the sufficiency range, resulting in maximum PPO activity.

GENERAL INTRODUCTION

Micronutrients, although required in smaller quantities, in the order of a few milligrams per kilogram of dry matter, play specific and essential functions on plant metabolism, and its deficiencies can lead to significant metabolic disorders enough to reduce the growth, production and quality of coffee beans.

In most acid soils at low pH and increasing redox potential, manganese (Mn) oxides can be easily reduced in the soil exchange sites (Kogelmann and Sharpe, 2006), increasing the concentration of soluble Mn^{2+} (Watmough et al., 2007), which is the predominant Mn form in the soil solution (Adriano, 2001) and the most available Mn form for plants (Marschner, 2011). Either, in highly weathered, humid tropical forest soils are often characterized by dominance of iron (Fe) rich minerals, so Fe and Mn will not be a problem in most of the coffee plantations. Regarding the Mo, Malavolta, (1980) states that in order to produce 1 ton/ ha of coffee beans it is necessary 0,25 g/ha of Mo, showing the extremely low requirement for Mo by coffee plants.

Based on the above stated we can infer that the biggest problems are related to B, Zn and Cu (Catani et al., 1967b), furthermore the amounts of micronutrients required for coffee trees follows the order Fe> Mn> B> Zn> Cu> Mo.

Several researches have shown that great number of coffee plantations are Zinc (Zn) deficient (Gallo et al., 1967; Gallo et al.; 1970; Martinez et al., 2004). The metabolic functions of Zn is based on its strong tendency to form tetrahedral complexes with N-, O- and particularly S-ligands through which plays a functional (catalytic) and a structural role in enzyme reactions (Vallee and Auld, 1990), stands out the Zn functions on the production of indoleacetic acid, on the ANR structure and protein synthesis, these functions may explain the lower cells division and elongation of Zn deficient plants.

Either for B, coffee is between the cultures more sensitive and more responsive to the Boron (B) fertilization (Brown and Shelp, 1997). The role of B on plant nutrition is not well understood, however, on molar basis, the requirement for B, by coffee plants is higher than any other micronutrient and its deficiency compromise process such as sugars transport, cell wall synthesis, lignification, cell wall structure, carbohydrates metabolism, RNA metabolism, respiration, phenols metabolism and membrane integrity (Parr and Loughman, 1983).

On the other hand, copper (Cu) deficiency usually is not common in coffee plantations where cupric fungicides makes part of the control program of coffee rust, but it can happen in soils that receive excessive liming i.e. rich in organic matter. Most of the functions of Cu as a plant nutrient are based on enzymatically bound Cu which catalyses redox reactions influencing on important physiological processes in plants, such as photosynthesis, respiration, metabolism of proteins and in activation processes of resistance of plants such as phyto-alexinas (Marchner, 2011).

Normally, B, Cu and Zn are supplied to coffee-plants by soil fertilization or foliar sprays. However, the mobility of B, Cu and Zn seems to be low, what explain more than two or three applications per year, increasing the production cost of the orchards, moreover, the effectiveness of foliar sprays depends on the thickness and composition of the cuticle, turgidity and age of leaves, chemical composition and surface tension of the solution, temperature and relative humidity.

On the other hand, the dynamics of micronutrients in soil is very complex and determined by several environmental factors, including pH, organic matter content, mineralogical composition and texture (Shuman, 1975; McBride and Blasiak, 1979; Martínez and Motto, 2000) or even competition among ions in adsorbing surfaces (Bibak, 1997; Mesquita, 1998; Echeverría et al., 1998).

An interesting alternative, to avoid these problems is the injections of solid substances into the trunk, since this method has higher use efficiency of product, reduces environmental contamination and is applicable when traditional methods (soil and foliar) are inefficient (Neves, 2011, and Fernández Sánchez-Zamora - Escobar, 2004).

Besides the known problems related to B, Cu and Zn supplying to coffee plants little is known about the effects of these nutrients on the quality of beverage, specially on the production of chemical compounds in the beans, moreover, there are no studies about reliable indicators of the nutritional status for coffee plants in B, Cu and Zn.

Thus, the general objective of this study was to characterize the nutritional status of coffee plants and evaluate their response in terms of productivity and production of chemical compounds related to the quality of coffee beans when subjected to solid injections and foliar sprays with B, Cu and Zn, and finally determine metabolic indicators of the nutritional status in B, Cu and Zn of coffee plants.

CAPÍTULO 1

CARACTERIZAÇÃO NUTRICIONAL DE PLANTAS SUBMETIDAS À FERTILIZAÇÃO DE BORO, COBRE E ZINCO FORNECIDOS VIA PULVERIZAÇÃO FOLIAR E INSERÇÃO DE COMPRIMIDOS NO TRONCO

RESUMO

O B, Cu e Zn são os micronutrientes mais limitantes à produção dos cafeeiros. As formas usuais de fornecimento às plantas são via solo ou via folha, no entanto, a eficácia de cada técnica normalmente é limitante por causa de característica químicas e físicas do solo, condições climáticas e mobilidade dos elementos no floema. Assim objetivou-se com este trabalho avaliar a viabilidade de inserção de comprimidos contendo sais de B, Cu e Zn, na haste ortotrópica do cafeeiro. Para isto, foram executados três experimentos em campo sob delineamento em blocos ao acaso com 5 repetições. Para avaliar o efeito do B foram aplicados os tratamentos sem o fornecimento de B, pulverização foliar contendo ácido bórico (0,4%) e inserção de comprimidos contendo sais de B, B+Cu, B+Zn e B+Cu+Zn; para avaliar o efeito do Cu foram aplicados os tratamentos sem o fornecimento de Cu, pulverização foliar contendo sulfato de cobre (0,4%) e inserção de comprimidos contendo sais de Cu, B+Cu, Cu+Zn e B+Cu+Zn e para avaliar o efeito do Zn foram aplicados os tratamentos sem o fornecimento de Zn, pulverização foliar contendo sulfato de zinco (0,4%) e inserção de comprimidos contendo sais de Zn, B+Zn, Cu+Zn e B+Cu+Zn. Avaliaram-se os teores de B, Cu e Zn, em folhas índice entre o período de floração e expansão rápida dos frutos nos dois anos de cultivo, e os conteúdos de B, Cu e Zn em folhas jovens durante 2 anos. A inserção de comprimidos no tronco, contendo sais de Cu e Zn foi eficaz no fornecimento dos nutrientes às plantas. O boro dos comprimidos foi liberado rapidamente e em doses excessivas, sendo assim necessários ajustes dos comprimidos quanto a sua composição e doses. Os conteúdos de B, Cu e Zn em folhas jovens de plantas que receberam esses nutrientes via pulverização ou inserção de comprimidos no tronco, estiveram relacionados ao estágio de desenvolvimento dos frutos.

NUTRITIONAL CARACTERIZATION OF PLANTS SUBMITTED TO FERTILIZATION WITH BORON, COPPER AND ZINC SUPPLIED VIA FOLIAR SPRAY AND SOLID INJECTIONS IN THE TRUNK

ABSTRACT

Boron, copper and zinc are the most limiting nutrient for coffee plants yield. The usual ways of micronutrients supply to the plants are by soil or leaf, however, the effectiveness of each technique is commonly limiting because of the chemical and physical characteristics of the soil, climatic conditions and mobility in the phloem. The objective of this work was to characterize the nutritional status of coffee plants submitted to foliar spray and trunk injections containing B, Cu and Zn salts, as well as, determine the feasibility of trunk injections into the orthotropic branch of coffee plants. Three experiments were designed, in the field, in randomized blocks with 5 replications. In order to assess the effect of B it was applied the treatments without B, foliar sprays containing boric acid at 0,4% and solid injections in the trunk containing B, B+Cu, B+Zn and B+Cu+Zn. To assess the effect of Cu it was applied the treatments without Cu, foliar sprays containing copper sulphate at 0,4% and solid injections in the trunk containing Cu, B+Cu, Cu+Zn and B+Cu+Zn. To assess the effect of Zn it was applied a treatment without Zn, foliar sprays containing zinc sulphate at 0.4% and solid injections in the trunk containing Zn, B+Zn, Cu+Zn and B+Cu+Zn. It was evaluated the B, Cu and Zn content, in index leaves and young leaves during 2 years. Trunk injections containing Cu and Zn were effective in the nutrients providing. Boron was overly released from the salts and adjustments in the composition and doses of the tablets inserted in the trunk are necessary. The contents of B, Cu and Zn in young leaves of plants supplied with these micronutrients by foliar spray or trunk injections were closely related to the fruit stage development.

INTRODUÇÃO

A cafeicultura no Brasil ocupa atualmente uma área equivalente a 2.267.577,8 hectares (Conab, 2014), sendo cultivada em ampla variedade de solos tornando improvável a existência de padrão único de correção das deficiências diversas.

A expansão da cafeicultura para solos de baixa fertilidade exige cada vez melhor entendimento da dinâmica dos nutrientes tanto no solo como na planta, de forma a se evitar problemas nutricionais futuros (Reis Jr. e Martinez, 2002). Condições adequadas de cultivo diminuem o risco da cultura, facilitam e minimizam as operações de manejo e por consequência auxiliam na obtenção de colheitas maiores e de melhor qualidade e com menor custo de produção (Matiello et al., 2005).

A recomendação torna-se mais complicada, pois a eficácia de cada método será influenciada por diversos fatores, com destaque para: fontes, tipo de solo, pH, solubilidade das fontes, efeito residual, mobilidade no solo e na planta, dentre outros. Dentre os vários métodos de aplicação de micronutrientes, destaca-se na cultura do cafeeiro a adubação via solo, incluindo adubação fluida, fertirrigação e a adubação foliar (Novais et al., 2007).

O B, Cu e Zn são os micronutrientes mais limitantes à cultura do cafeeiro, dado que os solos brasileiros têm quantidade suficiente para suprir as necessidades dos demais micronutrientes como o Fe, Mn e Mo.

A aplicação de Zn via solo, na maioria dos casos, só terá resposta positiva em solos de textura média ou arenosa. A compactação do solo, umidade, fonte de zinco, luz, temperatura, irrigação, também influenciam a eficiência da adubação com Zn (Souza, 1999). Destaca-se também que há diferenças quanto à capacidade de absorção, eficiência de uso e tolerância à deficiência de Zn entre variedades de café (Pedrosa, 2013).

Quanto ao B sabe-se que é um nutriente muito pouco móvel no floema e seu movimento do solo até a superfície radicular ocorre por fluxo em massa, assim em condições de estresse hídrico, mesmo que aplicadas doses elevadas, na tentativa de melhorar a produção, o transporte é diminuído, justamente por ser primordial, a água, para o fluxo transpiratório, dificultando a sua aquisição pelas raízes.

O Cu, assim como o Zn, pode ficar retido na superfície dos óxidos de ferro ou alumínio e mais consideravelmente na matéria orgânica do solo, ocorrendo não necessariamente falta do nutriente, mas sua indisponibilidade. O baixo teor de Cu na parte aérea

de algumas plantas deve-se parcialmente ao fato do Cu estar fortemente ligado a matéria orgânica do solo e em parte porque o Cu absorvido pelas raízes é pouco transportado para a parte aérea (Jarvis e Whitehead, 1981). A força de ligação do Cu com os ácidos húmicos diminui com o aumento da quantidade de Cu aplicada e aumenta também com o grau de humificação e com pH do solo (Abreu et al., 2001).

Nessas condições, a principal forma de suprimento de B, Cu e Zn ao cafeeiro é através da pulverização foliar, no entanto, sua eficácia depende da mobilidade do elemento no floema e da penetração através da cutícula sendo necessárias várias pulverizações foliares, onerando o custo da lavoura, e dependendo das condições climáticas e da planta esta técnica pode não ser eficaz.

Assim, para contornar esses problemas a inserção de sustâncias no tronco é uma alternativa interessante, dado que o método apresenta maior eficiência de uso do produto, reduz a contaminação ambiental e é aplicável quando os métodos tradicionais (solo e foliar) são ineficientes (Neves, 2011, Sánchez-Zamora e Fernández - Escobar, 2004).

Assim, o objetivo do trabalho foi caracterizar o estado nutricional de cafeeiros submetidos à pulverização foliar e à inserção de comprimidos no tronco contendo sais de boro, cobre e zinco no tronco, bem como avaliar a viabilidade da inserção de comprimidos no tronco como forma de suprimento desses micronutrientes.

Revisão de literatura

Boro na planta

A absorção de B pelas raízes ocorre principalmente na forma de ácido bórico (H_3BO_3 – Hu e Brown, 1997). O H_3BO_3 é um ácido fraco com pka de 9,24 e em condições normais do citoplasma 98% está como H_3BO_3 livre (Marschner, 2011; Power e Woods, 1997). Ainda segundo esse mesmo autor as principais funções fisiológicas do B estão ligadas a sua capacidade de formar ligações do tipo borato-éster com diversas moléculas biológicas, desde que tenham dois grupamentos hidroxil na configuração cis.

A capacidade do B complexar-se a resíduos apiose existentes em ramnogalacturanos II (Kobayashi et al., 1996), caracteriza a sua função fisiológica mais importante no desenvolvimento, elongação celular e manutenção da integridade estrutural da parede celular (Power e Woods, 1997; Hu et al., 1997), destaca-se que mais de 95 % do B encontra-se ligado às pectinas de parede. Acredita-se que o complexo é composto por duas cadeias de ramnogalacturonanos II monoméricos, as quais estão ligados de forma

cruzada com o ácido bórico através de ligação diéster-borato (Kobayashi et al., 1995). Ainda de acordo com Matoh et *al*., (1996) os ramnogalacturananos II são ligantes exclusivos para B e assim são capazes de ligar e formar uma rede de pectina nas paredes celulares.

A formação dessas ligações cruzadas do tipo diéster-borato é muito ativa durante a formação do tubo polínico no período reprodutivo (Loomis e Durst, 1992). Os órgãos reprodutivos apresentam intenso crescimento em tempo reduzido e suas paredes celulares são ricas em pectina, além disso, as estruturas reprodutivas transpiram pouco e são pobres em feixes vasculares, daí a extrema importância do B no pegamento floral e na retenção dos frutos (Marschner, 2011; Dell e Huang, 1997).

De acordo com Santinato et al., (1991) quando B foi aplicado após a florada fraca de cafeeiros e antes da florada forte, isoladamente ou junto ao Ca, houve aumento da produtividade devido a maior retenção de frutos (30% acima daquela apresentada pela testemunha sem a aplicação de B), embora não tenha sido observada a mesma resposta em anos de safra baixa.

Oertli, (1994) afirma que o B não está distribuído homogeneamente nas plantas, mas acumulado em áreas marginais, topos de folhas e entre nervuras, sendo translocado com a corrente de transpiração. A concentração de B dentro da mesma folha pode variar em 100 vezes e a análise foliar, então, representa somente um valor médio. O teor também aumenta com a idade da planta, e em casos extremos, o B pode atingir níveis tóxicos em folhas velhas e estar deficiente em pontos de crescimento na mesma planta. A taxa de transpiração afeta grandemente o transporte de B dentro das folhas e sua distribuição.

Shelp, (1988) relatou que a concentração de B na seiva do xilema de plantas de brócolis diminuiu para a metade quando o B não foi aplicado, ou foi removido após um período de suprimento adequado, enquanto que a concentração no floema não foi afetada, desaparecendo o gradiente de concentração entre os tecidos maduros e as folhas drenos jovens. O autor também sugere que, em níveis tóxicos, o B sofre extensa transferência lateral, provavelmente de xilema para xilema, aumentando, desse modo, a concentração de B nos drenos em desenvolvimento.

Furlani Junior et al., (2004) observaram que a produção de café em coco foi afetada pelas doses de B aplicadas, aumentando até a dose de 1g de B/planta e diminuindo após este valor. Segundo Fleischer et al., (1999) devido à função do B no arranjo das

pectinas de parede sua deficiência poderia causar alterações no tamanho dos poros da parede celular e posteriormente levar à morte da célula. Em trigo a deficiência de B causou queda na produção principalmente devido a macho esterilidade e consequente queda dos grãos (Rerkasem, et al., 2004).

Tem sido reportado que o B controla a expressão de várias enzimas modificadoras da parede celular tais como a xiloglucano endotransglicosilases/hidrolases (Camacho-Cristóbal et al., 2008). Sob condições de deficiência de B, o rearranjo das microfibrilas de xiloglucanos é comprometido com consequente diminuição da força tensil da parede celular (Ryden et al., 2003).

De acordo com Leite, (2002) a deficiência de B em cafeeiros implicou em vasos do xilema mais finos e menos resistentes, tortuosidades dos feixes vaculares, superfície irregular da parede celular de células do parênquima de folhas e menor número e formato anormal dos estômatos. As paredes mais finas do xilema das folhas das plantas deficientes foram atribuídas ao fato do xilema ser composto por células lignificadas nas quais não há mais deposição de cadeias pécticas e portanto, o aspecto esponjoso não pode ser observado.

Ainda de acordo com Malavolta, (1997) a deficiência de B é acompanhada pela produção e deposição de calose, obstruindo os tubos crivados, com consequente efeito adverso no transporte de seiva do floema.

Pesquisas também mostram a importância do B na manutenção da estrutura de membranas, especialmente, da membrana plasmática (Shelp, 1993; Cakmak e Römheld 1997; Goldbach et al., 2001). A existência de moléculas que contém ligantes hidroxilados como as glicoproteínas e glicolipídios são as possíveis explicações para as funções do B na membrana.

O efeito do B no metabolismo das auxinas, diferenciação e lignificação ainda não está claro, uma vez que os sintomas do excesso de fenóis se confundem com a inibição da atividade das auxinas, particularmente do ácido indolacético (AIA). Primeiramente, sabe-se que B é capaz de formar complexos estáveis com alguns fenóis e assim controlar sua concentração celular; quando há deficiência de B, o excesso de fenóis pode induzir a rota de síntese de lignina a partir de álcool fenóis (Pilbeam e Kirkby, 1983). Sugere-se que nestas condições alguns compostos fenólicos em excesso não só inibem o alongamento radicular mas também aumentam a divisão de células no sentido radial e posteriormente causam mudanças anatômicas (Marschner, 2011).

Acredita-se também, que em condições de deficiência de B a AIA oxidase tem sua atividade induzida por compostos fenólicos não complexados, induzindo a degradação do AIA e assim diminuindo a elongação celular em regiões meristemáticas (Lewis, 1980; Jacob e Uexkull, 1960; Marschner, 2011).

O papel do B como facilitador do transporte de açúcares a curta e longa distância é pouco provável pelo fato do principal açúcar de transporte de plantas de café no floema ser a sacarose, e com a sacarose o B não forma complexos estáveis. Embora, de acordo com Leite, (2002) o cafeeiro produza manitol e ácido cafeico, dois complexantes do B, que podem determinar a mobilidade do nutriente, sendo que a concentração destes compostos varia e exerce influência nas respostas de diferentes cultivares.

O efeito do B no metabolismo dos açúcares é baseado no fato da glicose-6-fosfato estar complexada com o ânion borato, em condições de suficiência e B, e assim restringe o fluxo de substrato respiratório para a rota das pentoses fosfatadas; em condições de deficiência, o fluxo é direcionado para a rota das pentoses fosfatadas e há aumento na biossíntese e acúmulo de fenóis e quinonas, potencialmente capazes de produzir radicais superóxido que danificam as membranas pela peroxidação de seus lipídios constitucionais (Camacho-Cristobal, et al., 2004).

A clorose e a necrose de folhas deficientes em B pode estar relacionada à inabilidade de síntese de parede celular ou mesmo pela ruptura da membrana (Marchner, 2011), no entanto, alguns autores também defendem a ideia de que plantas deficientes acumulam mais compostos fenólicos que posteriormente causam a necrose celular; segundo Goldbach e Amberger (1991) há acúmulo de substâncias melanóides como consequência do aumento da oxidação de quinonas originadas dos ácidos fenólicos e não como um efeito primário da síntese de fenóis.

Zinco na planta

O Zn é absorvido da solução do solo principalmente como Zn^{2+}, mas também pode estar complexado a ligantes orgânicos e ser, nessa forma, absorvido pelas raízes. Na planta existe exclusivamente na forma Zn^{2+} e não participa de reações redox (Marchner, 2011).

Suas funções relacionam-se às suas propriedades de cátion divalente, com forte tendência a formar complexos tetraédricos. O Zn age como componente metálico de algumas enzimas ou como co-fator de inúmeras delas. A desidrogenase alcoólica, supe-

róxido dismutase, anidrase carbônica e RNA-polimerase são exemplos de enzimas que o contêm.

A presença do Zn está intimamente relacionada à síntese de proteínas dado que é um nutriente essencial para a manutenção da integridade estrutural dos ribossomos e da RNA polimerase, enzima polimerizadora de nucleotídeos para a síntese do RNA. O aumento da degradação do RNA pela ação da RNAse, causa acúmulo de aminoácidos em detrimento da divisão celular, o que em parte explica boa parte da sintomatologia observada em folhas deficientes (Marschner, 2011, Sharma et al., 1982; Cakmak et al., 1989).

Ramaiah et al., (1964) observaram que os teores de proteínas formadas em folhas de cafeeiros com deficiência em Zn foram menores que em folhas normais. Malavolta, (1986) afirma que a produção de internódios curtos é resultado da menor síntese de proteínas em detrimento da divisão celular e formação de células com tamanho reduzido, além dos problemas gerados no metabolismo do nitrogênio, especialmente para a redução do nitrato.

O distúrbio no metabolismo do AIA também está intimamente relacionado com os mais típicos sintomas de deficiência de Zn, que são plantas enfezadas e com rosetas, isto é, a inibição da elongação dos internódios e a redução do tamanho da folha (Marschner, 2011; Mengel e Kirkby, 1987; Ferreira e Cruz, 1991). Ainda que obscuro, alguns autores acreditam que o Zn atue sobre a enzima de síntese do triptofano (sintetase do triptofano) que tem este elemento como grupo prostético.

A deficiência de Zn pode causar prejuízos tanto na manutenção enzimática como na enzima sintetase do triptofano, que causa a diminuição do volume celular e menor crescimento apical, devido à redução da síntese ou a própria degradação de auxinas (Epstein e Bloom, 2006).

Malta et al., (2002) observou redução na concentração de triptofano 72 horas após a pulverização foliar do cafeeiro com Zn, demonstrando que a síntese de AIA a partir do triptofano seria ou é inibida com a deficiência de Zn, ou mesmo que devido ao seu papel na síntese de proteínas, a falta de Zn faz com que diversos aminoácidos, inlcuindo triptofano, se acumulem.

Segundo Epstein, (1975), a concentração de AIA no tecido deficiente em Zn diminui mesmo antes do aparecimento dos sintomas visíveis, e fornecendo-se o Zn, o teor aumenta e a planta reinicia seu crescimento. De acordo com Römheld e Marschner, (1991) além da redução na síntese de AIA, a deficiência de Zn promove aumento na sua

degradação oxidativa como resultado do aumento da atividade de peroxidase e radicais livres de oxigênio.

Cafeeiros deficientes em Zn apresentam internódios curtos, folhas pequenas e estreitas, rosetas, gemas terminais mortas, ponteiros secos, superbrotamento, vingamento floral baixo, frutos pequenos, quedas dos frutos e consequentemente produção reduzida (Malavolta et al., 1997). Segundo Epstein, (1975), o efeito da redução na manutenção do AIA no tecido vegetal, além de promover o encurtamento dos internódios, proporciona também o secamento dos ponteiros e, em casos extremos, a morte da planta.

Muitas das injúrias das plantas ocorrem em função do estresse ambiental e estão associadas a danos oxidativos por ação direta ou indireta de espécies reativas de oxigênio (EROs), a exemplo do radical superóxido ($.O_2^-$), e do peróxido de hidrogênio (H_2O_2). As espécies reativas de oxigênio causam peroxidação de lipídeos de membranas, oxidação do AIA, desnaturação de proteínas e mutação do DNA (Bowler et al., 1992; Mittler, 2002).

A SOD apresenta-se em três isoformas possíveis dentro da célula, que se caracterizam pelo metal ligado ao seu sítio ativo. A Fe-SOD encontra-se em maior quantidade no citosol e cloroplastos, a Cu-SOD predominantemente no citosol e a Mn-SOD localiza-se principalmente nas mitocôndrias (Giannopolitis e Ries, 1977; Bowler et al., 1992; Del Río et al., 1998).

A produção de EROs aumenta, enquanto a desintoxicação decresce sob deficiência de Zn, resultando no aumento da atividade de processos oxidativos nas células, que levam ao aumento na permeabilidade de membranas, necroses foliares e inibição do crescimento da parte aérea das plantas. Embora o produto da reação da SOD seja o H_2O_2, sabe-se que este é menos prejudicial à célula e sua eliminação pode ser feita por peroxidases e catalases (Asada,1992).

Devido a inibição do crescimento em decorrência da inibição da síntese de proteínas sob deficiência de Zn há menor consumo de carboidratos, o que por sua vez, diminui a fotossíntese e favorece a produção de EROs, as quais não são removidas e levam ao aparecimento de sintomas de deficiência de Zn, particularmente sob alta intensidade luminosa.

Em três genótipos de trigo diferentes quanto a eficiência metabólica relacionada ao Zn, dois Zn-eficiente e um Zn-ineficiente detectou-se redução nas atividades da Cu-

Zn-SOD e anidrase carbônica quando as plantas foram cultivadas sob condições de deficiência de Zn (Hacisalihoglu et al., 2001).

Yu et al., (1999) em estudos com plantas de tabaco cultivadas em solução nutritiva, avaliaram plantas sem e com suprimento de Zn, tendo verificado que a atividade total da SOD foi reduzida sem o fornecimento de Zn. Esses autores concluíram ainda que a deficiência dos micronutrientes Cu, Zn e Mn pode, dependendo do grau de deficiência, alterar a atividade das enzimas antioxidantes em plantas de tabaco.

Kaya e Higgs, (2001) verificaram que em plantas de tomateiro deficientes em Zn houve redução dos teores de clorofila, aumento na permeabilidade de membranas e redução na produção de matéria seca em relação às plantas controle.

O Zn também é importante para a integridade estrutural das membranas celulares por se ligar a fosfolipídios de membrana, assim, plantas deficientes apresentam maior efluxo radicular de íons, aminoácidos e açúcares (Marschner, 2011).

Quanto à mobilidade do Zn no floema, Silva, (1979) afirma que o Zn é considerado parcialmente móvel dentro da folha na forma de Zn^{2+} ligado ao citrato. Já Malavolta, (1980) afirma que as plantas de café são muito pouco eficientes para absorver e translocar Zn. Martinez et al., (2005) afirmam que houve mobilidade mínima do Zn no floema de cafeeiros, e o caule das plantas é local de armazenamento de Zn, mesmo que temporário e não somente local de transporte do nutriente. Zhang e Brown, (1999) observaram que em condições de suprimento adequado do nutriente apenas parte do Zn é tranlocado no floema e a limitação na remobilização do Zn pode estar associada a alta afinidade do Zn^{2+} com as cargas livres dos vasos dos tecidos foliares.

Cobre na planta

O Cu é absorvido principalmente na forma de Cu^{2+} e é essencial à atividade de diversas enzimas que catalizam reações oxidativas em diversas rotas metabólicas (Lolkema e Vooijs, 1986). O Cu é constituinte de vários tipos de enzimas, dentre elas as enzimas azuis sem atividade de oxidase como a plastocianina que é uma importante transportadora de elétrons no fotossistema I e as proteínas multi-cobre, a exemplo da citocromo oxidase que é a oxidase terminal da cadeia de transporte de elétrons mitocondrial, a ascorbato oxidase que é catalisadora da oxidação do ácido ascórbico a ácido deidroascórbico, as oxidases de fenóis que são catalisadoras da oxidação dos fenóis, as amino oxidases que catalisam desaminações oxidativas e como exposto anteriormente é componente estrutural da CuZn-SOD (Marschner, 2011).

A polifenoloxidase (PPO) possui na estrutura o Cu como grupo prostético (Walker e McCallion, 1980), tendo o oxigênio molecular como co-substrato. Encontram-se latentes e se tornam ativas quando liberadas das membranas dos tilacóides devido ao rompimento causado por algum dano como ataque de insetos ou patógenos (Mayer e Harel, 1991). De acordo com Lax e Vaughn, (1991) polifenoloxidases são encontradas nos cloroplastos, mas são sintetizadas no citoplasma. É uma enzima envolvida em processos de oxi-redução (Mayer, 1987), promovendo a hidroxilação de monohidroxifenol para o-dihidroxifenol e a desidrogenação destes compostos, formando o-quinonas, que ao sofrerem polimerização não enzimática com aminoácidos ou proteínas, formam pigmentos marrons, vermelhos e pretos responsáveis pelo escurecimento dos tecidos (Underhill e Critchley, 1995).

Dado o seu papel na estrutura da plastocianina pode-se inferir que o Cu tem influência direta no processo fotossintético e consequentemente na translocação de açúcares; sob condições de deficiência há acúmulo de celulose e decréscimo nos teores de hemicelulose, lignina e aumento no teor de fenóis, devido à inibição da oxidase dos polifenóis que catalisa a oxidação de compostos fenólicos precursores da lignina e a oxidase das diaminas que catalisa a degradação das poliaminas formando H_2O_2 requerida para a oxidação pela peroxidase (Marschner, 2011; Walker e Loneragan, 1981). De acordo com Bussler, (1981) a diminuição da atividade destas enzimas diminui o processo de lignificação de plantas superiores.

Em cafeeiros, embora sejam raros os casos de deficiência, sabe-se que folhas deficientes tornam-se onduladas na parte superior, com nervuras salientes, evoluindo para clorose e necrose em manchas irregulares geralmente nas margens (Malavolta, 1997; Guimarães et al., 2010).

Fernandes e Henriques, (1991) afirmam que a alta concentração de Cu pode danificar a parede celular e a membrana plasmática e assim causar a perda de seletividade das membranas.

Existem evidências de que o Cu iniba fortemente a absorção de Zn e vice-versa (Bowen, 1969). Ouzounidou, (1994 b) observou que o acúmulo de Cu influencia a distribuição de Mg, Ca, Fe e K na planta. Jensen e Adalsteinsson, (1989) afirmam que o Cu é capaz de deslocar Ca da parede celular de raízes. Assim, sugere-se que o excesso deste nutriente tem efeito negativo na divisão e elongamento celular, evoluindo para a

morte radicular, clorose das folhas seguido de manchas aquosas, queda e secamento de folhas.

O Cu é considerado como prontamente imóvel na planta; de acordo com Loneragan, (1975) compostos nitrogenados solúveis como os aminoácidos atuam como carregadores deste elemento no xilema e floema, já que apresenta alta afinidade com o átomo de N do grupo amino, apesar de sua redistribuição ser dependente do nível de Cu nos tecidos. Liao et al., (2000) afirmam que 99,67% do Cu presente na seiva do xilema de plantas de tomate e chicória encontra-se na forma complexada.

A principal forma de fornecimento de Cu ao cafeeiro é via foliar, haja vista as pequenas quantidades exigidas; segundo Guimarães e Reis, (2010) o Cu pode ser satisfatoriamente suprido quando se adotam fungicidas cúpricos em duas ou mais aplicações para o controle da ferrugem.

MATERIAL E MÉTODOS

O experimento foi instalado em de julho de 2010 e avaliado até março de 2012, sendo conduzido em uma lavoura de *Coffea arabica* L. cv. Catuaí Vermelho IAC-99, em área da Universidade Federal de Viçosa situada à 581m de altitude, 20° 45' Sul e 42° 51' Oeste. O solo foi classificado como Latossolo Vermelho-Amarelo, com clima do tipo Cwa segundo Köppen, e temperatura e precipitação média anual de 19,4ºC e 1.221,4 mm, respectivamente.

Experimento 1 - Boro

Para avaliar o efeito do B, foram aplicados os seguintes tratamentos: controle sem o fornecimento de B, pulverização foliar com ácido bórico (0,4%) e cloreto de potássio (0,5%), inserção de comprimidos, na base do tronco, contendo sais de B, inserção de comprimidos contendo sais de B+Cu, inserção de comprimidos contendo sais de B+Zn e inserção de comprimidos contendo sais de B+Cu+Zn. O delineamento experimental foi o de blocos casualizados com 5 repetições e a parcela útil foi constituída das 4 plantas centrais em espaçamento de 3 x 1m e dispostas em três fileiras. Trabalhou-se com um total de 30 parcelas.

Experimento 2 - Cobre

Para avaliar o efeito do Cu, foram aplicados os seguintes tratamentos: controle sem o fornecimento de Cu, pulverização foliar com sulfato de cobre (0,4%) e cloreto de potássio (0,5%), inserção de comprimidos, na base do tronco, contendo sais de Cu, in-

serção de comprimidos contendo sais de B+Cu, inserção de comprimidos contendo sais de Cu+Zn e inserção de comprimidos contendo sais de B+Cu+Zn. O delineamento experimental foi o de blocos casualizados com 5 repetições e a parcela útil foi constituída das 4 plantas centrais em espaçamento de 3 x 1m e dispostas em três fileiras. Trabalhou-se com um total de 30 parcelas.

Experimento 3 - Zinco

Para avaliar o efeito do Zn, foram aplicados os seguintes tratamentos: controle sem o fornecimento de Zn, pulverização foliar com sulfato de zinco (0.4%) e cloreto de potássio (0.5%), inserção de comprimidos, na base do tronco, contendo sais de Zn, inserção de comprimidos contendo sais de B+Zn, inserção de comprimidos contendo sais de Cu+Zn e inserção de comprimidos contendo sais de B+Cu+Zn. O delineamento experimental foi o de blocos casualizados com 5 repetições e a parcela útil foi constituída das 4 plantas centrais em espaçamento de 3 x 1m e dispostas em três fileiras. Trabalhou-se com um total de 30 parcelas.

A combinação dos sais, em todos os experimentos, de B, Cu e Zn foi fornecida via comprimidos inseridos no alburno da haste ortotrópica do cafeeiro a 10 cm de altura em relação à superfície do solo. Os comprimidos foram elaborados no laboratório de Engenharia Civil da Universidade Federal de Viçosa utilizando-se de prensa hidráulica com força de 0,5 toneladas para todos os comprimidos. O Cu foi fornecido através de cápsulas sem qualquer compactação dos sais, devido à dificuldade desses sais formarem boa liga com os agentes excipientes.

Foram realizadas 3 pulverizações em cada ano agrícola entre os meses de setembro e fevereiro. A calagem e a adubação contendo nitrogênio, fósforo e potássio foram realizadas com base em análise de solo, na produtividade esperada e nas recomendações para o uso de corretivos e fertilizantes em Minas Gerais (Guimarães et al., 1999). As características químicas do solo dos anos 2010, 2011 e 2012 estão apresentados na tabela 1.

Tabela 1 - Composição química da camada de 0-20 cm do solo da área experimental nos anos 2010, 2011 e 2012

	pH	P	K	Ca^{2+}	Mg^{2+}	Al^{3+}	H+Al	SB	CTC (t)	CTC (T)	V	m	Prem
		$mg.dm^{-3}$		$cmol.dm^{3}$							%		$mg\ L^{-1}$
2010	4,9	25,8	70	1	0,3	0,5	3,96	1,48	1,98	5,44	27	25	-
2011	5,06	27,8	132	2,27	0,81	0,39	6,8	3,42	3,81	10,22	33,5	10,2	28,1
2012	7,01	17,1	60	3,6	1,4	0	2,15	5,15	5,15	7,3	71	0	28,9

*Análises realizadas segundo a metodologia da Empresa Brasileira de Pesquisa Agropecuária - EMBRAPA (1997). - pH em água, KCl e $CaCl_2$ - Relação 1:2,5; P e K - Extrator de Melich 1; Ca, Mg, Al - Extrator KCl 1 mol. L^{-1}. H + Al - Extrator Acetato de Cálcio 0,5 mol L^{-1} - pH 7,0; B - Extrator água quente; SB - Soma de Bases Trocáveis; CTC (t) - Capacidade de Troca Catiônica Efetiva; CTC (T) - Capacidade de Troca Catiônica a pH 7,0; V = Índice de Saturação de Bases; m = Índice de Saturação de Alumínio.

O estado nutricional das plantas foi avaliado coletando-se folhas do terceiro ou quarto nó, contado do ápice para a base, de ramos plagiotrópicos do terço médio das plantas no período entre a floração e a expansão rápida dos frutos, calculou-se também o desvio percentual do ótimo (DOP) a partir das concentrações da folha índice dos nutrientes estudados, segundo descrito por Montañes et al., (1991), e usando como norma o ponto central das faixas críticas obtidas para cafeeiro por Martinez et al., (2003). Calcularam-se também os índices DOP para as concentrações correspondentes aos limites inferior e superior da faixa crítica acima referida, obtendo-se os limites de índice DOP em que a nutrição das plantas poderia ser considerada adequada.

Para verificar a eficácia da inserção dos comprimidos no tronco, determinaram-se as concentrações, produção de matéria seca e os conteúdos foliares de B, Zn e Cu nas folhas novas localizadas no ápice dos ramos produtivos e na altura mediana das plantas nos anos agrícolas 2010/2011 e 2011/2012.

Durante todo o período de execução do experimento foram obtidos os valores médios da precipitação acumulada, através da soma das precipitações diárias, nos meses de amostragem (Figura 1).

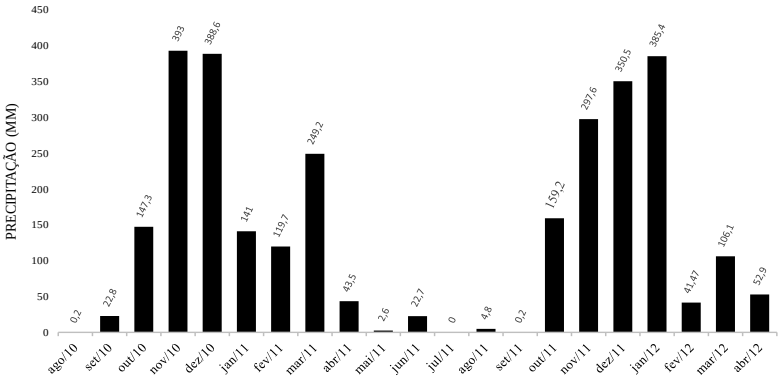


Figura 1 - Precipitação acumulada (mm) no período de execução do experimento.

As folhas coletadas foram lavadas em água desionizada e submetidas à secagem em estufa com circulação forçada de ar, a 70^{o}C, até peso constante, obtendo-se a massa de matéria seca.

Os teores de B foram determinados por digestão via seca, seguida de dosagem com azometina-H base (Malavolta et al., 1997). Os teores de Zn e Cu foram determinados por espectrometria de absorção atômica (AOAC, 1975) no extrato da digestão nítrico-perclórica (Johnson e Ulrich, 1959). De posse das concentrações e das massas de matéria seca das folhas jovens, calcularam-se os acúmulos em µg/folha.

Os dados obtidos nos experimentos foram submetidos a análise de regressão e os modelos escolhidos com base no coeficiente de determinação e no sentido biológico das respostas aos tratamentos segundo as variáveis analisadas.

RESULTADOS E DISCUSSÃO

Teores de Boro, Cobre e Zinco na folha índice

De acordo com Montañes et al., (1991) quando o valor médio do índice DOP for negativo, indica que há deficiência; valor positivo, indica excesso e valor zero, indica teor ótimo. Quanto maior o valor absoluto do índice, maior a severidade da deficiência ou excesso e a soma dos valores absolutos de todos os nutrientes da amostra, representa um índice do balanço ou equilíbrio nutricional da lavoura, o que permite a comparação de lavouras distintas entre si e o somatório com maior valor, representa também, maior desequilíbrio nutricional.

Os índices DOP do tratamento controle, que não recebeu B, Cu ou Zn, apresentaram-se negativos, abaixo do limite inferior da faixa de DOP calculada nos dois anos de avaliação para os três nutrientes avaliados (Tabela 2), baseando-se na faixa crítica estabelecida por Martinez et al., (2003) para cafeeiros da região de Viçosa-MG, que é de 29 a 52 mg/ kg de B, 13 a 29 mg/ kg de Cu e 6 a 12 mg/ kg de Zn.

O índice DOP do tratamento pulverizado, no ano de 2010, apresentou-se dentro do limite de confiança para os três nutrientes avaliados, lembrando que uma pulverização foliar foi feita 8 dias antes da amostragem de folhas. Já no ano de 2011, o índice DOP esteve ligeiramente abaixo da faixa de suficiência, apenas para o B, dado que uma das pulverizações foliares foi feita 87 dias antes da amostragem (Tabela 2).

Os índices DOP, dos tratamentos com inserção de comprimidos com um único nutriente estiveram dentro do intervalo de confiança para o Cu e Zn, já para B inserido

no tronco, observa-se que apenas em 2010 o índice DOP esteve dentro do intervalo de confiança, em 2011 houve excesso do nutriente. Em 2010, a inserção de comprimidos no tronco elevou os teores foliares para níveis adequados logo na primeira avaliação, exceto para o tratamento contendo B+Cu+Zn; nota-se que este resultado deve-se em parte à alta precipitação acumulada no período que antecede esta amostragem (Figura1).

No ano seguinte, embora as concentrações tenham sido inferiores, os teores apresentaram o mesmo comportamento, mostrando que ainda que as doses e a composição dos comprimidos precisem ser ajustadas, a inserção de comprimidos contendo B, Cu e Zn no tronco, foram eficazes para o fornecimento desses micronutrientes ao cafeeiro (Tabela 2).

As formulações contendo mais de um nutriente por comprimido apresentaram resultados variáveis. Comprimidos contendo B+Cu permitiram obter concentrações foliares adequadas de Cu nos dois anos e excessivas de B no segundo ano. Comprimidos contendo B+Zn permitiram obter concentrações adequadas de Zn nos dois anos e excessivas de B no segundo ano. Comprimidos contendo Cu+Zn resultaram em concentrações adequadas de Cu nos dois anos e acima do ótimo para Zn no segundo ano. A combinação dos três nutrientes em um só comprimido permitiu obter concentrações adequadas de Cu e Zn nos dois anos e excessivas de B no segundo ano. Tais resultados indicam que o método, se bem ajustado poderá ser empregado para o fornecimento conjunto de mais de um micronutriente ao cafeeiro.

Tabela 2 - Desvio percentual do ótimo (DOP) em folhas índices de *Coffea arabica* que receberam B, Cu e Zn via inserção de comprimidos no tronco e via pulverização foliar

Faixa de suficiência para B	Limite inferior	Índice DOP	Limite superior	Índice DOP
	31 mg/ kg	-8,823	37 mg/ kg	8,823
	Teor de B 2010	Índice DOP	Teor de B 2011	Índice DOP
TC	24,07	-29,19	27,46	-19,22
TP	35,04	3,06	30,35	-10,73
B	35,508	4,44	50,51	48,56
B+Cu	35,036	3,05	64,64	90,14
B+Zn	33,25	-2,18	72,31	112,68
B+Cu+Zn	23,93	-29,60	78,64	131,29
Faixa de suficiência para Cu	Limite inferior	Índice DOP	Limite superior	Índice DOP
	13 mg/ kg	-38,095	29 mg/ kg	38,095
	Teor de Cu 2010	Índice DOP	Teor de Cu 2011	Índice DOP
TC	9,64	-54,10	5,24	-75,05
TP	13,50	-35,68	13,13	-37,44
Cu	17,05	-18,77	18,61	-11,35
B+Cu	14,94	-28,84	15,79	-24,81
Cu+Zn	18,96	-9,69	19,40	-7,58
B+Cu+Zn	16,462	-21,61	16,52	-21,33
	Limite inferior	Índice DOP	Limite superior	Índice DOP
Faixa de suficiência para Zn	6 mg/ kg	-33,33	12 mg/ kg	33,33
	Teor de Zn 2010	Índice DOP	Teor de Zn 2011	Índice DOP
TC	6,52	-27,53	4,68	-48
TP	10,06	11,8	7,42	-17,56
Zn	10,85	20,59	11,58	28,67
B+Zn	10,63	18,15	10,89	21,0
Cu+Zn	11,75	30,6	13,66	51,78
B+Cu+Zn	9,85	9,45	9,98	10,89

T0 - Tratamento controle, sem aplicação de Boro, Cobre ou Zinco,
TP - Tratamento pulverizado com ácido bórico, sulfato de zinco e sulfato de cobre,
B - Inserção de comprimido no tronco contendo sais de Boro,
B+Cu - Inserção de comprimido no tronco contendo sais de Boro e Cobre,
B+Zn - Inserção de comprimido no tronco contendo sais de Boro e Zinco,
Cu+Zn - Inserção de comprimido no tronco contendo sais de Cobre e Zinco,
B+Cu+Zn - Inserção de comprimido no tronco contendo sais de Boro, Cobre e Zinco.

Conteúdos de Boro, Cobre e Zinco nas folhas jovens

Quando a taxa de crescimento relativo da matéria seca é superior à taxa de absorção relativa do nutriente ocorre a diluição da concentração do nutriente, fato que é amplamente relatado na literatura (Jarrell e Beverly, 1981). Considerando que os sintomas de deficiência ou toxidez de B e Zn incluem a redução do tamanho da folha (Malavolta et al., 1997; Epstein e Bloom, 2006), a apresentação dos resultados do conteúdo de

micronutrientes por folha, dá melhor informação da disponibilização e alocação dos micronutrientes em estudo nos tecidos jovens em crescimento.

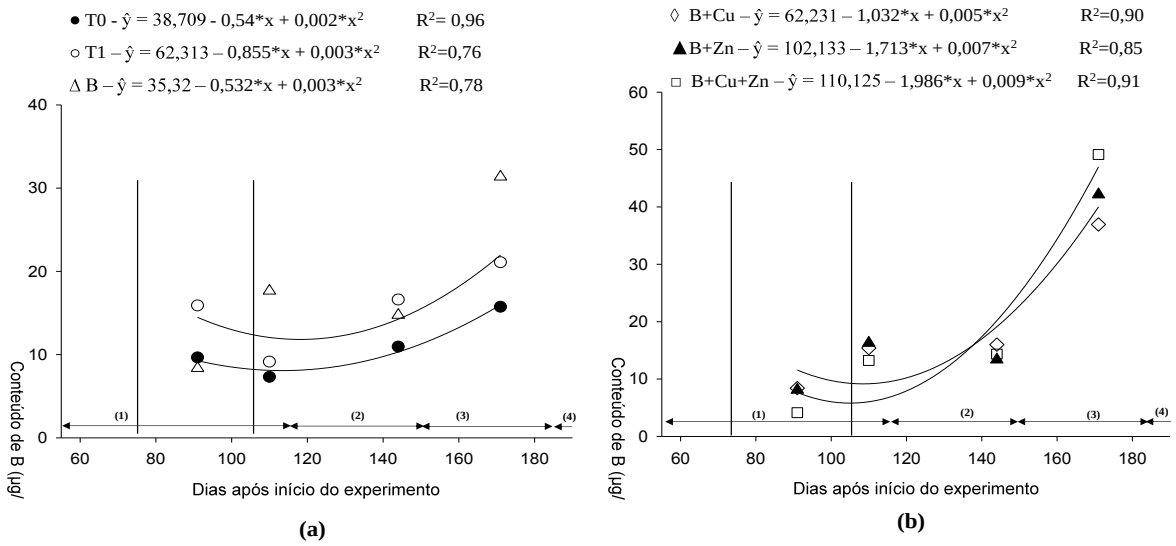
Vale ressaltar que a floração aconteceu aos 55 e 422 dias após início do experimento e que segundo Laviola et al., (2009) em cafeeiros cultivados a 651m de altitude, semelhante à altitude da região de Viçosa, a duração da fase de chumbinho é de 66 dias, a expansão rápida de 35 dias, o crescimento suspenso de 32 dias e a granação e maturação aproximadamente de 91 dias.

Boro

No ano de 2010/2011, observa-se que o conteúdo de B em folhas jovens do tratamento pulverizado e dos tratamentos que receberam B, B+Cu, B+Zn e B+Cu+Zn inseridos no tronco apresentaram comportamento quadrático, sendo os pontos de mínimo estimados aproximadamente aos 118, 92, 101, 108 e 105 dias após início do experimento, respectivamente, ou seja quando os frutos estavam no final do estágio de chumbinho. Nota-se que ao final da primeira expansão rápida o conteúdo dos tratamentos que receberam B via inserção de comprimidos era consideravelmente maior que os do tratamento pulverizado e do tratamento controle (Figura 2a, 2b).

O aumento do conteúdo foliar ao longo do ciclo, em todos os tratamentos provavelmente decorra dos avanços da estação das águas e suas consequências na água disponível, o que impulsionou a maior transpiração e consequentes absorção e transporte do B para a parte aérea ao longo do tempo (Figura 1, 2a, 2b).

Embora, o conteúdo foliar de B do tratamento controle, sem a aplicação de B, Cu ou Zn, esteja abaixo do observado nos tratamentos em que foi inserido comprimido no tronco, o modelo que melhor se ajustou aos dados também foi o quadrático, o ligeiro aumento a partir dos 112 dias deve-se certamente a reservas do nutriente no caule e no solo e que com o aumento do fluxo transpiracional foi para a parte aérea (Figura 2a).



*****As linhas verticais representam as datas em que foram realizadas as pulverizações foliares.
[1] Chumbinho; [2] Expansão Rápida; [3] Crescimento Suspenso; [4] Granação e Maturação

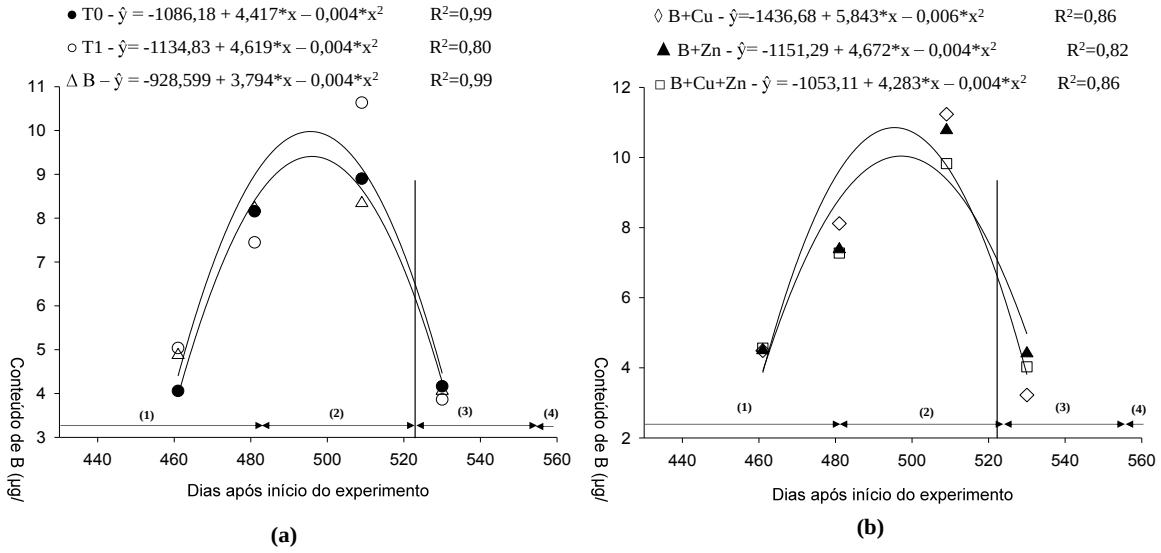
Figura 2 – Conteúdo de B em folhas jovens de plantas que não receberam B, Cu e Zn e de plantas submetidas à pulverização foliar e inserção de comprimidos no tronco contendo sais de B (a), e inserção de comprimidos no tronco contendo sais de B+Cu, B+Zn e B+Cu+Zn (b), no ano de 2010.

De acordo com Laviola et al., (2009), no estádio de chumbinho, a proporção de acúmulo, tanto de B como de Zn, nos frutos é maior, quando comparada à de acúmulo dos outros micronutrientes devendo-se à grande importância destes nutrientes nos processos de divisão celular e na estabilização de membranas das novas células formadas (Marschner, 2011; Marenco e Lopes, 2005).

A pulverização foliar é uma prática utilizada para o fornecimento de micronutrientes ao cafeeiro, entretanto, por ser o B e o Zn elementos de baixa mobilidade no floema (Faquin, 1994) várias pulverizações anuais são necessárias pelo fato de não haver translocação significativa para as folhas novas que crescem após a pulverização (Malavolta, 1980; Favaro, 1992).

No ano 2011, os tratamentos que receberam B via inserção de comprimidos e o tratamento pulverizado apresentaram comportamento quadrático, sendo os pontos de máximo estimados aos 491, 499, 495, 497 e 498 dias, esses resultados sugerem que houve grande exigência em B pelos frutos que estavam em fase de chumbinho e expansão rápida incialmente, mesmo que, para o tratamento pulverizado, foi feita uma aplicação aos 524 dias após início do experimento. Ao final das amostragens possivelmente houve esgotamento do B contido nos comprimidos já que os conteúdos foliares dos tra-

tamentos que receberam B via inserção de comprimidos foram próximos aos do tratamento controle, sem aplicação de B, Cu ou Zn, aliado ao fato de que nesse período às precipitações constantes favoreciam a absorção e transporte do nutriente para a parte aérea (Figura 3a, 3b).



*****As linhas verticais representam as datas em que foram realizadas as pulverizações foliares.
[1] Chumbinho; [2] Expansão Rápida; [3] Crescimento Suspenso; [4] Granação e Maturação

Figura 3 – Conteúdo de B em folhas jovens de plantas que não receberam B, Cu e Zn e de plantas submetidas à pulverização foliar e inserção de comprimidos no tronco contendo sais de B (a), e inserção de comprimidos no tronco contendo sais de B+Cu, B+Zn e B+Cu+Zn (b), no ano de 2011.

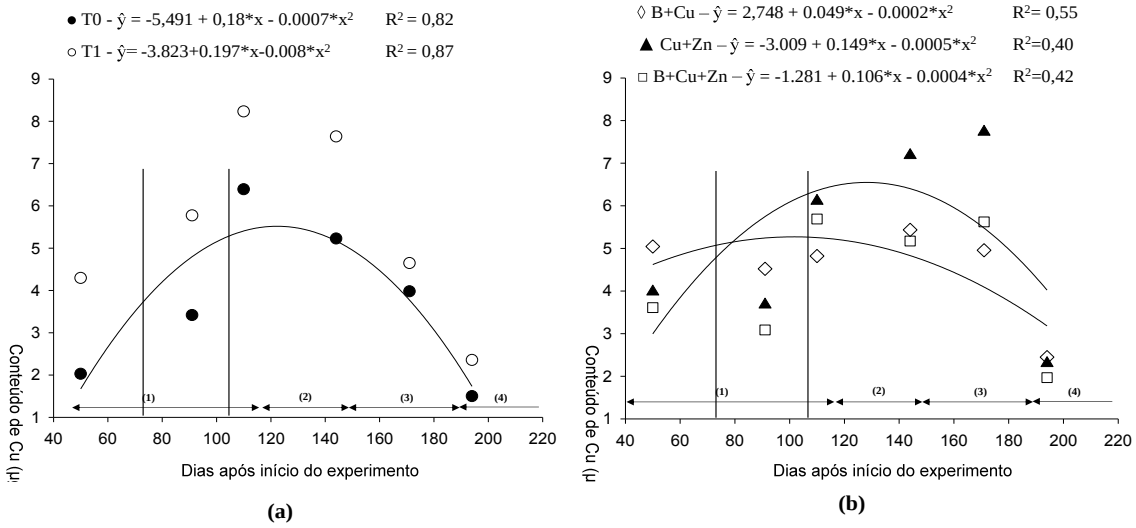
Cobre

Os conteúdos foliares no tratamento pulverizado e os tratamentos B+Cu, Cu+Zn e B+Cu+Zn inseridos no tronco apresentaram comportamento quadrático, no primeiro ano de avaliação, sendo os pontos de máximo estimados aos 109, 124, 124, e 133 dias após início do experimento, respectivamente, o tratamento contendo apenas Cu inserido no tronco não variou com o tempo sendo a média geral de 4,79 µg/ folha (Figura 4a, 4b). De modo geral esses pontos situam-se no final da fase de chumbinho e início da primeira expansão rápida, o que provavelmente elevou a demanda pelo micronutriente e fez com que os conteúdos foliares se reduzissem.

As precipitações pluviométricas frequentes possivelmente são a razão da absorção crescente até essas datas. Por outro lado, ao final da fase de expansão rápida os conteúdos foliares já eram baixos o que mostra uma possível competição fruto/folha, aliado

à liberação inicial rápida do nutriente devido o fornecimento do Cu via inserção de cápsulas sem a devida compactação para que a liberação fosse gradual. De acordo com Laviola et al., (2009) o Cu apresenta mínima concentração nas folhas em fase de granação e maturação.

O tratamento pulverizado apresentou comportamento parecido, mas ao início da fase de granação e maturação os conteúdos foliares também eram baixos, provavelmente devido ao baixo efeito residual das pulverizações foliares (Figura 4a).

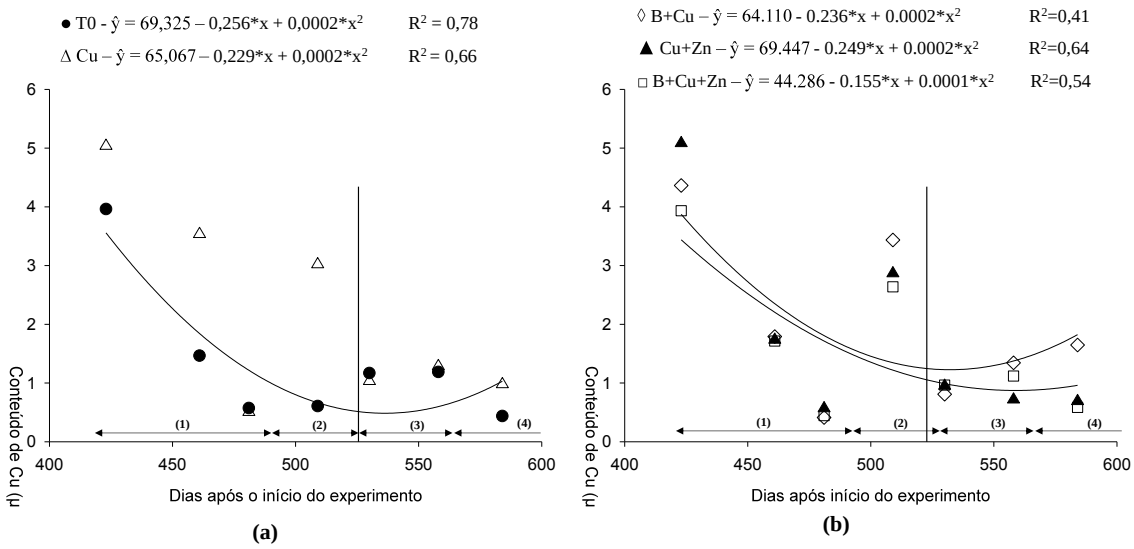


*****As linhas verticais representam as datas em que foram realizadas as pulverizações foliares.
[1] Chumbinho; [2] Expansão Rápida; [3] Crescimento Suspenso; [4] Granação e Maturação

Figura 4 – Conteúdo de Cu em folhas jovens de plantas que não receberam B, Cu e Zn e de plantas submetidas à pulverização foliar, inserção de comprimidos no tronco contendo sais de Cu (a), e inserção de comprimidos no tronco contendo sais de B+Cu, Cu+Zn e B+Cu+Zn, no ano de 2010.

No ano de 2011, o conteúdo foliar de Cu dos tratamentos contendo Cu, B+Cu, Cu+Zn e B+Cu+Zn, apresentaram comportamento quadrático sendo os pontos de mínimo estimados aos 572, 590, 623 e 777 dias, respectivamente, sugerindo que houve liberação rápida do nutriente logo no primeiro ano de avaliação e que nesse ano houve maior translocação do nutriente para os frutos em formação em detrimentos das folhas jovens, o conteúdo foliar do tratamento pulverizado não variou ao longo do tempo e a média geral foi de 2,34 µg/ folha (Figura 5a, 5b).

Em ambos os anos, o tratamento controle foi inferior aos tratamentos que receberam Cu via pulverização ou via inserção de comprimidos no tronco (Figura 5a).

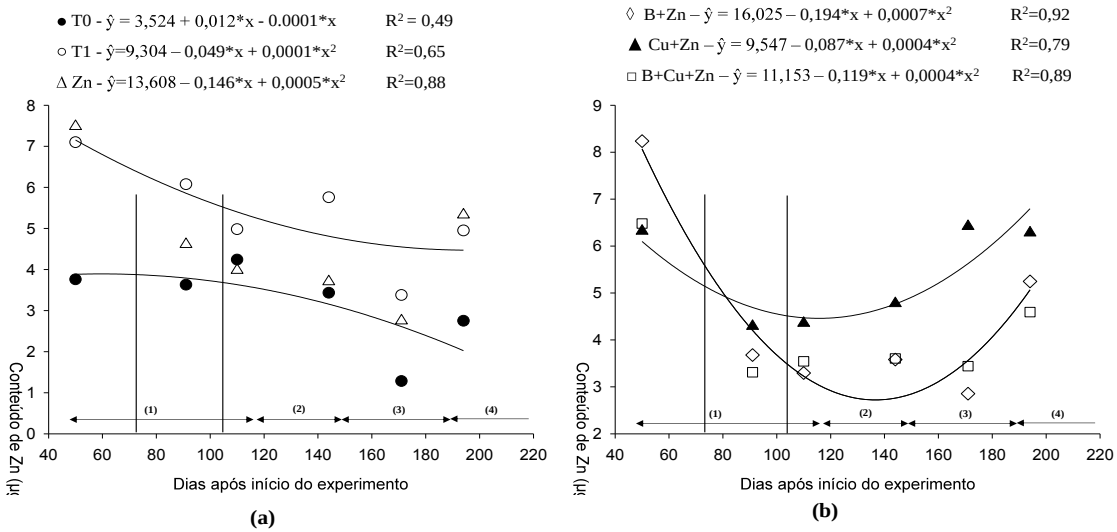


*****As linhas verticais representam as datas em que foram realizadas as pulverizações foliares.
[1] Chumbinho; [2] Expansão Rápida; [3] Crescimento Suspenso; [4] Granação e Maturação

Figura 5 – Conteúdo de Cu em folhas jovens de plantas que não receberam B, Cu e Zn e de plantas submetidas à pulverização foliar, inserção de comprimidos no tronco contendo sais de Cu (a), e inserção de comprimidos no tronco contendo sais de B+Cu, Cu+Zn e B+Cu+Zn, ano de 2011.

Zinco

O conteúdo foliar de Zn do tratamento pulverizado e daqueles contendo Zn, B+Zn, Cu+Zn e B+Cu+Zn inserido no tronco apresentaram comportamento quadrático sendo os pontos de mínimo estimados aos 246, 146, 139, 110 e 149 dias após início do experimento (Figura 6a, 6b), respectivamente, indicando que até essas datas, mesmo com bom volume de chuvas, houve maior translocação do nutriente para os frutos em detrimento das folhas jovens concordando com os resultados de Laviola et al., (2009). Segundo esse autor, o Zn foi o elemento que apresentou maior acúmulo relativo, em frutos, no estádio de expansão rápida, denotando a importância do Zn na síntese de triptofano, aminoácido precursor da biossíntese da auxina, ácido indol acético (AIA), essencial para o processo de alongamento celular.

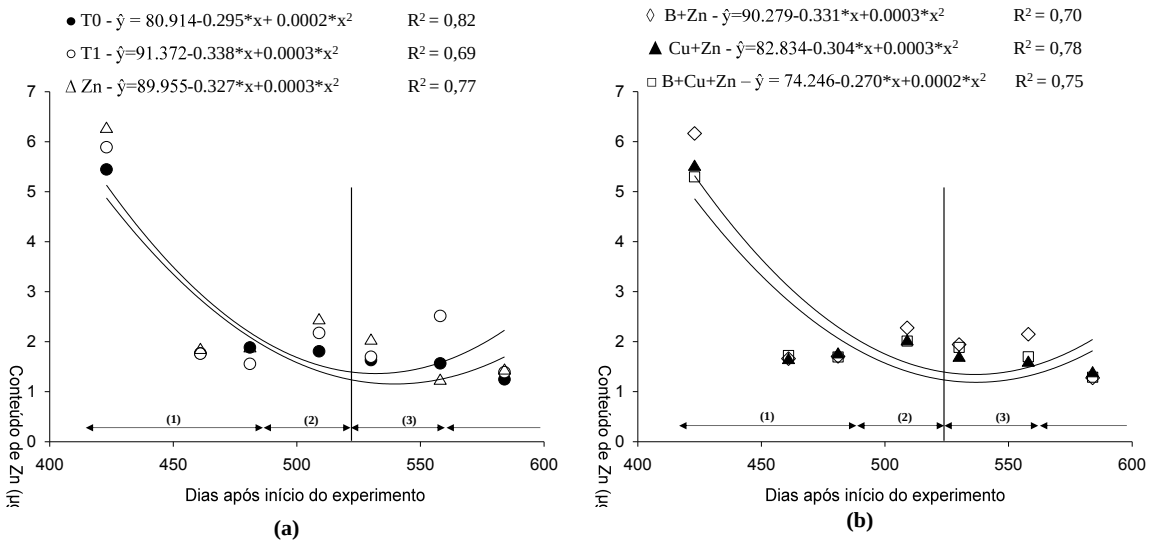


*****As linhas verticais representam as datas em que foram realizadas as pulverizações foliares.
[1] Chumbinho; [2] Expansão Rápida; [3] Crescimento Suspenso; [4] Granação e Maturação

Figura 6 – Conteúdo de Zn em folhas jovens de plantas que não receberam B, Cu e Zn e de plantas submetidas à pulverização foliar, inserção de comprimidos no tronco contendo sais de Zn (a), e inserção de comprimidos no tronco contendo sais de B+Zn, Cu+Zn e B+Cu+Zn, ano de 2010.

No segundo ano de avaliação, observa-se o mesmo comportamento, o tratamento pulverizado e aqueles contendo Zn, B+Zn, Cu+Zn e B+Cu+Zn apresentaram comportamento quadrático e atingiram concentrações mínimas aos 564, 545, 552, 507 e 451 dias após início do experimento, respectivamente, todas próximas, levando a crer que tenham tido grande influência da fase de desenvolvimento dos frutos; o tratamento controle, sem a aplicação de B, Cu ou Zn, também apresentou o mesmo comportamento possivelmente em função de reservas do nutriente no solo (Figura 7a, 7b).

Uma pulverização foliar foi feita aos 374 dias e por isto os conteúdos de Zn são altos inicialmente. A tendência de aumento dos conteúdos foliares a partir dessas datas provavelmente é função do início da fase de granação e maturação com redução da demanda por Zn.



*****As linhas verticais representam as datas em que foram realizadas as pulverizações foliares.
[1] Chumbinho; [2] Expansão Rápida; [3] Crescimento Suspenso; [4] Granação e Maturação

Figura 7 – Conteúdo de Zn em folhas jovens de plantas que não receberam B, Cu e Zn e de plantas submetidas à pulverização foliar, inserção de comprimidos no tronco contendo sais de Zn (a), e inserção de comprimidos no tronco contendo sais de B+Zn, Cu+Zn e B+Cu+Zn, ano de 2011.

CONCLUSÕES

A inserção de comprimidos no tronco, contendo sais de Cu e Zn é eficaz no fornecimento dos nutrientes às plantas. Quanto ao B é necessário ajustar a composição e as doses para que a liberação seja gradual.

Os conteúdos de B, Cu e Zn em folhas jovens foram influenciados pelo estágio de desenvolvimento dos frutos, independentemente da forma de fornecimento, por serem os drenos preferenciais.

CHAPTER 2

BORON, COPPER AND ZINC AFFECTING THE PRODUCTIVITY, CUPPING QUALITY AND CHEMICAL COMPOUNDS IN COFFEE BEANS

ABSTRACT

Micronutrients, although required in small quantities, play specific and essential functions on plant metabolism, and its deficiency can lead to significant metabolic disorders, enough to reduce the production and the quality of coffee beverage. The role of micronutrients for coffee production is widely known, however, little is known about the influence of B, Cu and Zn on coffee brew quality. In order to verify the feasibility of supplying B, Cu and Zn by solid injections into the orthotropic branch of *Coffea arabica* L., as well as, evaluate the response of the coffee tree in terms of productivity and beverage quality, three field experiments were performed. All the experiments were arranged in a randomized blocks design with 5 replications. In order to assess the effect of B it was applied a treatment without B, foliar sprays containing boric acid at 0.4% and solid injections in the trunk containing B, B+Cu, B+Zn and B+Cu+Zn. To assess the effect of Cu it was applied a treatment without Cu, foliar sprays containing copper sulphate at 0.4% and solid injections in the trunk containing Cu, Cu+B, Cu+Zn and B+Cu+Zn. To assess the effect of Zn it was applied a treatment without Zn, foliar sprays containing zinc sulphate at 0.4% and solid injections in the trunk containing Zn, Zn+B, Zn+Cu and B+Cu+Zn. The productivity, cupping quality and some chemical indicators of beans quality were evaluated in two crop seasons. Boron, copper and zinc supplied by foliar spray or solid injections in the trunk influenced the chemical composition and quality of the coffee beans, characterized by the levels of caffeine, trigonelline, sucrose, glucose, arabinose, mannose, 3-caffeoylquinic acid, 5-caffeoylquinic acid, polyphenol oxidase activity and total phenolic compounds. Only Cu nutrition influenced the cupping quality. Copper and zinc supplied by solid injections was equivalent in either forms of supply in production and in quality.

BORO, COBRE E ZINCO AFETANDO A PRODUTIVIDADE, QUALIDADE DA BEBIDA E COMPOSTOS QUÍMICOS DE GRÃOS DE CAFÉ

RESUMO

Os micronutrientes, embora exigidos em pequenas quantidades, desempenham funções específicas e essenciais no metabolismo das plantas, sendo que a deficiência pode ocasionar distúrbios metabólicos importantes o suficiente para reduzir a produção e a qualidade do cafeeiro. A importância dos micronutrientes para a produção do cafeeiro é largamente conhecida, entretanto, pouco se sabe sobre a influência do B, Cu e Zn na qualidade da bebida do café. Para verificar a viabilidade de suprimento de B, Cu e Zn via inserção de comprimidos na haste ortotrópica do *Coffea arabica* L., bem como, avaliar a resposta do cafeeiro em termos de produtividade e qualidade da bebida, foram executados três experimentos em campo. Todos os experimentos foram dispostos como blocos casualizados com 5 repetições. Para avaliar o efeito do B foram aplicados os tratamentos sem o fornecimento de B, pulverização foliar contendo ácido bórico (0,4%) e inserção de comprimidos contendo sais de B, B+Cu, B+Zn e B+Cu+Zn; para avaliar o efeito do Cu foram aplicados os tratamentos sem o fornecimento de Cu, pulverização foliar contendo sulfato de cobre (0,4%) e inserção de comprimidos contendo sais de Cu, B+Cu, Cu+Zn e B+Cu+Zn e para avaliar o efeito do Zn foram aplicados os tratamentos sem o fornecimento de Zn, pulverização foliar contendo sulfato de zinco (0,4%) e inserção de comprimidos contendo sais de Zn, B+Zn, Cu+Zn e B+Cu+Zn. Avaliaram-se a produtividade e a prova de xícara e alguns atributos químicos indicadores da qualidade dos grãos em dois anos consecutivos. O B, Cu e Zn fornecidos via pulverização foliar ou inserção de comprimidos na base do tronco influenciaram a qualidade dos grãos de café, caracterizada pelos teores de cafeína, trigonelina, sacarose, glicose, arabinose, manose, ácido 3-cafeoilquínico, ácido 5- cafeoilquínico, atividade da polifenoloxidase e compostos fenólicos totais. Apenas o Cu influenciou a qualidade da bebida avaliada pela prova de xícara. Ambas as formas de fornecimento de Cu e Zn foram equivalentes tanto para a produção quanto para a qualidade da bebida.

INTRODUCTION

Brazil is the largest world producer of coffee. As a world leader in production and exportation, the country needs to attend market requirements, innovating and adopting technologies to produce good-quality types of coffee.

The necessity of offering good coffees is rising up, due to the enhancement of consuming coffees with refined taste and aroma, which are related to the chemical composition of the coffee beans. The flavor and the aroma are the main criteria to evaluate the quality of beverage, as well the most important attributes for consuming coffee (Cantergiani et al., 1999; Clarke, 1987). The cup test has been considered the best way to evaluate the flavor and aroma of coffee, however quite often it is criticized by its subjective nature (Carvalho et al., 1994). Therefore, it is essential to study accurated methods that correlate the chemical characteristics and the quality of beverage.

The production of bioactive compounds related to the desirable flavor and aroma involves extremely complex chemical reactions, however, until now, little or not is known about the effect of the mineral nutrients B, Cu and Zn on the production of chemical compounds that define the good-quality of coffee. Most of the studies about mineral nutrition and coffee fertilization are focused on sources and doses of these nutrients, in order to optimize the productivity.

This work aimed evaluate the production of bioactive compounds and the quality of raw coffee beans harvested from plants fertilized with B, Cu and Zn via foliar sprays or solid injections of salts in the trunk and correlate these variables with the nutritional status of the plants.

Background review

Zinc

According to Fageria et al., (2002) the lack of Zn impairs the world agriculture production. Around 50% of the cereal farmland over the world have low Zn availability, which reduce the production and nutritional quality of grains.

Symptoms of Zn deficiency are related, mainly, to disturbance in auxin metabolism, as for example the indolacetic acid (IAA) that is a phytohormone responsible to the cell elongation and plant growth regulation.

Although, the function of Zn on auxin metabolism is not clarified (Epstein, 1975; Faquin, 1994; Marschner, 2011), it is accepted that it plays essential role in the

synthesis of tryptophan, an amino acid precursor of IAA (Válio, 1979; Mengel and Kirkby, 1987).

The photosynthetic activity of coffee plants is hugely diminished under Zn lack, given its importance on enzymes involved in carbon fixation (Hansch and Mendel, 2009). Besides this, Zn regulates or makes part of the structure of several other enzymes involved in protein synthesis, and in nitrogen metabolism (Marschner, 2011, Cakmak et al., 1989).

Neves et al., (2011) observed an increase of 30.9% on coffee production with the injection of tablets containing 1.8 g of Zn salts in the trunk of coffee-plants, comparatively to the control treatment without Zn application.

Melo et al., (1999) states that the production of coffee responds to increasing doses of zinc sulphate supplied by foliar spray, and that the spraying at low Zn concentrations four times during the growth season provided significant increase on coffee production.

Studying increasingly zinc sulphate doses supplied as foliar spray in coffee plants cultivated in a Latossol, Silva, (1979) obtained an average of increase in production of 73% in three years, when applying 4 kg/ ha/ year and 82% when applying 6 kg/ ha/ year. However, when applying 8 kg/ ha/ year the production increase was only of 39% comparatively to the control treatment without Zn supply.

Boron

Boron deficiency is common in most of the Brazilian soils (Malavolta, 1986; Malavolta et al., 1981). In coffee plants, its deficiency has been attributed to the natural loss of soil fertility, as well as the wide use of highly demanding varieties. Boron deficiency in coffee plants can reduce the root system growth; cause the death of thin root tips and consequently the decrease in water and mineral absorption. In consequence of that, the plants become sensible to the drought and less responsive to fertilizations. Due to B functions on pollinic tube formation, its deficiency can cause flower abortion, pith and low productivity (Franco, 1982).

Santinato et al., (1991) state that the supply of B, as organic material, 6 to 12 times a year, which do not necessarily coincide with the flowering, promoted greater fruit retention (30% higher when compared to the control treatment without B application) and an increase in productivity, as well as the maintenance of good foliar content.

According to Araújo and Silva, (2012), the production of cottonseeds was posi-

tively influenced by B and Zn interaction, responding linearly to the concentrations of these nutrients. Similar results were found by Lima, (2006) in which the production of sesame seeds was significantly affected by the B and Zn interaction. Hosseini et al., (2003) also state the synergic effect between B and Zn in maize plants.

Copper

In regions with soils rich in organic matter, most of the Cu is linked to this fraction (Sanders et al., 1986). Even not being as common as the deficiencies of B and Zn, in coffee plants Cu deficiency compromise the activity of several enzymes that catalyze oxidative reactions of several metabolic routes, specially the plastocyanin, dismutase superoxide and polyphenol oxidase (Marschner, 2011). The common form used for Cu supplying to coffee plants is through foliar spray, however, when Zn is present, they compete to the same absorption site and prevail the one in higher concentration. The Cu deficiency can cause irreversible metabolic disturbances in coffee plants, and possibly compromise the production of chemicals related to the beverage quality, however, there is a lack of studies about this subject.

Coffees of superior quality are those that have in adequate amounts chemical compounds responsible for the flavor and aroma such as caffeine, trigonelline, aldeides, furans, ketones, sugars, proteins, amino acids, pirroles, pyridines, pyrazines, oxazoles, carboxilic acids, fatty acids, phenolic compounds, etc. Altogether, in adequate proportions, they are responsible to the body, acidity and smooth of the beverage.

Chemical compounds of coffee beans

Production of secondary metabolites, such as polyphenols, caffeine, trigonelline, alcohols and aldehydes depends on the primary metabolism and its catabolic reactions that produce energy and carbonic skeleton, such as the sucrose. Therefore, if any factor affects photosynthates production during the fruits development, also affects negatively the quality of beverage (Fagan et al., 2011).

Caffeine, commonly known as 1, 3, 7 trimethylxanthine, belongs to the methylxanthine class and gives bitterness to the coffee taste (Clifford, 1985). During the roast process, 10% of the caffeine volatilizes, simultaneously the bean mass decrease due to the water evaporation, and thus the caffeine content in the beans increase. Mazzafera, (1999) observed in leaves that K deficiency induces and P lack reduces the the caffeine production.

Trigonelline is an alkaloid biologically derived from enzymatic methylation of

nicotinic acid. It contributes to the bitterness of the brew and is a precursor for the formation of different classes of volatile compounds during roasting such as pyrroles and pyridines (Farah, 2012), some of which according to Flament, (1968) may confer an "objectionable flavor".

Non-reducing sugars predominates in coffee beans, mainly sucrose. During the roast process the reducing sugars react with amino acids (Mailard reaction and/or caramelization) leading to the formation of colorful volatile compounds that present effect on the flavor of the final product (Carvalho, Chalfoun and Chagas, 1989).

Chlorogenic acids (CGA) comprise a major class of phenolic compounds, which are derived primarily from esterification of trans-cinnamic acids (e.g., caffeic, ferulic, and p-coumaric) with quinic acid and may account up to 14% of their dried mass (Trugo and Macrae, 1984). CGA have a marked influence in determining coffee quality and play an important role in the formation of coffee flavor (Carelli et al., 1974; Clifford and Wight, 1976; Trugo and Macrae, 1984a, Variyar et al., 2003, Farah, 2006a). These compounds confer astringency, bitterness and acidity to the coffee brew, nevertheless high amounts in green coffee, particularly caffeoylquinic and feruloylquinic acids may produce undesirable flavor possibly due to oxidation and degradation products formed before roasting.

During the roast process, they are strongly degraded generating acids, lactones and volatile compounds such as the phenol, guaiacol and 4-vinyl guaiacol (Silwar et al., 1986; Bichi et al., 1995; Nogueira and Trugo, 2003), which contribute to the flavor and aroma of the coffee, especially for the astringency of the beverage; the proanthocyanidins along to the polyphenols also provides astringent flavor (Morais et al., 2009). Within acceptable limits chlorogenic acids have a positive effect on the beverage body.

When in presence of microorganisms or under anaerobic conditions the sugars present in the coffee mucilage can be fermented and produce alcohols that may be broken down successively in acetic, lactic, propionic and butyric acids, the deleterious effects of the two last ones on the coffee quality are well known. On the other hand, the production of citric and malic acids by endogens routes can produce a desirable acidic flavor, characteristic of good coffees. Pinto et al., (2002) studying the quality of beans used to prepare espresso coffee, observed that the ones of low quality, such as the types "rio" and "rioysh", presented higher acidity than the types "strictly soft" and "soft" .

Clemente, (2010) studied the effect of K doses combined with two N doses in

coffee bean quality and observed that the N:K ratio influenced the quality of the beverage, evaluated by the cupping test, being the best quality observed when the N:K was 1:1.56. High N dose did not improve the production of cherry coffee and the quality of the beverage, meanwhile, the increase of K doses resulted in quadratic response for all qualitative variables studied, except PPO activity and total phenolic compounds.

Because of the subjective nature of the cupping test, several studies have been made to evaluate, among other, the PPO activity, electrical conductivity or potassium leached of beans and total titratable acidity in order to complete and validate the evaluation by the cup test.

PPO is a cupric enzyme linked to the cellular membranes, and as discussed by the literature is directly involved with the quality of the coffee beverage, (Amorim and Silva, 1968; Amorim, 1978; Leite, 1991; Carvalho et al., 1994; Pimenta, 1995; Chagas et al., 1996a; Chalfoun, 1996; Pereira, 1997). It is established that coffee beans strongly damaged or Cu deficient may have low PPO activity and low quality.

It is also clear that, in higher plants the metabolism of phenolic compounds is activated when B is deficient (Lee and Aronoff, 1967; Dear and Aronoff, 1965, Perkins and Aronoff, 1956). Shkolnik, (1984) states that B is able to form complex with free phenolic compounds and consequently delay or prevent the oxidation of such compounds. Cakmak et al., (1995) suggest that the contribution of B to the membrane stabilization is due not only to the complexation of glycolipids and/or glycoproteins, but also to the complexation of phenolic compounds. When oxidized phenolic compounds can originate quinones and reactive oxygen species, able to cause irreversible oxidative damage to cell membranes.

Amorim and Teixeira, (1975) state that the loss of quality of coffee is strongly linked to biochemical transformations on enzymatic nature, highlighting changes in the activity of PPO, as well the lipases and proteases activity. When active, these enzymes catalyze reactions that produce compounds able to degrade cell walls and membranes, negatively affecting the physical and chemical characteristics of the coffee beans.

Carvalho et al., (1994) performed pioneering works, with physical and chemical evaluations of processed coffee beans previously classified as “strictly soft”, “soft”, “softish”, “hard”, “ryoshy” and “rio”, and verified that coloration index and PPO allows the separation of beans with different coffee quality types .

Potassium leached or the electrical conductivity has been used in researches as

consistent indicators of cellular membrane integrity. Beans presenting disrupted membranes leach greater amount of soluble solutes and present higher values of potassium leached and electrical conductivity according to Krzyzanowsky et al., (1991).

MATERIALS AND METHODS

The experiments were settled in July, 2010, in a field crop area of the Universidade Federal de Viçosa, Viçosa, Minas Gerais State, Brazil, using an adult orchard of *Coffea arabica* L. cv. Catuaí IAC-99 and has been conducted and evaluated until March, 2012. The experimental field is located at 20°45 south and 42°51 west, at 541 m above sea level. The soil of the experimental field is classified as a Red–Yellow Latosol and the climate as Cwa, according to the Köppen classification, with annual temperature and precipitation averages of 292.55 K and 1221.4 mm, respectively.

Treatments and experimental design

In the first experiment the following treatments were performed to evaluate Boron effect: control without B supply, foliar sprays with boric acid at 0.4%, injection of tablets containing B salts at the base of the trunk, injection of tablets containing B+Cu salts at the base of the trunk , injection of tablets containing B+Zn salts at the base of the trunk and injection of tablets containing B+Cu+Zn salts at the base of the trunk. The experiment was assigned as randomized blocks with 5 replications and the useful plot constituted of the 4 central plants at the spacing 3X1 m and disposed in three rows. We worked with 30 total plots.

The second experiment, to evaluate Copper effect, was performed in the same way than the first receiving the following treatments: control without Cu supply, foliar sprays with copper sulfate at 0,4%, injection of tablets containing Cu salts at the base of the trunk, injection of tablets containing Cu+B salts at the base of the trunk , injection of tablets containing Cu+Zn salts at the base of the trunk and injection of tablets containing Cu+B+Zn salts at the base of the trunk.

The third experiment, to evaluate Zinc effect, was performed like the two priors, receiving the following treatments: control without Zn supply, foliar sprays with zinc sulfate at 0.4%, injection of tablets containing Zn salts at the base of the trunk, injection of tablets containing Zn+B salts at the base of the trunk , injection of tablets containing Zn+Cu salts at the base of the trunk and injection of tablets containing Zn+B+Cu salts at the base of the trunk.

The tablets were prepared at the Laboratory of Civil Engineering, of the Universidade Federal de Vicosa, using an hydraulic press with a force of 0.5 tons. The copper was supplied through capsules without any compression of the salts, due to the difficulty of these for forming compact mass with excipient agents. The tablets were implanted into the orthotropic branch of the coffee tree at 10 cm above ground.

In each crop season the foliar sprays were applied between September and February. Liming and fertilization with nitrogen, phosphorus and potassium were performed based on soil analysis, and the expected productivity, following the recommendations of Guimarães et al., (1999).

In order to determine the nutritional status of the plants subjected to the different treatments, in the two crop seasons, coffee leaves were taken from the third or fourth nodes counted from the apex to the base of plagiotropic branches, at a median height in the canopy and in the period between flowering and first rapid expansion of the fruits. The leaves were washed in deionized water and dried in an oven with forced air at 70 °C, until constant weight.

Boron content was determined using the azomethine-H method after dry digestion of the plant material (Malavolta et al., 1997). The content of Cu and Zn were determined by atomic absorption spectrophotometry (AOAC, 1975) in the extract of the nitric-perchloric digestion (Johnson and Ulrich, 1959).

Evaluations

Production

The harvesting of the four usable plants of the plot was carried out, when the plants had approximately 95% coffee cherries. The coffee cherries were hand-picked and dried on a bench in a greenhouse until achieve 11% moisture content. After drying, they were hulled and used for the chemical analysis.

Cupping quality

Cupping test was performed by professional tasters, using the CoE (cup of excellence) method. Each attribute (clean beverage, sweet, acidity, body, taste, flavor, and reminiscent taste) received a score based on the taste intensity exhibited in the samples (Brasil, 2003 - Table 1).

Table 1- Numerical scores for coffee cupping test

Taste	Nota
Strictly soft (Specialty coffee)	≥ 87
Soft	80 - 86
Softish	74 - 79
Hard	≤ 74

Chemical analysis of the coffee beans

Total sugars, non-reducing sugars, coloration index, total titratable acidity, pH, electrical conductivity and leached potassium were evaluated in beans harvested in the crop season 2010/2011. Coloration index, potassium leached, total titratable acidity, pH, electrical conductivity, caffeine, trigonelline, total phenolic compounds, sucrose, glucose, mannose, arabinose, galactose, proanthocyanidin, 3-caffeoylquinic acid, 4 - caffeoylquinic acid, 5-caffeoylquinic acid and PPO activity were evaluated in beans harvested in the crop season 2011/2012 as described below.

Sugars

Total sugars were extracted by Lane Enyon method as described by AOAC, (1990), and determined by Somogy technique, adjusted by Nelson, (1944).

In 5 grams of ground coffee beans, 5 mL of ethanol at 80% were added and stored at $4^{\circ}C$ during 24 hours, later it was filtered in quantitative filter paper, and an aliquot of 250 µL of the alcoholic extract was taken. Then, 250µL of phenol at 5% and 1.25 mL of sulfuric acid concentrated were added. Subsequently, the samples were stirred and left on water bath at $30^{o}C$ during 20 minutes. The readings of total sugars were performed in spectrophotometer at 490 nm. The reducing sugars were determined collecting 200µL of the filtered, in which were added 200µL of the Nelson reagent (Nelson, 1944). The samples were stirred and read in spectrophotometer at 540 nm. The non-reducing sugars were determined by the difference between total and reducing sugars. The extraction and readings were performed in duplicates and the results expressed in percentage (%).

Coloration index

Coloration index was determined by the Singleton method (1966), adapted for coffee. Samples of 2 g of ground coffee received 50 mL of distilled water being homogenized in electric stirrer during 1 hour. Then, the homogenate was filtered in filter paper; an aliquot of 5 mL of the filtered was taken and mixed with 10 mL of distilled water. The samples were left to rest during 20 minutes and the readings were performed in spectrophotometer at 425 nm. The readings were performed in duplicates and the results

expressed in DO.425 nm.

Total titratable acidity and pH

Total titratable acidity and the pH were determined as described by AOAC, (1990). In 2 grams of ground coffee beans was added 50 mL of distilled water and the mix was stirred in an electric stirrer at 150 rpm during 1 hour. The material was, then filtered in quantitative filter paper. An aliquot of 5 mL of the filtered solution was added in an erlenmeyer, diluted in 50 mL of distilled water, received 3 drops of phenolphthalein at 1%, and was titrated with NaOH (0.1 mol/L). The pH was measured in the same raw extract using a pH meter DIGIMED-DMPH-2. The readings were performed in duplicates and the results of total titratable acidity expressed in mL of NaOH/ 100 grams of sample.

Electrical conductivity and potassium leached

Electrical conductivity was determined according to the method described by Loeffler et al., (1988). Fifty grams of ground coffee were placed in plastic cups receiving 75 mL of distilled water. The samples were placed in ventilated oven during 5 hours and next the electrical conductivity was measured using a portable conductivity meter. From each sample, an aliquot was picked, in order to determine the potassium leached, using a flame photometer, as described by Prete, (1995). The readings were performed in duplicates and the results expressed in mL of NaOH/ 100 grams of sample. The readings were performed in duplicates and the results of electrical conductivity were expressed in µS/ cm/ g and potassium leached in g/ kg.

Polyphenol oxidase

Three milliliters of sodium phosphate 0.1 mol/L, pH 7, at 4ºC containing 1% of ascorbic acid and 1% of polyvinylpyrrolidone were added to 0.3 grams of ground coffee. The mix was macerated in ice bath and the extract was centrifuged at 4ºC during 15 minutes. After, the PPO activity was determined as described by Ponting and Joslyn, (1948), using the sample extract without DOPA as blank. The results were expressed in U/ minute/ gram of sample. The extraction and readings were performed in duplicates and the results expressed U/ minute/ gram of sample.

3-Caffeoylquinic acid, 4- Caffeoylquinic acid and 5- Caffeoylquinic acid

Chlorogenic acids were extracted according to Farah et al., (2005) and Trugo and Macrae, (1984). Finely ground instant coffee (0.5 g) was dissolved in 80 mL of an aqueous methanol solution (40%) and transferred into a 100 mL calibrated flask. Carrez

solution (2 mL of each component) was added and the mixture was allowed to stand for 10 minutes after making up to volume. The precipitate was removed by filtration under gravity and the filtrate was used directly for chromatography.

The assay conditions for detection of the different isomers were: flow rate of 1 mL/ minute, the mobile phase contained a gradient of 10 mM sodium citrate solution (pH adjusted to 2.5) and methanol. Analyses were performed using high performance liquid chromatography using a C18-ODS reverse phase column and a UV detector operating at 325 nm.

A mixture of 3-CQA, 4-CQA, and 5-CQA was prepared from 5-CQA using the isomerization method of Trugo and Macrae, (1984) and quantification of the isomers took into account the area of 5-CQA standard and molar extinction coefficients of each acid (Farah et al., 2005 and Ruback, 1969). The extraction and readings were performed in duplicates and the results expressed in percentage (%).

Caffeine and trigonelline

Caffeine was determined according to Mazzafera et al., (1994) with additional modifications as described by Vitorino et al., (2001), and the trigonelline according to Vitorino et al., (2001). Ten mL of methanol at 80 % were added to 0.1 grams of ground coffee beans and the mix remained during 1 hour in water bath at 80°C, with occasional stirring. After cool down at room temperature, an aliquot of 2 mL was centrifuged during 10 minutes. The supernatant was filtered in a membrane filter and injected in HPLC tubes. The analysis were performed using a HPLC with a reversible phase, Acclaim 120 C18 column. For caffeine the flow rate was of 1 mL/min, the mobile phase was composed of methanol and water (40:60) and the used wavelength was 272 nm. For trigonelline the flow rate was of 1 mL/minute, the mobile phase was composed of methanol, water and acetic acid (20:79:1), and the used wavelength was 265 nm. The extraction and readings were performed in duplicates and the results expressed in percentage (%).

Total Phenolic Compounds

Phenolic compounds were determined by Folin Denis method as described by AOAC (1990). Thirty mL of methanol at 50% were added in a ground coffee sample of 0.5 grams under continuous stirring during 15 minutes, followed by filtration. Then, 0.1 mL of the extract was picked, receiving 2.5 mL of an aqueous solution of Folin-Ciocalteu reagent at 10% and 2.0 mL of a freshly prepared sodium carbonate solution at 7.5%. This mixture was placed in water bath at a temperature of 50°C during 5 minutes.

After cooling down at room temperature, the absorbance was recorded in a spectrophotometer UV/ VIS at 760 nm. The calibration curve was made with aqueous solution of gallic acid at increasing concentrations. The extraction and readings were performed in duplicates and the results expressed in percentage (%).

Proanthocyanidins

An amount of 0.3 g of ground coffee was extracted with 10 mL of methanol (80%) under continuous stirring during 24 hours at room temperature. It was transferred 0.1 mL of the raw extract to a test tube and added 2 mL of a freshly vanillin solution at 70% in sulfuric acid in a concentration of 1g/ 100 mL. The resulting solution was placed in water bath at 50°C during 15 minutes. Readings were performed using a spectrophotometer at 500 nm, being used a standard curve of catechin (Hagerman, 1997; Haslam, 1989). The readings were performed in duplicates and the results expressed in percentage (%).

Sucrose

A sample of 0.05 g of ground coffee plus 1 mL of ethanol (80%) was placed in centrifuge tubes, and immersed in water bath (80°C) during 20 minutes. Subsequently, the samples were centrifuged and the supernatant transferred to another centrifuge tube. On the remaining residue, it was poured 1 mL of ethanol (80%) and the procedure was repeated. The supernatant was centrifuged during one minute, filtered in a membrane filter and injected into HPLC tubes. In order to determine sucrose, it was used a refractive index detector at 50°C and a SP 0810 (300mm x 8 mm) column at a temperature of 80°C, ultra pure water as the mobile phase and a flow rate of 1 mL/ minute (Sluiter et al., 2008). The extraction and readings were performed in duplicates and the results expressed in percentage (%).

Glucose, mannose, galactose and arabinose

Ground coffee samples of 0.5 grams were subjected to acid hydrolysis with 3 mL of sulfuric acid at 72% and maintained in water bath at 50◦C during 7 minutes. Then, on the resulting solution was poured 84 mL of ultra pure water followed by autoclaving at 121◦C during 45 minutes. After cooling at room temperature, an aliquot of 10 mL was transferred to a flask and the pH adjusted between 4 to 6 with pure calcium carbonate. The supernatant was collected, filtered and injected into HPLC tubes. In order to determine these sugars, we used a refractive index detector at 50◦C and a SP 0810 (300 mm x 8 mm) column at 80◦C, ultra pure water as the mobile phase and a

flow rate of 0.6 mL/minute (Sluiter et al., 2008). The extraction and readings were performed in duplicates and the results expressed in percentage (%).

Statistics

Data were submitted to variance analysis and the means compared by Dunnet`s test at 10% of probability. It was considered the treatment without B, Cu and Zn as the first control because it is the control of all treatments and the sprayed treatment because this is the usual form to supply B, Cu and Zn to coffee plants.

RESULTS AND DISCUSSION

Boron content in index leaves

In the crop season 2010/2011, the indexes leaves of the coffee-plants that received B as foliar spray or solid injections with B, B+Cu and B+Zn presented higher amounts of B than the control treatment (WB). For this same crop season, the contents of Cu and Zn even as spray or solid injections presented higher contents of these nutrients than that of the control treatments WCu, WZn (Table 2).

In that same season, when considering the sprayed treatment as the control, it was observed that the content of B of the treatments B, B+Zn and B+Cu were statistically similar to the sprayed treatment and B+Cu+Zn statistically lower than those (Table 2).

The content of Cu of the treatments Cu, Cu+Zn and B+Cu+Zn were statistically higher than the sprayed, suggesting fast releasing of the nutrient in the treatments that received Cu as solid injections. For the Zn content, only the treatment Cu+Zn was significantly higher than the sprayed (Table 2).

Considering the sufficiency ranges of 29 to 52 mg/kg for B, 13 to 29 mg/ kg for Cu and 6 to 12 mg/kg for Zn as determined by Martinez et *al*, (2003) for the region of Vicosa, only the plants of the control treatment (WB - WCu) were deficient in B and Cu and the treatments B+Cu+Zn were deficient in B.

In the crop season 2011/2012, solid injections of B, Cu and Zn in the trunk resulted in higher contents of these nutrients in the indexes leaves than the control treatments WB, WCu and WZn, resulting in concentrations, in the case of B, that can be considered toxic to the plants. Considering the sprayed treatment as the control, it can be noted that the foliar content of all treatments receiving solid injections of B, Cu and

Zn were significantly high (Table 2).

In all treatments, except the control without B, Cu and Zn, the concentrations of Cu and Zn were considered adequate according to Martinez et *al*., (2003). The supply of Cu by solid injections, using capsules without any compression coupled with high rainy precipitation by the sampling occasions may be the reason for the concentrations at satisfactory levels in the first year of assessment.

The results suggest that both forms, in both crop seasons, were efficient to increase the contents of B, Cu and Zn in index leaves of coffee plants, even with the necessity to review the composition and doses of B salts.

Table 2 - Contents of B, Cu and Zn (mg/kg) of indexes leaves of coffee-plants that received B, Cu and Zn as solid injections or foliar sprays (FS)

Crop season	**2010/2011**	**2011/2012**
	Boron	
WB	24.076 *	27.464

FS	35.042 +	30.352
B	35.508 +	50.412 * +
B+Cu	35.036 +	64.648 * +
B+Zn	33.257 +	72.312 * +
B+Cu+Zn	23.936 *	78.64 * +
CV (%)	9.10	13.19
	Copper	
WCu	9.64 *	5.24 *
FS	13.507 +	13.138 +
Cu	17.057 * +	18.617 * +
B+Cu	14.942 +	15.79 * +
Cu+Zn	18.965 * +	19.408 * +
B+Cu+Zn	16.462 * +	16.521 * +
CV (%)	8.25	6.15
	Zinc	
WZn	6.522 *	4.680 *
FS	10.062 +	7.420 +
Zn	10.853 +	11.58 * +
B+Zn	10.633 +	10.89 * +
Cu+Zn	11.754 * +	13.66 * +
B+Cu+Zn	9.85 +	9.980 * +
CV (%)	6.25	16.31

WB, WCu and WZn - without application of B, Cu or Zn,
FS – foliar spray with Boric acid, Copper sulphate and Zinc sulphate (0,4%),
B – trunk injection of tablets containing B salts,
Cu - trunk injection of tablets containing Cu salts,
Zn – trunk injection of tablets containing Zn salts,
B+Cu - trunk injection of tablets containing B and Cu salts,
B+Zn - trunk injection of tablets containing B and Zn salts,
Cu + Zn - trunk injection of tablets containing Cu and Zn salts,
B+Cu+Zn - trunk injection of tablets containing B, Cu and Zn salts.
Means followed by * differ statistically from the control treatment (FS) at the 10% significance level, according to Dunnett's test.
Means followed by + differ statistically from the control treatment (WB – WCu – WZn) at the 10% significance level, according to Dunnett's test.

Production

Considering the treatments without B or sprayed as controls, there was no effect of B on coffee production in the first crop season, being the averages of 3.59 kg of cherry coffee per plant (2992 kg/ha of processed coffee). This result can be explained by the fact that coffee plants produce in nodes formed in the previous growing season, i.e., the nodes in which the fructification have occurred were already formed by the occasion of the experiment beginning (Table 3).

In the crop season 2011/2012 there was significant difference in coffee production among the treatments at 10.9% of probability (Table 3), being the productions of the treatments containing B+Cu and B+Zn 35.01% and 22.82% greater than the control

treatment WB, corresponding to a difference of 1328 kg/ha and 866 kg/ha of processed coffee respectively, even with the index leaves presenting excessive concentrations of B (Tables 2 and 3).

The level of probability of 10.9% can be considered acceptable for coffee experiments conducted in commercial orchards, because the experimental conditions are very heterogeneous and each coffee-plant of the plant population has great variability. These findings indicate that solid injections is a good way to supply B, even if the doses and composition of the tablets need to be improved.

For Cu, in the first crop season, there was no effect of Cu on cherry coffee production, being the average of 3.607 kg per plant, which corresponds to 3005 kg /ha of processed coffee. In the crop season 2011/2012, the treatment B+Cu was statistically different of both controls WCu or the sprayed treatment (Table 3).

These results suggest that tablets containing B+Cu salts supplied Cu in adequate amounts. It can be highlighted the good combination of B and Cu in the same tablet, since the treatments containing only Cu, Cu+Zn, B+Cu+Zn and the sprayed treatment were statistically equal compared to the control, without B, Cu and Zn. In such treatments maybe the plants were slightly sensitive to high concentrations of Cu, as can be concluded taking in account the Cu content in the indexes leaves.

There was no significant effects of Zn on production, in the two crop seasons, even in view of the variations occurred in the contents of Zn in indexes leaves, being the mean values of the production 3.54 (2947 kg/ha of processed coffee) and 4.99 (4157 kg/ha of processed coffee), respectively for the two seasons evaluated (Table 3).

According to Brown and Hu, (1998) in species which do not produce polyols, as coffee plants, B is immobile in phloem because of the low capacity of forming stable complexes with sucrose, therefore, the foliar sprays correct the deficiency only in the leaves that received the fertilizers, the leaves that grow after fertilizers application will present low B concentrations, demanding a greater number of applications.

Table 3 – Coffee cherry production of coffee-plants submitted to the fertilization via solid salts injections in the trunk and foliar sprays with B, Cu and Zn

Treatments	**Production**	
	Boron	
	2010/2011	**2011/2012**
WB	3.469	4.552
FS	3.566	5.469

B	3.829	4.305
B+Cu	3.744	6.146 * +
B+Zn	3.474	5.591 * +
B+Cu+Zn	3.454	3.929
CV (%)	22.88	26.66
	Copper	
WCu	3.469	4.552
FS	3.566	5.469
Cu	3.991	5.561
B+Cu	3.744	6.146 * +
Cu+Zn	3.420	5.147
B+Cu+Zn	3.454	3.929
CV (%)	22.85	20.38
	Zinc	
WZn	3.469	4.552
FS	3.566	5.469
Zn	3.833	5.244
B+Zn	3.474	5.591
Cu+Zn	3.420	5.147
B+Cu+Zn	3.454	3.929
CV (%)	28.56	27.03

WB, WCu and WZn - without application of B, Cu or Zn,
FS – foliar spray with Boric acid, Copper sulphate and Zinc sulphate (0,4%),
B – trunk injection of tablets containing B salts,
Cu - trunk injection of tablets containing Cu salts,
Zn – trunk injection of tablets containing Zn salts,
B+Cu - trunk injection of tablets containing B and Cu salts,
B+Zn - trunk injection of tablets containing B and Zn salts,
Cu + Zn - trunk injection of tablets containing Cu and Zn salts,
B+Cu+Zn - trunk injection of tablets containing B, Cu and Zn salts.
Means followed by * differ statistically from the control treatment (FS) at the 10% significance level, according to Dunnett's test.
Means followed by + differ statistically from the control treatment (WB – WCu – WZn) at the 10% significance level, according to Dunnett's test

Santinato et al., (2012) working with high doses of boric acid applied in the soil, observed that, despite the plant did not presented toxicity symptoms, excessive doses caused reduction of 600 kg/ha of processed coffee.

Positive correlations between B availability in soil and harvest index was found by Lima Filho and Malavolta, (1992) for *Coffea arabica* cv. Catuaí Amarelo. They have observed great correlation among harvest index and B content in leaves, branches length, number of leaves and branches, and low correlation among harvest index and dry matter of the roots, stem, branches and leaves. These variables are important for coffee production, because, according to Rena and Maestri, (1985), the vertical growth of coffee-plant determine the formation of nodes, and from buds of these nodes emerge plagiotropic branches, in whose nodes will develop leaves and inflorescences. There-

fore, the flowering depends on the plagiotropic branches growth, number of nodes, and number of leaves, since it is verify that many nodes without leaves do not flower.

According to Santinato et al., (1994) 6 to 12 applications per year with organic solutions of B at 10% not coinciding with the flowering, provided high productivity and maintained good correlation between B content in leaves and production, although, there was no significant difference between the treatments regarding the B content in leaves. Barros et al., (1996) observed that boric acid application at 0.3% twice a year resulted in productions only 8% higher than the control treatment without B application. On the other hand, Santinato et al., (1991), Marubayashi et al., (1994), Lima Filho and Malavolta, (1992) and Barros et al., (1996) reported that not always the increase in B concentration provides an increase in coffee productivity.

According to Andrade, (1973) in adult coffee plants receiving sprays containing Cu, high leaf Cu content did not reduce production, possibly because Cu is located on the leaf surface or even because the element remain largely in the cuticle not reaching the cytoplasm.

Loneragan, (1975) states that Cu movement into the plants depends on its concentration. During the initial stages of growth, Cu in excess causes reduction on the branching, thickening and abnormal coloration of rootlets (Reuther and Labanauskas, 1966).

Regarding Zn in a field experiment, Guimarães et al., (1983) observed an increase of 60 to 360 kg/ ha of processed coffee with Zn supplementation by foliar spray, followed by the increase in the concentration of Zn from 8 to 21 mg/kg in indexes leaves. In turn, Lima Filho Malavolta, (1998) proved the positive interaction between B and Zn studying the dry matter production in seedlings of coffee varieties and when the nutrients were supplied together, the dry matter production was 21% higher.

In a field experiment performed in the same conditions that the experiment reported here, Martinez et al., (2013) studied the effect of Zn on the production and on some quality attributes of coffee beans and did not observe effect of the nutrient on the production, however, there was effect of Zn on beans size, moreover, plants supplemented with Zn had the highest percentage of exportable grains, in other words, retained in the sieves 17 and 18. Still according to the authors, there was no significant effect of Zn on the cup quality of coffee beans, however while the scores related to the

cup quality of the beans harvested from plants that did not received Zn was 60, in beans harvested from plants that received Zn it was 72.5.

In the same orchard, Neves et al., (2011) studied the effect of Zn, supplied by trunk injection and foliar sprays, on the production and on some attributes of quality. The cumulative production of two crop seasons for treatments that received tablets of Zn inserted in the trunk and the treatment without Zn were 11292 and 7810 kg/ha of processed coffee, respectively. The difference among them was 3482 kg/ha, which corresponds at 30.9 %. Still according to the authors, the beans were classified as "hard" type and there was no significant effect of Zn on coffee bean quality evaluated by cup test.

Cupping quality

There was no significant effects of B, on cupping test in both years, being the overall means 83.73 and 80.4 in the respective assessed years; in general the coffee beans were classified as “soft” (Table 4).

Considering the treatment WCu or the sprayed as controls, only the treatment containing Cu+Zn had score statistically low in the crop season 2011/2012, evidencing the effect of the way of Cu supply and the effect of the nutrient on the cupping quality. As previously reported, the indexes leaves of the plants subjected to this treatment had slightly high Cu content. In case of Cu and Zn tending to the excess there is a negative interaction with other cationic micronutrients that when in appropriate concentrations certainly would influence positively the route of production of compounds associated to desirable flavors and aromas (Tables 2 and 4).

There was no effect of Zn on cupping test, reaching scores of 83.46 and 79.97 in the two crop seasons respectively, the beans would be classified as “soft” (Table 4).

In spite of the fact that the precision and validity of the cupping is much discussed because of its subjective nature and limitations imposed by the tasters abilities, this result points out to the major importance of the pos-harvest procedures on coffee quality, comparatively to B, Cu or Zn effects.

Table 4 – Cupping test of coffee beans harvested from plants submitted to fertilization via solid salts injections and foliar spray with B, Cu and Zn

Treatments	**2010/2011**	**2011/2012**
	Cupping test	
	Boron	
WB	82.4	82.7

FS	84.8	83.9
B	84.4	72.8
B+Cu	83.4	83.25
B+Zn	83.6	79.35
B+Cu+Zn	83.8	80.4
CV (%)	3.76	8.20
	Copper	
WCu	82.4	82.7
FS	84.8	83.9
Cu	85.0	81.5
B+Cu	83.4	83.25
Cu+Zn	82.0	74.6 * +
B+Cu+Zn	83.8	80.4
CV (%)	4.27	5.81
	Zinc	
WZn	82.4	82.7
FS	84.8	83.9
Zn	84.2	78.9
B+Zn	83.6	79.35
Cu+Zn	82.0	74.6
B+Cu+Zn	83.8	80.4
CV (%)	4.34	10.17

WB, WCu and WZn - without application of B, Cu or Zn,
FS – foliar spray with Boric acid, Copper sulphate and Zinc sulphate (0.4%),
B – trunk injection of tablets containing B salts,
Cu - trunk injection of tablets containing Cu salts,
Zn – trunk injection of tablets containing Zn salts,
B+Cu - trunk injection of tablets containing B and Cu salts,
B+Zn - trunk injection of tablets containing B and Zn salts,
Cu + Zn - trunk injection of tablets containing Cu and Zn salts,
B+Cu+Zn - trunk injection of tablets containing B, Cu and Zn salts.
Means followed by * differ statistically from the control treatment (FS) at the 10% significance level, according to Dunnett's test.
Means followed by + differ statistically from the control treatment (WB – WCu – WZn) at the 10% significance level, according to Dunnett's test

Electrical conductivity, potassium leached and coloration index in the crop season 2010/2011

In the first crop season, there was no effect of B on CI (0.886 DO.425 nm), TTA (10.66 mL of NaOH/ 100 grams of sample), pH (5.59), EC (41.21 µS/ cm/ g), KL (1.29 g/ kg), TS (11.3 %) and RS (0.227 %) of the beans considering the treatment WB or the sprayed as controls (Table 5).

Either, for Cu, the CI (0.887 DO.425 nm), TTA (10.86 mL of NaOH/ 100 grams of sample), pH (5.60), EC (40.82 µS/ cm/ g), KL (1.29 g/ kg), TS (11.70 %) and RS (0.218 %) of the beans did not differ among the treatments and controls (Table 5).

There was no effect of Zn on the CI (0.887 DO.425 nm), TTA (11.06 mL of

NaOH/ 100 grams of sample), pH (5.59), EC (40.55 µS/ cm/ g), KL (1.27 g/ kg), TS (11.41 %) and RS (0.229 %) of the beans (Table 5).

Table 5 – Coloration index (CI – D.O. 425 nm), total titratable acidity (TTA - mL NaOH/ 100g), pH, electrical conductivity (EC - µS/cm/ g), potassium leached (KL – g/kg), total sugars (TS - %) and reducing sugars (RS - %) in coffee beans of *Coffea arabica* treated with different forms of B, Cu and Zn supply, in the crop season 2010/2011

Crop season 2010/2011							
Boron							
Treatments	CI	TTA	pH	EC	KL	TS	RS
WB	0.806	11.2	5.61	41.39	1.29	11.73	0.216
FS	0.894	10.4	5.60	42.71	1.40	11.41	0.197
B	0.842	10.0	5.60	41.36	1.30	11.63	0.212
B+Cu	0.933	10.4	5.58	41.84	1.29	12.09	0.199
B+Zn	0.909	10.8	5.50	41.09	1.23	9.70	0.279
B+Cu+Zn	0.935	11.2	5.61	38.87	1.23	11.28	0.264
CV (%)	12.03	20.05	1.35	14.26	12.67	13.91	34.39
Copper							
Treatments	CI	TTA	pH	EC	KL	TS	RS
WCu	0.806	11.2	5.61	41.39	1.29	11.73	0.216
FS	0.894	10.4	5.61	42.71	1.40	11.41	0.197
Cu	0.874	10.4	5.60	41.98	1.36	11.48	0.221
Cu+B	0.933	10.4	5.58	41.84	1.29	12.09	0.199
Cu+Zn	0.881	11.6	5.58	38.14	1.18	12.20	0.211
B+Cu+Zn	0.935	11.2	5.61	38.87	1.23	11.28	0.264
CV (%)	10.76	24.48	0.75	13.8	13.67	14.16	20.22
Zinc							
Treatments	CI	TTA	pH	EC	KL	TS	RS
WZn	0.806	11.2	5.61	41.39	1.29	11.73	0.216
FS	0.894	10.4	5.60	42.71	1.40	11.41	0.197
Zn	0.899	11.2	5.62	41.09	1.32	12.13	0.209
Zn+B	0.909	10.8	5.50	41.09	1.23	9.70	0.279
Zn+Cu	0.881	11.6	5.58	38.14	1.18	12.20	0.211
B+Cu+Zn	0.935	11.2	5.61	38.87	1.23	11.28	0.264
CV (%)	11.06	17.27	1.45	16.7	14.13	17.24	35.64

WB, WCu and WZn - without application of B, Cu or Zn,
FS – foliar spray with Boric acid, Copper sulphate and Zinc sulphate (0.4%),
B – trunk injection of tablets containing B salts,
Cu - trunk injection of tablets containing Cu salts,
Zn – trunk injection of tablets containing Zn salts,
B+Cu - trunk injection of tablets containing B and Cu salts,
B+Zn - trunk injection of tablets containing B and Zn salts,
Cu + Zn - trunk injection of tablets containing Cu and Zn salts,
B+Cu+Zn - trunk injection of tablets containing B, Cu and Zn salts.
Means followed by * differ statistically from the control treatment (FS) at the 10% significance level, according to Dunnett's test.
Means followed by + differ statistically from the control treatment (WB – WCu – WZn) at the 10% significance level, according to Dunnett's test

In the second crop season there was no effect of B on the TTA (45.95 mL NaOH/ 100g), EC (57.26 µS/cm/ g) and KL (2.06 g/kg), only the treatments B+Cu+Zn and B+Cu differed from the sprayed treatment, but did not differ from the treatment without B, indicating the effect of the way of B supply on this variable but not effect of the nutrient (Table 6).

There was no effect of Cu on TTA (49.41 mL NaOH/ 100g of sample), pH (5.68), EC (57.93 µS/cm/ g) and KL (2.05 g/kg). The treatments that received Cu, Cu+B, Cu+Zn and B+Cu+Zn via solid injections differed from the sprayed, and considering the treatment WCu as control only the sprayed was different, indicating the effect of Cu and the ways of Cu supply on CI (Table 6).

Zinc did not influence the CI (0.561 DO.425 nm), TTA (47.43 mL of NaOH/ 100 grams of sample), pH (5.68), EC (57.8 µS/ cm/ g) and KL (2.08 g/ kg) in the crop season 2011/2012 (Table 6).

Table 6 – Coloration index (CI – D.O. 425 nm), total titratable acidity (TTA - mL NaOH/ 100g), pH, electrical conductivity (EC - µS/cm/ g) and potassium leached (KL – g/kg) of coffee beans of *Coffea arabica* treated with different forms of B, Cu and Zn supply, in the crop season 2011/2012

Crop season 2011/2012

Treatments	CI	TTA	pH	EC	KL
			Boron		
WB	0.513	49.41	5.72	57.52	2.15
FS	0.759	48.42	5.63 +	50.18	2.01
B	0.744	41.50	5.63 +	56.77	2.27
B+Cu	0.368 *	40.52	5.68	60.98	2.05
B+Zn	0.661	45.46	5.69	58.83	1.86
B+Cu+Zn	0.411 *	50.40	5.74 *	59.27	2.02
CV (%)	31.89	31.12	0.86	13.47	14.28
			Copper		
WCu	0.513	49.41	5.72	57.52	2.15
FS	0.759 +	48.42	5.63	50.18	2.01
Cu	0.497 *	59.29	5.64	56.92	1.93
Cu+B	0.368 *	40.52	5.68	60.98	2.05
Cu+Zn	0.499 *	48.42	5.70	62.73	2.12
B+Cu+Zn	0.411 *	50.40	5.74	59.27	2.02
VC (%)	29.25	31.61	1.26	12.79	14.15
			Zinc		
WZn	0.513	49.41	5.71	57.52	2.15
FS	0.759	48.42	5.63	50.18	2.01
Zn	0.525	42.49	5.65	58.29	2.36
Zn+B	0.661	45.46	5.69	58.83	1.86
Zn+Cu	0.499	48.42	5.70	62.73	2.12
B+Cu+Zn	0.411	50.40	5.74	59.27	2.02
VC (%)	31.29	30.14	1.17	15.86	15.91

WB, WCu and WZn - without application of B, Cu or Zn,
FS – foliar spray with Boric acid, Copper sulphate and Zinc sulphate (0.4%),
B – trunk injection of tablets containing B salts,
Cu - trunk injection of tablets containing Cu salts,
Zn – trunk injection of tablets containing Zn salts,
B+Cu - trunk injection of tablets containing B and Cu salts,
B+Zn - trunk injection of tablets containing B and Zn salts,
Cu + Zn - trunk injection of tablets containing Cu and Zn salts,
B+Cu+Zn - trunk injection of tablets containing B, Cu and Zn salts.
Means followed by * differ statistically from the control treatment (FS) at the 10% significance level, according to Dunnett's test.
Means followed by + differ statistically from the control treatment (WB – WCu – WZn) at the 10% significance level, according to Dunnett's test

According to Carvalho et al., (1994), the coloration index allows separation of different types of coffees, such as "rioysh" and "rio", i.e, not acceptable drink with coloration indexes lower than 0.650 D. O. 425 nm. For those classified as "hard" type (acceptable), "soft", "softish" (fine) and "strictly soft" (extra fine), the coloration indexes would be equal or greater than 0.650 D.O. 425 nm.

The results obtained for the second crop season of evaluation, suggest that coloration index may not be a good indicator of quality assessed by cupping test, since in spite of having coloration indexes very close, the cup quality score for the beans produced by the sprayed treatment was 15.24% higher than the obtained by the treatment

receiving B tablets inserted in the trunk (Table 4 and 6).

The coloration index of the sprayed treatment is in agreement with those reported by Carvalho et al., (1994) in which the coffees of best quality were darker and the dark coloration attributed to the formation of essential compounds to develop desirable flavors and aromas (Table 6).

On the other hand, the treatments containing B+Cu and B+Cu+Zn presented coloration index quite low (0.368 and 0.411 DO. 425 nm, respectively) and high cupping quality scores (83.25 and 80.4, respectively), being, in this case, classified as "softish", probably because of the positive effects of Cu and Zn, contradicting the findings of Carvalho et al., (1994). Júnior and Corrêa et al., (2002) also reported that higher CI could be attributed to the occurrence of biochemical alterations and oxidative reactions caused by the dry conditions or inadequate storage.

Martinez et al., (2013) studying Zn effect on the production and cupping quality of coffee did not observe Zn effect on coloration index, being the mean values of 0.95 D.O 425 nm, which is in agreement with the results found by Lima et al., (2008) for good coffees and, also, with the means found in this work.

Cellular membrane damage and the subsequent loss of permeability control was proposed by Heydecker, (1972) and Harrington, (1973) as the early step in seed deterioration process. According to Amorim, (1978), since the potassium leached is proportional to the loss of bean quality, it can measured the loss of membrane permeability caused damage in coffee beans.

Malta et al., (2002) studying some attributes related to the quality in different coffee varieties noted that Catuaí Vermelho presented electrical conductivity of 104 µS/cm/g, considerably higher than those reported in the present experiment, even for the control treatment.

Lima et al., (2013) determined the electrical conductivity of beans subjected to B doses, and verified that both, absence or toxic concentrations the nutrient are harmful to the quality of bean seeds, enhancing the electrical conductivity. Moreover, high physiological quality of bean seeds was obtained in a consortium with beans and castor bean, when supplied with adequate doses of B.

Evaluating the physiological quality of bean seeds over different doses of Mn and Zn, Teixeira et al., (2005) did not observe effect of Zn on the electrical conductivity, however, adequate doses of Mn improve seed quality resulting in low electrical con-

ductivity (65.8 μS/cm/g). The seed quality was the lowest in the control treatment, without application of Zn and Mn. Amorim, (1978), Prete, (1995) and Lima et al., (2008) observed inverse correlation between potassium leached, electrical conductivity and cupping quality of coffee.

According to Amorim et al., (1976) and Chagas et al., (1996 b), good coffees have high contents of sugars, around 8% according to Navellier, (1970), and around 5 to 10% according to Prete, (1995). In general, the results of the first crop season were above the mean values reported by the literature.

It can also be noted, that total acidity has remained below 211.2 g NaOH/100 mL sample, considered by Carvalho et al., (1994) as a parameter for good coffees. Turning to the pH, it can also be observed that the mean of both years of assessment are quite close from those found by Barrios, (2001) that were between 5.73 and 5.88. According to Sivetz and Desrosier, (1979), roasted beans of palatable coffees, without bitter or acidity, must have pH between 4.95 and 5.2. The results of the present work are slightly above of the range established by this author.

Neves et al., (2011) studying the effect of different doses of Zn supplied to coffee plants by trunk injections of tablets containing Zn salts observed that the electrical conductivities of the control treatment, without Zn application, were 22.61 and 88.42 μS/cm/g in two consecutive crop seasons, respectively. For the treatments with Zn inserted into the trunk the means of the electrical conductivities were 16.84 e 66.16 μS/cm/g. The average values of potassium leached were 1.13 e 0.95 g/kg, in two consecutive crop seasons, for the treatments without Zn application and 0.83 and 0.65 g/kg for the treatments with Zn application. The difference among the treatments was attributed to the Zn functions on cell membrane integrity of coffee beans.

Neves et al., (2011), also, have not observed variations on acidity of beans harvested from plants that received Zn by injection of tablets in the trunk, being the average values of 156.3 mL de NaOH/100g, in coffees that were classified as “hard” in the cup test. In the same orchard, Martinez et al., (2013) did not observe effect of the nutrient on the total titratable acidity and pH, being the average values of 14.7 mL de NaOH/100g and 5.4, respectively.

Caffeine, trigonelline, glucose, galactose, arabinose and mannose

In this study, the effect of B, Cu and Zn on the caffeine, trigonelline, sucrose

and glucose productions was evident by the significant differences between the sprayed treatment and the treatments that received the nutrients via solid injections compared to the control treatments WB, WCu and WZn (Table 7).

Either, it is possible to observe effect of the different ways of B, Cu and Zn supply on the contents of caffeine, trigonelline and sucrose by the difference between the treatments B+Cu+Zn and the sprayed treatment (Table 7). The results suggest that the lack of B, Cu and Zn influenced the route of caffeine and trigonelline synthesis, but probably the excessive concentrations of B, like in the treatment containing B+Cu+Zn, also did.

There was no effect of B on galactose production. The arabinose of the treatments containing B+Cu and B+Zn presented means statistically higher than the presented by the control treatment (WB). Regarding mannose, only the treatment containing B+Cu was statistically different from the control treatment (WB) suggesting effect of B on its production (Table 7).

The effect of Cu on mannose levels in the coffee beans was evidenced by the significant difference among the beans produced from the plants of the sprayed treatment and those receiving tablets of Cu, Cu+B and Cu+Zn inserted in the trunk comparatively to the control treatment (WCu). Regarding the levels of galactose only the sprayed treatment differed from the control treatment (WCu), and arabinose was significantly greater only in the treatment containing Cu+B inserted in the trunk (Table 7).

Regarding to the galactose it was not observed significant differences between the treatments with Zn applications. The arabinose content in beans of the treatments containing Zn and Zn+B were statistically greater than that of the control treatment (WZn). Mannose of the sprayed treatment and those receiving Zn and Cu+Zn inserted in the trunk differed from that of the control treatment (WZn), showing, the Zn effect on monosaccharides synthesis and close relationship between its production and the content of Zn in index leaves (Table 7).

Mazzafera, (1999), working with nutritive solution and young coffee plants, did not found significant effects of B, Cu and Zn deprivation on the caffeine production by coffee leaves. The author states that the effect of mineral nutrients on the activity of methyltransferases involved in caffeine synthesis is still unclear.

The contents of trigonelline observed in this work, in general are in agreement with those established by the literature that varies from 0.6 to 1.2% for *Coffea arabica*

(Illy and Viani, 1995) and the caffeine contents are close from those determined by Screenath, (1997) of about 1.2% for *Coffea arabica*.

Table 7 - Caffeine (Caf - %), trigonelline (Trig - %), sucrose (Suc - %), glucose (Glu - %), galactose (Gal - %), arabinose (Ara - %) and mannose (Man - %) of coffee beans harvested from plants submitted to the fertilization via solid salts injections in the trunk or foliar sprays with B, Cu and Zn, in the crop season 2011/2012

Treatmentss	Caf	Trig	Suc	Glu	Gal	Ara	Man
			Boron				
WB	1.010 *	0.828 *	5.140 *	0.182 *	0.105	0.027	0.144
FS	1.511 +	0.959 +	6.452 +	0.265 +	0.144	0.028	0.154
B	1.510 +	0.977 +	6.607 +	0.284 +	0.112	0.034	0.151
B+Cu	1.458 +	0.963 +	6.451 +	0.278 +	0.109 *	0.049 *+	0.166 *+
B+Zn	1.505 +	0.987 +	6.808 +	0.347 *+	0.162 *	0.039 *+	0.152
B+Cu+Zn	0.935 *	0.852 *	5.102 *	0.243 +	0.106	0.026	0.144
CV (%)	5.85	5.54	6.04	14.32	42.61	19.23	6.59
			Copper				
WCu	1.010 *	0.828 *	5.140 *	0.182 *	0.105 *	0.027	0.144 *
FS	1.511 +	0.959 +	6.452 +	0.265 +	0.143 +	0.028	0.154 +
Cu	1.496 +	0.989 +	6.007 +	0.288 +	0.102 *	0.024	0.164 *+
B+Cu	1.458 +	0.963 +	6.450 +	0.278 +	0.109 *	0.049 *+	0.167 *+
Cu+Zn	1.463 +	0.967 +	6.531 +	0.298 +	0.122	0.026	0.163 +
B+Cu+Zn	0.935 *	0.852 *	5.102 *	0.243	0.106 *	0.027	0.144 *
CV (%)	6.16	4.63	6.14	16.78	17.06	19.39	4.05
			Zinc				
WZn	1.010 *	0.828 *	5.140 *	0.182 *	0.105	0.027	0.144 *
FS	1.510 +	0.959 +	6.451 +	0.265 +	0.143	0.028	0.154 +
Zn	1.554 +	0.996 +	6.619 +	0.312 +	0.104	0.041 *+	0.156 +
B+Zn	1.505 +	0.987 +	6.808 +	0.347 *+	0.162	0.039 *+	0.152
Cu+Zn	1.463 +	0.966 +	6.531 +	0.298 +	0.122	0.026	0.163 +
B+Cu+Zn	0.935 *	0.852 *	5.102 *	0.243 +	0.106	0.026	0.144 *
CV (%)	5.95	5.03	6.41	13.35	49.26	12.54	4.14

WB, WCu and WZn - without application of B, Cu or Zn,
FS – foliar spray with Boric acid, Copper sulphate and Zinc sulphate (0.4%),
B – trunk injection of tablets containing B salts,
Cu - trunk injection of tablets containing Cu salts,
Zn – trunk injection of tablets containing Zn salts,
B+Cu - trunk injection of tablets containing B and Cu salts,
B+Zn - trunk injection of tablets containing B and Zn salts,
Cu + Zn - trunk injection of tablets containing Cu and Zn salts,
B+Cu+Zn - trunk injection of tablets containing B, Cu and Zn salts.
Means followed by * differ statistically from the control treatment (FS) at the 10% significance level, according to Dunnett's test.
Means followed by + differ statistically from the control treatment (WB – WCu – WZn) at the 10% significance level, according to Dunnett's test

Within the mono and oligosaccharides, sucrose is a non-reducing sugar in greater quantity in coffee beans, varying from 1.9 to 10% of the dry matter (Lockhart, 1957; Wolfrom et al., 1960; Feldman et al., 1969). According to Knopp et al., (2006) sucrose represents more than 90% of the total low molecular weight carbohydrates and corresponds to 7.07% of the dry matter of coffee beans; this value is very close from those found in this experiment.

According to Camacho-Cristobal, et al., (2004) glucose 6-phosphate, an enzyme involved in the glycolysis route, in conditions of B sufficiency appears complexed with borate anion, and thus, restricts the flow of respiratory substrate to the pentose phosphate pathway, therefore, when B is adequate the sucrose production is greater.

Brown and Clark, (1977) noted that plants of wheat deficient in Cu presented soluble carbohydrates content considerably lower than plants well-nourished in Cu. The lower levels of plastocyanin, as consequence of Cu deficiency, may decrease the efficiency of photosynthetic electron transport in photosystem I, and thus impair the CO_2 fixation rate, in such a way that starch and soluble sugars content (especially sucrose) are reduced.

According to Fischer et al., (2001) mannose, gallactose, glucose and arabinose contents were 4.75, 2.46, 1.68 and 0.99% in *Coffeea arabica* beans, being slightly greater of those obtained in this experiment.

Caffeoylquinic acids (3-CQA, 4-CQA e 5-CQA), PPO activity and phenolic compounds

Among the phenolic compounds, caffeoylquinic acids, dicaffeoylquinic acids, feruloylquinic acids, are the main chlorogenic acid subgroups present in coffee. In general, these compounds react during the roasting process producing free phenolic acids, and therefore, volatile phenolic compounds that contribute to the aroma of coffee beans (Farah et al., 2004 and Farah et al., 2005).

The effect of B, Cu and Zn on 3-CQA and 5-CQA contents have evidenced by the significant difference between the treatments that received the nutrients via solid injections or foliar spray and the control treatment (WB - WCu - WZn). Considering the sprayed as the control only the treatment B+Cu+Zn differed significantly, for 5-CQA content, showing ~~the~~ response to different ways of B, Cu and Zn supply (Table 8).

The 4-CQA (6.32%), proanthocyanidin (6.55%) and total phenolic compounds

(5.33%) were not affected by B treatments. The effect of Cu, also, was not significant for the contents of 4-CQA (0.681%) and proanthocyanidin (6.15%). Either for Zn, 4-CQA (0.675%) and proanthocyanidin (6.74%) were not significant (Table 8).

The effect of B on PPO activity was evidenced by the significant difference of the treatments B+Cu and the sprayed treatment compared to the control (WB), even when B concentration in the indexes leaves of the treatment that received B by trunk injections were above the adequate range established by Martinez et al., (2003).

It is suggested that the content of B that is good for great growth and production is below those that maximize the PPO activity, feature that has been directly related to cupping quality (Tables 2 and 8).

It was observed significant difference between the sprayed and the treatments Cu, Cu+B and Cu+Zn comparatively to the control treatment (WCu) for the PPO activity, with a particular focus on the clear inverse relationship between the PPO activity and the concentration of 5-CQA in the beans (Table 8).

The concentration of Cu in leaves of the sprayed treatment were lower than those of the treatments receiving tablets injections, however, still considered within the adequate range according to Martinez et al., (2003). It suggests that the composition of caffeoylquinic acids and PPO activity remained constant within the range of Cu concentration in the indexes leaves that indicates adequate nutrition (Tables 2 and 8).

Regarding the Zn nutrition, the PPO activity and contents of total phenolic compounds, only the treatment Zn+Cu and the sprayed treatment differed from the control (WZn), being the highest activity observed when phenolic compounds concentration were low (Table 8).

For the PPO activity, considering the sprayed treatment as the control, all treatments that received B, Cu and Zn via solid injections, except B+Cu+Zn, are statistically similar to the sprayed treatment, confirming the similar (?) effect of different ways of supply the micronutrients (Table 8).

Several works, in the literature, relate the accumulation of caffeoylquinic acids in B deficiency. Camacho-Cristobal et al., (2004) reported that the main effect of B deficiency is the accumulation of glucose, fructose and starch, followed by increasing in the 3-CQA, 4-CQA and 5-CQA contents in tobacco leaves. Therefore, the high concentration of phenolic compounds, in B deficient plants, could be either, a result of the soluble sugars accumulation (Baumert et al., 2001; Marschner, 2011).

Table 8 - 3-Caffeoylquinic acid (3-CQA - %), 4-caffeoylquinic acid 4-CQA - %), 5-caffeoylquinic acid (5-CQA- %), proanthocyanidin (Pro - %), polyphenol oxidase activity (PPO - U/ min/ g) and phenolic compounds (TP - %) of coffee beans harvested from plants submitted to the fertilization via solid salts injections in the trunk or foliar sprays with B, Cu and Zn, in the crop season 2011/2012

Treatments	3-CQA	4-CQA	5-CQA	Pro	PPO	TP
			Boron			
WB	0.654 *	0.729	1.695 *	6.36	74.52 *	6.37
FS	0.445 +	0.722	1.406 +	5.70	85.93 +	5.15
B	0.440 +	0.579	1.346 +	5.53	82.07	4.21
B+Cu	0.474 +	0.659	1.396 +	6.78	85.50 +	4.61
B +Zn	0.452 +	0.638	1.384 +	7.14	78.38	5.50
B+Cu+Zn	0.522	0.607	1.580 *	6.41	74.68 *	6.13
CV (%)	19.89	22.27	6.36	38.15	9.01	24.59
			Copper			
WCu	0.654 *	0.730	1.695 *	6.36	74.53 *	6.38
FS	0.445 +	0.722	1.406 +	5.70	85.93 +	5.15
Cu	0.474 +	0.752	1.398 +	6.23	85.15 +	4.06 +
B+Cu	0.474 +	0.659	1.396 +	6.78	85.51 +	4.61
Cu+Zn	0.438 +	0.617	1.300 +	7.59	85.19 +	4.87
B+Cu+Zn	0.522 +	0.607	1.581 *	6.41	74.68 *	6.14
CV (%)	16.10	14.37	5.45	37.40	6.25	22.57
			Zinc			
WZn	0.654 *	0.730	1.695 *	6.36	74.52 *	6.37 *
FS	0.445 +	0.722	1.406 +	5.70	85.93 +	5.15 +
Zn	0.483 +	0.740	1.417 +	7.21	79.08	5.70
B+Zn	0.452 +	0.638	1.384 +	7.14	78.38	5.50
Cu+Zn	0.438 +	0.617	1.300 +	7.59	85.19 +	4.87 +
B+Cu+Zn	0.522 +	0.607	1.581 *	6.41	74.68 *	6.13
CV (%)	16.25	18.28	6.08	31.93	7.11	14.01

WB, WCu and WZn - without application of B, Cu or Zn,
FS – foliar spray with Boric acid, Copper sulphate and Zinc sulphate (0.4%),
B – trunk injection of tablets containing B salts,
Cu - trunk injection of tablets containing Cu salts,
Zn – trunk injection of tablets containing Zn salts,
B+Cu - trunk injection of tablets containing B and Cu salts,
B+Zn - trunk injection of tablets containing B and Zn salts,
Cu + Zn - trunk injection of tablets containing Cu and Zn salts,
B+Cu+Zn - trunk injection of tablets containing B, Cu and Zn salts.
Means followed by * differ statistically from the control treatment (FS) at the 10% significance level, according to Dunnett's test.
Means followed by + differ statistically from the control treatment (WB – WCu – WZn) at the 10% significance level, according to Dunnett's test

Camacho-Cristobal et al., (2002) attributed this effect to the enhancement of the phenylalanine ammonia lyase activity and consequent increase in the phenolic com-

pounds synthesis. Additionally, according to this author, when in high concentration, B and glucose 6-phosphate form complexes and therefore restrict the flow of respiratory substrate for the pentose phosphate pathway. Such behavior may explain the enhanced concentrations of the 3-CQA and 5-CQA in the control treatment WB, WCu and WZn.

In addition, phenolic compounds accumulation is a feature of plants B deficient because the formation of borate complexes with some phenols that can be involved in the regulation of free phenol concentration and in the alcohol phenol synthesis, which are direct precursors of the lignin (Pilbeam and Kirkby, 1983 cited by Marschner, 2011).

In the present work it was not possible establish a direct relationship between caffeoylquinic acid contents and cupping quality, since the control treatment (WB - WCu – WZn) presented average score of 82.7, therefore classified as “soft”.

Polyphenol oxidase

Hajiboland and Farhanghi, (2010) studying the effect of adequate and low doses of B in turnip plants observed that PPO activity, as well as, phenolic compounds increased in roots and shoot when B supply was low. The PPO activity in leaves and roots of deficient plants were 6.3 and 4.6 folds higher, respectively, than that of the control plants receiving sufficient B.

According to Karabal et al., (2003) excess of B alters the cell membrane integrity, thus, it is observed initially progressive increase in PPO activity followed by falling, because of quinones production that inhibit the enzyme, which may explain the low PPO activity of the treatment containing B+Cu+Zn inserted into the trunk.

Carvalho et al., (1994) proposed a way to access the coffee quality using PPO activity levels. According to them, “rio” and “rioysh” types are well correlated to PPO activities below 55.99 U/ min/ g of sample; hard type is correlated to activities between 55.99 and 62.99 U/ min/ g of sample; soft type, is correlated to activities between 62.99 and 67.66 U/ min/ g of sample, and strictly soft type, is correlated to activities above 67.99 U/ min/ g of sample.

In the present work, it was not possible to establish a good relationship between cupping quality and PPO activity, since the control treatment WB, WCu, WZn and B+Cu+Zn have had high cupping test scores (82.7 and 80.4 – “softish” type) followed by low PPO activity. It can be highlighted, however, that low PPO activity in these treatments was accompanied by high concentrations of 3-CQA and 5-CQA.

According to Mazzafera and Robinson, (2000) the 5-CQA is likely the main substrate of the PPO. Farah et al., (2006b) state there is an increase in the coloration intensity of coffee beans in response to the PPO action on 5-CQA, thus the authors associated the oxidation products, to the low quality of coffee beans rich in caffeoylquinic acid. Amorim, et al., (1977) reported that PPO action, along the structural changes of the membrane, is a possible cause for the formation of beans classified as "rioysh" type.

It is often mentioned by the literature that Cu is the PPO catalyst (Malavolta, 1980; Robinson and Eskin, 1991), thus the evaluation of PPO activity can be a good indicator of the nutritional status in Cu. Taking in account the importance of Cu on the PPO structure and because of this enzyme is involved on the control of the concentration of free phenolic compounds, the efficiency in Cu supplementation to the plants is, therefore, a requirement of great importance in order to obtain coffees with high quality.

In a nutritive solution experiment Lacerda, (2014) observed that Zn doses affected the contents of chlorogenic acids of *Coffea arabica* beans. Total phenolic compounds, 5-CQA and 4-CQA reached minimum points when the indexes leaves presented 10 mg/kg of Zn, i.e. at about in the center of the sufficiency range established by Martinez et al., (2003). The grains produced in conditions of deficiency or excess of Zn presented higher values of these compounds. The curves for PPO and 3-CQA presented exactly inverse shape, reaching the maximum points in grains of plants with 10 mg/kg of Zn in indexes leaves. Due to the direct relationship among 3-CQA and PPO, the author questioned if the content 3-CQA, in a different way than that of 5-CQA, could be related to good quality of coffee beans.

Although, in this work, there was no good agreement between the cupping test and chemical attributes of coffee quality, it should be emphasized that the cupping test is subjective and new methods must be studied in order to evaluate properly the coffee bean quality.

CONCLUSIONS

Boron, copper and zinc supplied by foliar sprays or solid trunk injections influence the chemical composition and quality of the coffee beans, characterized by cupping test and the contents of caffeine, trigonelline, sucrose, glucose, arabinose, mannose, 3-caffeoylquinic acid, 5-caffeoylquinic acid, polyphenol oxidase activity and total phenolic compounds.

Copper and Zn supplying by solid trunk injections give equivalent results to the foliar sprays both in production and quality. The trunk injections containing B salts resulted in toxicity and affected negatively the production, while some attributes related to the quality of the grains were higher with high B supply, capable of limiting the growth and yield of coffee plants.

CHAPTER 3

PHOTOSYNTHESIS AND ENZYMATIC ACTIVITY IN INDEX LEAVES OF *Coffea arabica* AS A FUNCTION OF B, Cu AND Zn CONTENTS

ABSTRACT

Boron, copper and zinc are the most limiting nutrients in coffee production. However, studies relating highly sensitive metabolic indicators with yield and nutritional status of coffee plants are scarce. Therefore, the objective of this study was to determine the concentrations of B, Cu and Zn in the index leaf that are associated with the maximum photosynthetic efficiency and relate them to the activities of the enzymes polyphenol oxidase and dismutase superoxide. Different rates of the nutrients Cu (0.4, 0.8, 1.6 and 3.2 µmol/L), Zn (1.0, 2.0, 3.0 and 4.0 µmol/L) and B (10, 20, 30 and 40 µmol/L) were the factors analyzed in the study. All treatments also received nutrient solution containing: 6; 0.5; 2.25; 1; 1.75 mmol/L of N, P, Ca, Mg and S and 12; 0.3 and 40 µmol/L of Mn, Mo and Fe, respectively. The parameters photosynthetic efficiency of the photosystem II (Fv/Fm), rate of electron transport (ETR), dismutase superoxide activity (SOD) and polyphenol oxidase activity (PPO), as well as the levels of B, Cu and Zn, in index leaf were evaluated at 0, 60 and 90 days after the application of the treatments. The polyphenoloxidase activity can be a good indicator of the nutritional status of coffee plants for B and Cu. The activity of dismutase superoxide was proportional to leaf concentrations of Cu and Zn. The photosynthetic activity is optimal when the leaf content of B and Cu are high, also, resulting in maximum polyphenol oxidase activity.

FOTOSSÍNTESE E ATIVIDADE ENZIMÁTICA EM FOLHAS ÍNDICES DE *Coffea arabica* EM FUNÇÃO DE CONCENTRAÇÕES DE B, Cu E Zn.

RESUMO

O B, Cu e Zn são os micronutrientes mais limitantes à produção dos cafeeiros. No entanto, estudos relacionando indicadores metabólicos de alta sensibilidade, relacionados à produtividade, e ao estado nutricional de cafeeiros ainda são escassos. Assim, o objetivo do trabalho foi determinar as concentrações de B, Cu e Zn da folha índice que se relaciona com a máxima eficiência fotossintética e relacionar à atividade das enzimas polifenoloxidase e superóxido dismutase ao estado nutricional do cafeeiro quanto ao B, Cu e Zn. O experimento foi executado em casa-de-vegetação sob delineamento inteiramente casualizado com três repetições. As doses de 0.4, 0.8, 1.6 e 3.2 µmol/L de Cu; de 1, 2, 3 e 4 µmol/L de Zn e 10, 20, 30 e 40 µmol/L de B constituíram os fatores em estudo. A solução nutritiva de todos os tratamentos também continha: 6, 0.5, 2.25, 1, 1.75 mmol/L de N, P, Ca, Mg e S e 12; 0.3 e 40 µmol/L de Mn, Mo e Fe. Avaliaram-se a eficiência do fotossistema II (Fv/ Fm), a taxa de transporte de elétrons (ETR), a atividade da superóxido dismutase (SOD) e a atividade da polifenoloxidase (PPO), bem como, os teores de B, Cu e Zn, da folha índice, aos 90 dias após a aplicação dos tratamentos. A atividade da polifenoloxidase pode ser um indicador do estado nutricional das plantas em B e Cu. A atividade da superóxido dismutase é proporcional aos teores foliares de Cu e Zn. A atividade fotossintética é ótima quando os teores foliares de B e Cu também são ótimos, resultando, também, em máxima atividade da polifenoloxidase.

INTRODUCTION

The productivity of crops is predominantly determined by the carbon metabolism. The energy required for the assimilation of nutrients derives directly or indirectly from photosynthesis, which in its turn, is a function of the adequate nutrient supply.

The emission of chlorophyll fluorescence is often used for photosynthetic measurements (Genty et al., 1989), especially in environments that promote stress conditions (Zhang, et al., 2003). The current measurement methods provide rapid information

and enable the non-destructive and non-invasive determination of conversion, transference and dissipation of light energy in the photosystem II (Bilger et al., 1995, Baker, 2008), under laboratory and/or field conditions.

The best approach to obtain information on fluorescence emission is by following its kinetics over time. Studies on fluorescence have been related with fluorescence responses when a photosynthetic tissue is quickly lit after it had been kept in dark conditions during some time.

At the time of an environmental or biotic stress, changes in the functional state of the thylakoid membranes of chloroplasts cause changes in the fluorescence signal, characteristics which can be quantified in leaves (Ribeiro et *al.,* 2003; Baker and Rosenqvst, 2004).

The maximum quantum yield of photosystem II, which is estimated by the F_v/F_m rate, indicates dissipation of photochemical energy and expresses the efficiency of capture of this excitation energy by the opened reaction centers of photosystem II (Baker, 1991; Krause and Weis, 1991). The electron transport rate (ETR) numerically indicates the absolute value of electrons transported per square meter in an interval of one second and is directly related to the photosynthetically active radiation incident on the leaf.

In coffee plants, the saturation for sun or shade leaves are approximately from 600 to 300 µmol (photons)/ m^2/ s, respectively (Kumar and Tieszen, 1980; Fahl et al., 1994). Irradiances higher than those desired can cause photosynthesis photoinhibition and very often lead to a decrease in the net rate of electron transport through the photosystem II and a strong increase in the rate of rotation of the main polypeptide present in photosystem II reaction centers (Makin and Niyogi, 2000).

With respect to mineral nutrition, it is known that the order of micronutrient requirement by coffee plants is Fe>Mn>B>Zn>Cu>Mo, which may infer that the main problems are related with B, Cu and Zn, since Brazilian soils are rich in Fe and Mn. Adequate availability of micronutrients provides well-developed and vigorous coffee plants, which in a second moment determines good productivity.

Copper and Zn play various physiological and biochemical functions in plants, like the structural constitution of enzymes such as superoxide dismutase (Hansch and Mendel, 2009). Cu-Zn superoxide dismutase plays an important role on protecting the plant against oxidative damage caused by reactive oxygen species (ROS) and the enzyme activity correlates positively with active Zn in plants. ROS are continuously gen-

erated as products of photosynthesis and other cellular metabolites (Foyer and Noctor, 2000).

Due to the B ability to complex with free phenolic compounds, it is well known that it is indirectly involved in quinone formation and hence in the synthesis of phenolic alcohols which are precursors of the lignin biosynthesis, however generating ROS.

Increases in the concentration of phenols in B deficient tissues may result from restrictions in the biosynthesis of phenolic alcohols (Pilbeam and Kirkby, 1983). Polyphenoloxidase is a cupric enzyme that processes the hidroxilation of monophenols to diphenols and the oxidation of these diphenols to o-quinones. Based on this, it is expected that PPO activity may be a good indicator of the nutritional status of coffee plants, as the B and Cu.

However, there are few studies on these highly sensitive metabolic indicators related to productivity, thus with the photosynthesis and the nutritional status of coffee plants. Therefore, the objective of this study was to determine the concentrations of B, Cu and Zn in the index leaves that correlate with the maximum photosynthetic efficiency and relate the PPO activity and dismutase superoxide to the nutritional status of B, Cu and Zn.

MATERIAL AND METHODS

The experiment was conducted with plants of *Coffea arabica* L. cv. Catuaí IAC-99 grown in a greenhouse belonging to the Plant Science Department of the Federal University of Viçosa, during 5 years. The experimental arrangement was a completely randomized design with five replications. These coffee plants were grafted using different plagiotropic branches of this same variety. After the establishment, the plants were transferred to greenhouse and were conducted in nutrient solution as described by Clemente et al., (2013).

Then, the plants were transferred to buckets (20 L) containing washed sand and thereafter conducted in hydroponic cultivation system. The buckets were fitted with drains at the bottom that were connected to a discharge pipe that was connected to a reservoir (50L) of nutrient solution. The nutrient solution was pumped to the buckets using a timer-controlled electric pump.

The nutrient solution was stored in 50 L reservoirs. During cultivation, the volume of the nutrient solution was supplemented daily with water to the initial volume;

the pH was monitored daily and maintained between 5.5-6.5 using HCl and NaOH (1mol/ L). The electrical conductivity of the nutrient solution was monitored daily and always adjusted when the values reached depletions of 30% compared with the initial values. The nutrient solution was completely renewed every two months.

In this system, the plants were irrigated seven times over a period of 24 hours, receiving the solution during 3 minutes every three hours during the day and every 6 hours during the night, using a timer-controlled electric pump.

Different rates of the nutrients Cu (0.4, 0.8, 1.6 and 3.2 µmol/L), Zn (1.0, 2.0, 3.0 and 4.0 µmol/L) and B (10, 20, 30 and 40 µmol/L) were the factors analyzed in the study. All treatments also received nutrient solution containing: 6; 0.5; 2.25; 1; 1.75 mmol/L of N, P, Ca, Mg and S and 12; 0.3 and 40 µmol/L of Mn, Mo and Fe, respectively. The plants received the treatment during 120 days.

For the diagnosis of plant nutritional status, leaves were collected from the third or fourth node, from the apex to the base of the branches, in the middle third of the plants.

Boron concentration in plant tissue was determined by dried digestion, followed by the azomethine-H colorimetric method (Malavolta et al., 1997). The concentrations of Zn and Cu were determined by atomic absorption spectrophotometry (AOAC, 1975) after nitric-perchloric digestion of the samples (Johnson and Ulrich, 1959).

Polyphenol oxidase and superoxide dismutase activities were determined by collecting three foliar discs from the leaves of the third node counted from the apex to the base of the middle third of the plants. The samples were wrapped in aluminium foil, immediately frozen in liquid nitrogen and stored in a freezer (-45°C) until analysis.

Determination of superoxide dismutase activity was performed according to Giannopolitis and Ries (1977), based on the amount of enzyme required to cause 50% inhibition of nitroblue tetrazolium photoreduction. PPO was extracted according to Concellón et al., (2004) and the activity was determined according to Kavrayan and Aydemir, (2001).

The relative chlorophyll content was determined using a SPAD-502 chlorophyll meter, taking three readings in 30 leaves per plot.

The variables of chlorophyll *a* fluorescence were determined with a portable modulated fluorometer MINI-PAM (Walz, Germany), obtaining the maximum quantum yield of photosystem II and the relative rate of electron transport.

Data were subjected to regression analysis. Regression models were chosen based on the biological sense of the responses and coefficients of determination. Statistical analyses were performed using the software SAEG (Euclydes, 1983).

RESULTS AND DISCUSSION

B, Cu and Zn contents

By end of the experiment, it was observed a linear increase in the B content of index leaves. Copper and Zn contents in index leaves increased linearly with the rates applied (Figures 1a, 1b and 1c).

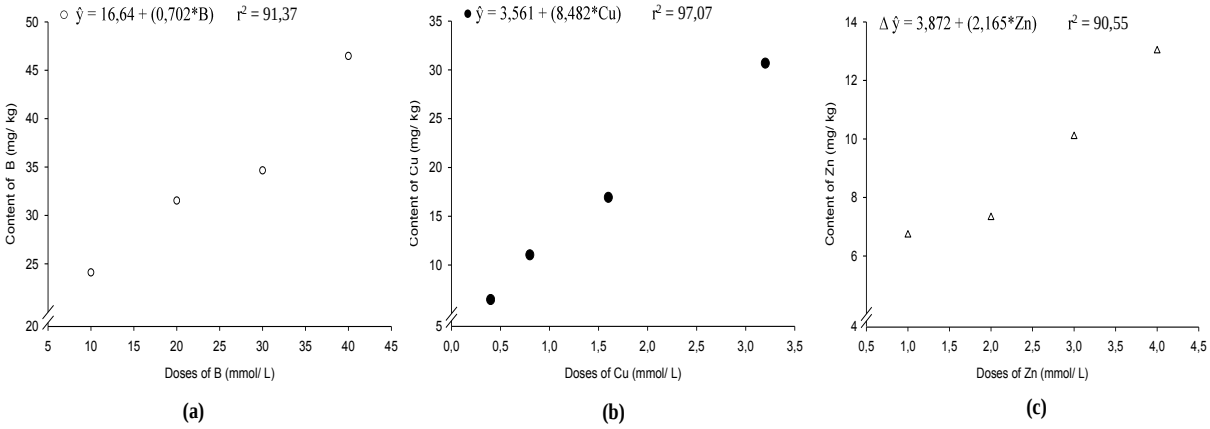


Figure 1- Contents of B (a), Cu (b) and Zn (c) as a function of increasing rates of B, Cu and Zn applied in the nutrient solution.

Hu and Brown, (1997) reported that the B absorption in higher plants is a passive, non-metabolic process and occur as response to the external concentration of boric acid, membrane permeability, formation of complexes inside the cell and transpiration rate. In barley (Nable et al., 1990 b), sunflower, squash and tobacco cells (Brown and Hu, 1994), the accumulation of B was linear with increasing concentrations of B and was not saturable even when plants were subjected to a wide range of B concentration.

Copper absorption occurs through an active process and there is strong evidence that inhibits Zn uptake and vice-versa (Bowen, 1969); on the other hand, when in excess, it can interact with K (Daliparthy et al., 1994), P (Rhoads et al., 1992) and N (Soon et al., 1997), resulting in toxicity to the cells.

Excessive doses of Zn can inhibit the absorption of other cations and according to Favaro, (1992) although sometimes the foliar content are excessively high, most of

this Zn can be removed from the sites of greater metabolic activity without causing damage to the cellular metabolism, at least in a short term.

It is known that Zn in excess inhibits photosynthesis by the reduction of RuBP carboxylase/oxygenase (Rubisco), essentially, because Zn and Mg compete for the same active site on Rubisco and decrease the photosystem II activity by displacing Mn (Misra and Ramani, 1991; Marschner, 2011; Fageria, 2001; Kaya and Higgs, 2001; Kaya et al., 2001).

Chlorophyll fluorescence and SPAD index

At the end of the experiment Fv/ Fm and ETR showed quadratic behavior even with the increasing contents of B in the index leaves, reaching the maximum points at 25.48 and 26.41 µmol/ L of B, respectively (Figure 2). The contents of B in the index leaf at the maximum points were 34.52 and 35.17 mg/ kg for Fv/ Fm and ETR, respectively, therefore slightly below the center of the range considered adequate for coffee, according to Martinez et al., (2003).

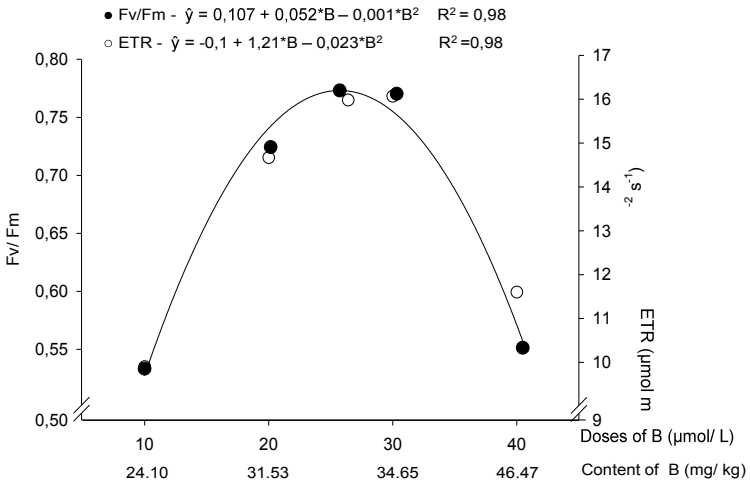


Figure 2 - Quantum yield of photosynthesis (Fv/Fm) and electron transport rate (ETR) in response to increasing rates of B in nutrient solution.

The SPAD index presented a quadratic response reaching the maximum at 32.54 mmol/ L (Figures 3). The content of B in the index leaf, at this point was 39.48 mg/ kg, which is within the range described by Martinez et al., (2003). It can be noted that, this level of B is very close from the levels that provided maximum Fv/ Fm and ETR.

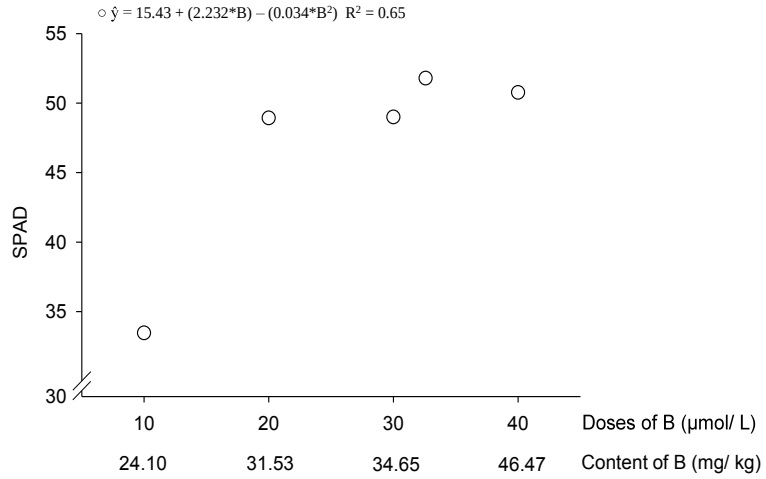


Figure 3- SPAD index as a function of increasing rates of B in nutritive solution.

According to Hajiboland and Farhanghi, (2010), Fv/ Fm and ETR were not significantly affected by B deficiency. However, El-Shintinawy, (1999) stated that B deficiency can affect the Hill reaction, the activity of electron transfer and the evolution of oxygen in the photosynthesis process of sunflower plants.

Hajiboland and Farhanghi, (2010) observed that there was more photoinhibition in B deficient leaves, since the chlorophyll content was lower and leaves with less chlorophyll are more susceptible to photoinhibition. Another likely cause of greater photoinhibition in leaves B deficient is the excess of reducing power (NADPH) by the lower activity of some enzymes related to the photosynthetic system.

On the other hand, excess B affects the photosynthesis because of damages on the thylakoid structure and CO_2 capture. These effects include disruption of electron transport and oxygen molecules come to function as acceptors of unused electrons, favoring the formation of ROS (Papadakis et al., 2004; Molassiotis et al., 2006).

Ardic et al., (2009) reported that the chlorophyll fluorescence in chickpea tolerant to water stress was not significantly affected by treatments containing B, however in some drought tolerant varieties the Fv/ Fm decreased 10 % after B application at a concentration of 6.4 mM in nutrient solution, which is a dose considered excessive for the culture.

Papadakis et al., (2004) observed a significant decrease in Fv/ Fm of orange leaves grown under conditions of excess B. This decrease was attributed to the oxidation of chlorophyll and chloroplastidic membranes, which can be exacerbated by excess B; a fact also reported in apple rootstocks (Sotiropoulos et al., 2006).

In conditions of B deficiency, alterations may occur in the formation of crossed bonds of the cell wall, rather than the growth of young leaves (Hu and Brown, 1994).

In contrast to the deficiency, when B is in excess, there is reduction of the leaf area, loss of chlorophyll and morphological disorders (Nable et al., 1997). This effect on chlorophyll content still is not clear, but there is inhibition of uptake of metallic ions, including Mg ions, thus there is loss of chlorophyll structure in conditions of B excess (Reinbott et al., 1997; Power e Woods, 1997), which explains the results of the SPAD index observed in the present study.

Inbaraj and Muthuchelian, (2011) reported that under conditions of B excess there was a reduction of 44% in protein synthesis compared to the treatment with appropriate B supply. They also observed that the stresses due to excess or lack of B caused significant decrease in the content of chlorophyll a, chlorophyll b and carotenoids in leaves of cowpea. The authors attributed this effect to the reduced synthesis of proteins and lower RNA synthesis and/or higher RNAse activity.

Fv/ Fm and ETR presented a quadratic response, with the increasing doses of Cu in the nutritive solution and with the increasing Cu content in the index leaves, and reached the maximum at the rate of 1.91 and 1.87 µmol/ L, (Figures 4); at these levels the concentration of Cu in the index leaf was 19.76 mg/ kg and 19.43 mg/ kg (Figure 4).

It can be noted that the content of Cu, in the index leaves, was linear and the leaf level at the maximum point is within the adequate range according to Martinez et al., (2003).

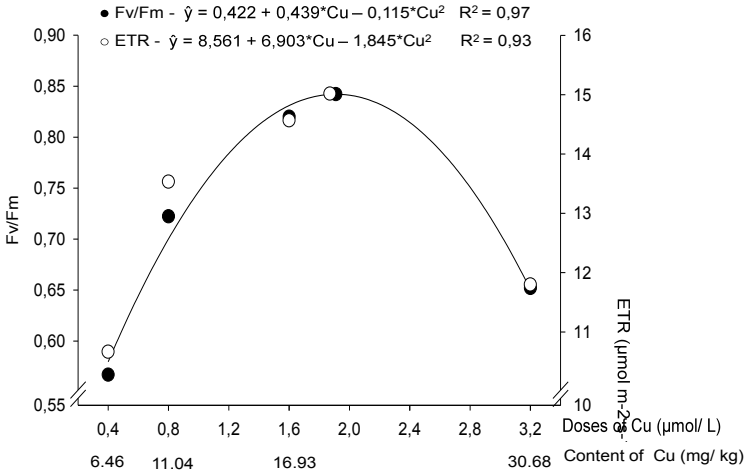


Figure 4 – Quantum yield of photosynthesis (Fv/Fm) and electron transport rate (ETR) in response to increasing rates of Cu in nutrient solution.

The model that best fitted to the SPAD index as a function of Cu rates was the square root (Figures 5).

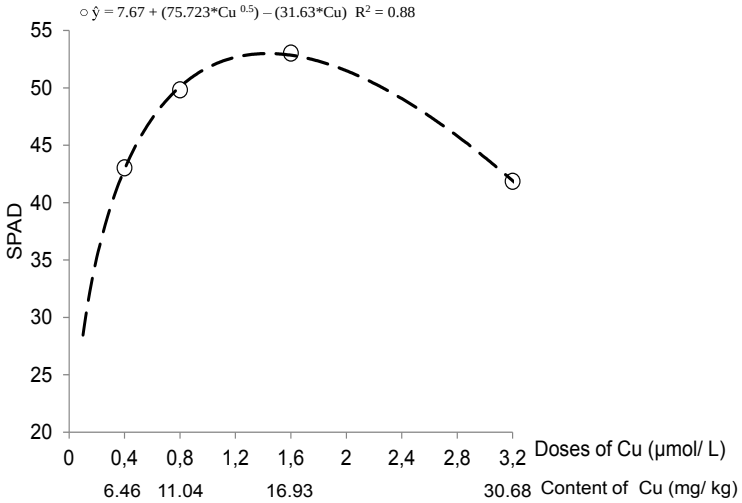


Figure 5- SPAD index as a function of increasing Cu rates in nutritive solution.

Lower activity of photosystem II in Cu-deficient plants may be related to the essentiality of Cu in the synthesis of quinones. In Cu-deficient chloroplasts, the electron transport is affected by the lack of two specific membrane polypeptides in the membrane of chloroplasts, which are probably essential to maintain adequate fluidity of the membrane and, thus, ensure mobility of the molecules of electron transport between the two photosystems (Marschner, 2011; Droppa et al., 1984a).

Cambrollé et al., (2011; 2012) observed that high Cu concentrations diminished the yield and the efficiency of the photosystem II and as consequence induced photoinhibition by light stress in plants of *Glaucium flavum*, *Halimione portulacoides* and *Limoniastrum monopetalum*.

Cuchiara et al., (2013) studying the effect of increasing doses of Cu in plants of *Alternanthera tenella* noted that excess Cu caused a decrease in the reservoir size of electron acceptors at the photosystem I, reducing the number of electrons transported per reaction center and reducing the parameters related to the flow, production and reduction efficiency of the photosystem I, thus excess Cu affected predominantly the structure and functionality of the photosystem I.

Fv/Fm and ETR showed a quadratic response, for Zn, reaching the maximum at the doses of 2.61 and 2.63 µmol/ L and the Zn levels at these points were 9.54 and 9.57 mg/ kg. Stands out that Zn content, in the index leaf, increased with Zn applied in the nutritive solution (Figure 6).

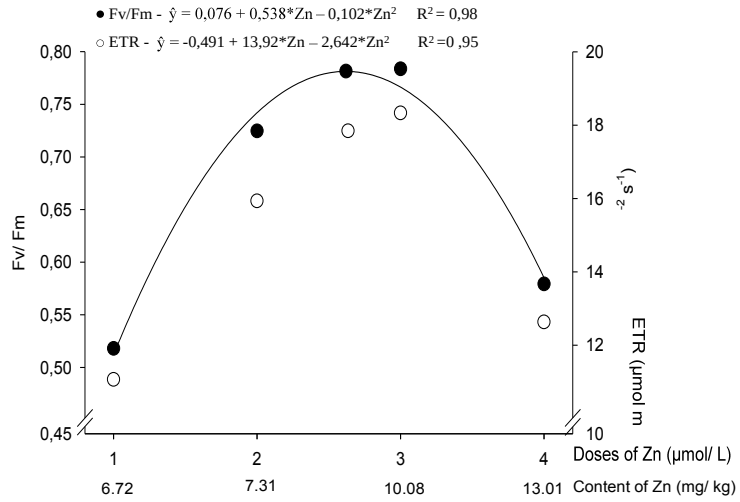


Figure 6 – Quantum yield of photosynthesis (Fv/Fm) and electron transport rate (ETR) in response to increasing Zn rates in nutrient solution.

The SPAD index showed a quadratic response reaching maximum at the dose of 2.32 µmol/ L, being the Zn content at this point 8.89 mg/ kg (Figures 7). The concentrations of Zn in the index leaf are within the range considered adequate as described by Martinez et al., (2003).

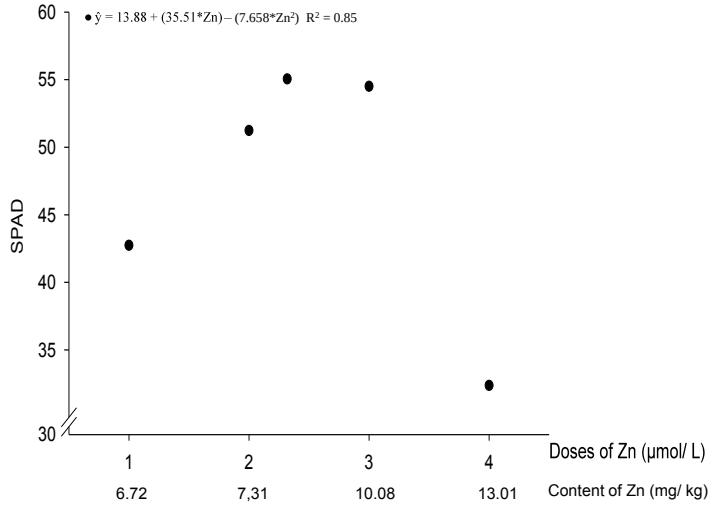


Figure 7- SPAD index as function of increasing Zn rates.

According to Bonnet et al., (2000), in plants subjected to excessive rates of zinc sulphate there was a strong decrease in the maximum capacity of photosynthetic activity, as well as in the ETR. Yruela et al., (1996) found similar results.

Enzymatic activity of PPO and SOD

The PPO activity presented a quadratic response, reaching the maximum activity at 33.53 µmol/ L (Figure 8). The content of B, in the index leaf, at this rate was 40.18

mg/ kg, a mean value which is in the center of the range considered adequate according to Martinez et *al*., (2003).

It was observed that PPO activity is closely related to the nutritional status of leaves, for B, because the content of B, in the index leaf, increased with the B applied in the nutritive solution, probably it was accumulated and impaired the PPO activity by the end of the experiment (Figure 8).

At this time, it is observed that PPO activity between the maximum point and the higher limit of the sufficient range, as previously established by Martinez et al., (2003) (52 mg/ kg), varies from 8.46 to 10.76 UA/ min/ mg of protein, indicating that in this range the PPO activity can be an indicator of the nutritional status of coffee plants.

The dismutase superoxide activity increased linearly with the B rates applied (Figures 8). At the higher limit of the sufficient range for B (52 mg/ kg) as established by Martinez et al., (2003), the SOD activity estimated is 4843.41UA/ mg of fresh weight, indicating that when the SOD activity is higher than this value, the nutrient may be in toxic concentrations.

It also can be noted that the foliar content associated to the maximum PPO activity (40.18 mg/ kg) is very close from those in which Fv/Fm and ETR are maximum (34.52 and 35.17 mg/ kg, respectively) and the SOD activity increased with the content of B in the index leaf.

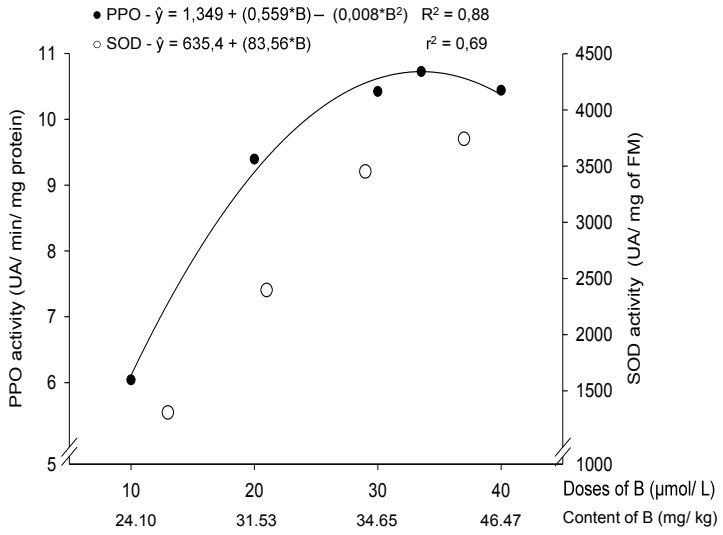


Figure 8- Polyphenol oxidase (PPO) and dismutase superoxide (SOD) activities as a function of increasing B rates.

Hajiboland and Farhanghi, (2010) observed that the PPO activity, as well as the content of total phenolic compounds increased in roots and shoots under conditions of low B supply, being the PPO activity in B-deficient leaves and roots 6.3 and 4.6 times higher, respectively, compared to the control plants in adequate B supply.

Among other effects, B deficiency changes the functions of the plasma membrane due to changes in the metabolism of phenolic compounds. Thus, the pentose phosphate pathway and not glycolysis becomes the predominant pathway for carbohydrate degradation, leading to the formation of phenols (and tryptophan) via the shikimic acid pathway (Marschner, 2011).

On the other hand, Shkolnik, (1984) stated that high B rates cause significant changes in the activity of several enzymes and, consequently, change the metabolism of higher plants. Furthermore, the excess of B may alter membrane integrity (Karabal et al., 2003), which partially explains the decreasing in PPO activity from the dose of 33.53 µmol/ L.

Kocacalıskan and Ölcer, (2006) reported that the application of 10 and 20 mM of B, which is considered excessive, reduced the PPO activity in germinated seeds of maize. The PPO activity increased with B deficiency in leaves of sunflower (Pfeffer et *al*., 1998) and tobacco (Camacho-Cristobal et *al*., 2002), while in squash roots was the opposite (Cara et al., 2002). It was observed that both high and low B rates can reduce the PPO activity in chickpea leaves (Chatterjee et al., 2005).

The consequent accumulation of phenols and the increase in PPO activity lead to the formation of highly reactive intermediates, such as quinones, which like the photoactivated phenols, are highly effective in the production of superoxide radicals, which are potentially capable of damaging chlorophyll and membranes due to lipid peroxidation and consequently inhibit the PPO activity.

Boron deficiency, according to Hajiboland and Farhanghi, (2010) increased the SOD activity in 89% in roots and shoot of turnip. Cakmak et al., (1995) discussed that B stabilizes membranes by complexing phenolic compounds and, thus, prevents or limits the oxidation of phenolic compounds to quinones with consequent formation of reactive oxygen species. In this study, the third evaluation showed that the ROS production increased probably due to excess B, occurring a linear increase in SOD activity, which corroborates the results found by Garcia et al., (2001); Karabal et al., (2003); Molassiotis et al., (2006); Sotiropoulos et al., (2006) and Cervilla et al., (2007).

Cakmak and Römheld, (1997) stated that excessive concentration of B inhibits the synthesis of tocopherol and creates a favorable condition for ROS production, given that α-tocopherol acts along with the SOD antioxidant system in plants. Also, Keles et al., (2004) reported that citrus leaves with high B concentrations presented SOD activity 14% lower than leaves with adequate concentrations.

The PPO activity showed quadratic response reaching the maximum at 1.97 µmol/ L in nutrient solution (Figures 9). The Cu contents in the index leaf, at this rate, was 20.31 mg/ kg, respectively. It can be noted that this leaf content is very close to those that shows the highest values for Fv/ Fm and ETR (19.76 mg/ kg and 19.43 mg/ kg, respectively). According to Martinez et al., (2003) the critical range for Cu varies from 13 to 29 mg/ kg, therefore maximum activities occur within this critical range.

The PPO activity between the maximum point and the upper limit of the sufficiency range (29 mg/ kg) varied from 9.44 to 11.81 UA/ min/ mg of protein, showing that the PPO activity in this range indicates a good nutritional status of coffee plants for Cu.

The SOD activity increased linearly with the Cu rates (Figures 9) and at the upper limit of the sufficiency range for Cu (29 mg/ kg), the SOD activity was 3579.45 UA/ mg of fresh weight, indicating that in higher SOD activities, Cu can be in toxic concentrations.

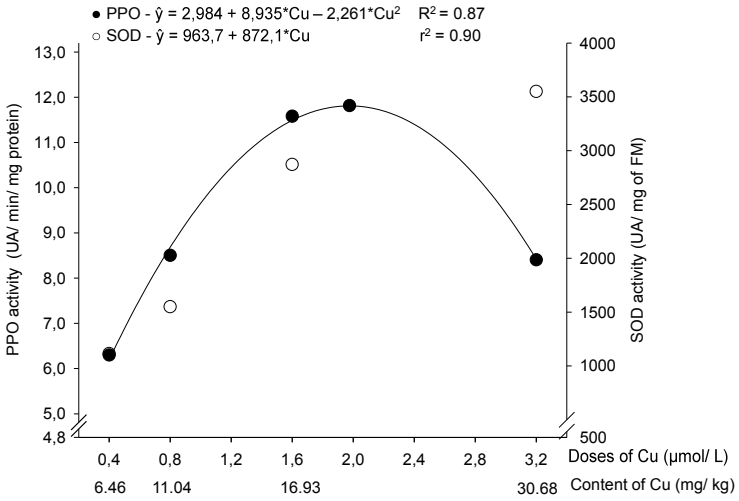


Figure 9- PPO and SOD activities as a function of increasing Cu rates.

According to Schoot Uiterkamp and Mason, (1973) and Lerch, (1976), there are a couple of Cu atoms involved in the reactions catalyzed by PPO and their valences vary during the hydroxylation to the cuprous state. Also, Mendonça and Guerra, (2003) discussed that PPO belongs to the oxidoreductase groups and contain Cu as a prosthetic

group, hence the proportionality observed between the activity of this enzyme and Cu concentration in index leaves, up to the maximum points.

Some studies show that deficiency and toxicity of Cu can reduce the PPO activity in beet leaves, while only its excess increases the PPO activity in roots of *Panax ginseng* (Ali et al., 2006).

Aust et al., (1989) and De Vos et al., (1992) stated that Cu is a potential generator of reactive oxygen species such as superoxide radicals, hydroxyl and hydrogen peroxide. Consequently, this metal can cause severe damages by adding sulfhydryl groups to proteins and induce lipid peroxidation of cell membranes.

Alves et al., (2003) studying the effect of the rates 0.12 and 0.16 mg/ L of Cu on the activity of metalloenzymes in aquatic plants observed an increase of approximately 100% in the SOD activity of plants with the highest Cu rate. Rama Devi and Prasad, (1998), working with the aquatic plant *Ceratophyllum demersum* observed an increase in SOD activity when segments of these plants were kept during 24 hours in nutrient solution containing excess Cu.

The PPO activity decreased with the rates of Zn and the SOD activity increased with Zn rates (Figures 10). However, the increasing in the Zn content may have been accompanied by the reduction in growth of leaves, making them more susceptible to oxidative stress.

At the upper limit of the sufficient range of Zn (12 mg/ kg) the SOD activity was 2792.47 UA/ mg of fresh weight, indicating that when the SOD activity is higher than this value, Zn may be in toxic concentrations. According to Malavolta et al., (1974), Zn deficiency can cause an increase in the polyphenol oxidase and peroxidase activities.

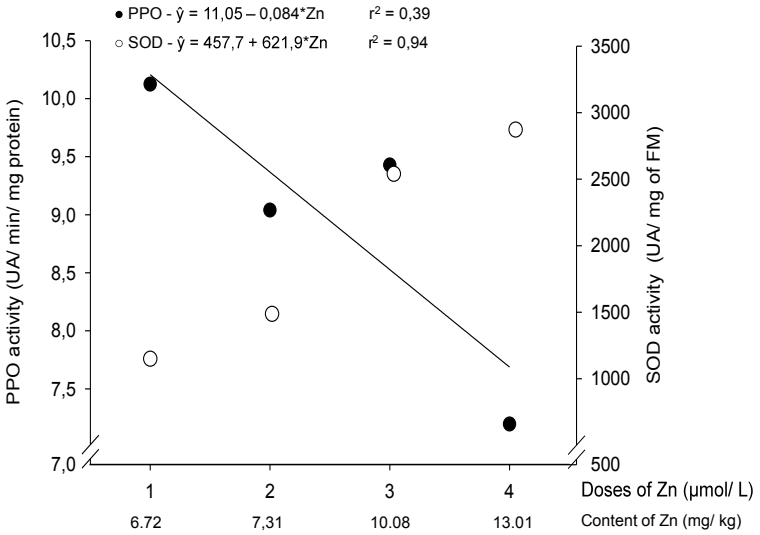


Figure 10- PPO and SOD activities as a function of Zn application at 90 days after the beginning of the experiment.

Pandey et al., (2002) argued that the Cu-Zn SOD activity is closely related to Zn supply and its activity is a good indicator of the nutritional status of *Vigna mungo* plants. Yu et al., (1999) noted that the SOD activity reduced in conditions of no Zn supply. These authors concluded that deficiency of Cu, Zn and Mn, depending on the intensity, alters the activity of antioxidant enzymes in tobacco plants.

Cakmak and Marschner, (1993) discussed that the reduction of protein synthesis may explain the low antioxidative activity of the enzyme in the case of Zn deficiency. Also according to these authors, the concentrations of proteins respond quickly to Zn resupply in the solution.

Kappus, (1985) stated that like other metals, Zn in excess causes toxicity in leaves, and it is well known for acting as ROS generator and inhibitor of the electron transport chain.

Some studies also show that ROS production may be a cause for SOD inactivation (Casano et al., 1997; Panda and Patra, 2010). Panda and Khan, (2004) found that SOD activity in *Hydrilla verticulata* reduced in Zn excessive conditions, not corroborating with the results found in this experiment.

The contents observed at the maximum for ETR and Fv/ Fm were close to those of the SPAD index, showing that from intermediate rates, there was impairment of the photosynthetic system, together with this, the SOD activity increased with applications of Cu and Zn in nutrient solution, suggesting that there was ROS production which

probably harmed the photosystem II activity and hence the transport of electrons to the photosystem I.

The SOD activity increased with of the Zn rates, however, chlorophyll, measured indirectly by the SPAD index, decreased from intermediate rates, confirming the deleterious effects of the ROS action. It seems that the reduction in the SPAD index from intermediate rates is related to nutritional imbalances with other cationic micronutrients Zn.

The imbalance between the photochemistry and biochemistry activity of photosynthesis favors the generation of ROS (Souza et al., 2005), resulting in a greater demand for an efficient antioxidant defense mechanism to prevent oxidative stress (Cavalcanti et. al., 2004).

CONCLUSIONS

The concentrations in the index leaves of B varying from 34.52 mg/ kg to 39.49 mg/ kg, Cu from 15 to 19.5 mg/ kg and Zn from 8 to 9.5 mg/ kg related with the maximum photosynthetic activity determined by the SPAD index, electron transport rate and photosynthetic efficiency of the photosystem II.

The polyphenol oxidase activity can be an indicator of the nutritional status of coffee plants for B and Cu. The activity of dismutase superoxide is proportional to the contents of Cu and Zn.

FINAL CONSIDERATIONS

Boron, Copper and Zinc have primordial function on the production of organic compounds, rich in energy, from its functions on photosynthesis of coffee trees, as well, on the production of compounds of secondary metabolism and on the activity of antioxidative enzymes, indicating the lack of any of them, despite of the small quantities required, can cause losses on productivity and quality of beans and beverage.

Solid injections into the trunk can be workable to supply micronutrients to coffee trees, however, additional studies will be required to adjust the composition and

doses of these micronutrients in the tablets, in order of obtain a release in accordance with the demand of the plants and thus, do not reach toxic concentrations in periods of increased transpiration. After that, new studies will be required to turn this practice useful to wide areas of coffee plantations.

Boron supplied via solid injections was released in excess, and increased B contents reaching toxic concentrations in the second crop season, nevertheless it was possible to verify its effect on the production of caffeine, trigonelline, sucrose, phenolic compounds, polyphenol oxidase activity and caffeoylquinic acids.

Copper and zinc also influenced the production of the same coffee compounds, although only Cu influenced the production and cupping quality evaluated by cupping test.

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APPENDIX

Table 1A – Table of variance analysis for the content of B, Cu and Zn of the first pair of leaves

		Means square					
		Boron[1]					
	DF	WB	FS	B	B+Cu	B+Zn	B+Cu+Zn
Regression	3	171.163	346.958	507.03	627.835	728.315	903.031
Residue	1	1.246	16.789	60.891	44.48	100.055	102.646
		Boron[2]					
		WB	FS	B	B+Cu	B+Zn	B+Cu+Zn
Regression	3	59.789	67.830	59.152	72.482	68.531	61.166
Residue	1	0.143	5.36	0.049	5.149	4.689	3.009
		Copper[1]					
	DF	WCu	FS	Cu	B+Cu	Cu+Zn	B+Cu+Zn
Regression	3	33.001	67.398	-	42.235	56.532	36.826
Residue	3	1.035	0.973	-	0.847	4.654	2.253
		Copper[2]					
	DF	WCu	FS	Cu	B+Cu	Cu+Zn	B+Cu+Zn
Regression	3	6.547	-	15.055	10.771	11.150	7.924
Residue	3	0.468	-	1.441	1.770	1.457	1.077
		Zinc[1]					
	DF	WZn	FS	Zn	B+Zn	Cu+Zn	B+Cu+Zn
Regression	3	21.181	59.514	47.124	46.407	59.923	36.902
Residue	4	0.925	0.908	0.520	0.507	0.372	0.251
		Zinc[2]					
	DF	WZn	FS	Zn	B+Zn	Cu+Zn	B+Cu+Zn
Regression	3	14.677	17.179	18.419	17.959	14.749	14.442
Residue	4	0.548	1.128	1.031	1.248	0.693	0.680

[1] First crop season.

[2] Second crop season.

Table 2A - Table of variance analysis for the contents of B, Cu and Zn in the index leaf, in the crop season 2010/2011 and 2011/2012

		Boron	
	DF	2010/2011	2011/2012
Treatment	4	155.766**	2331.39**
Blocks	5	14.645	84.388
Residue	20	8.034	50.713
CV (%)		9.10	13.19
		Copper	
	DF	2010	2011
Treatment	4	52.999**	133.90**
Blocks	5	1.606	0.470
Residue	20	31.014	0.826
CV (%)		8.25	6.15

Zinc			
	DF	2010	2011
Treatment	4	16.308 **	51.109 **
Blocks	5	1.795	6.810
Residue	20	0.386	2.504
CV (%)		6.25	16.31

ns - F not significant.
** - F significant at 1% of probability.
* - F significant at 5% of probability.

Table 3A - Table of variance analysis for production per plant and cupping test

Means square					
Boron					
		Production		Cupping test	
	DF	2010/2011	2011/2012	2010/2011	2011/2012
Treatment	5	0.128 ns	3.714 ns	3.493	84.525 ns
Blocks	4	1.033	8.191	10.55	52.878
Residue	20	0.675	1.776	9.91	43.471
CV (%)		22.88	26.66	3.76	8.20
Copper					
		Production		Cupping test	
		2010/2011	2011/2012	2010/2011	2011/2012
Treatment	5	0.245 ns	3.110 *	7.473 ns	57.912 *
Blocks	4	0.651	7.555	12.216	47.154
Residue	20	0.680	1.095	12.756	22.214
CV (%)		22.85	20.38	4.27	5.81
Zinc					
		Production		Cupping test	
		2010/2011	2011/2012	2010/2011	2011/2012
Treatment	5	0.117 ns	1.998 ns	5.733 ns	53.448 ns
Blocks	4	0.599	7.039	8.450	49.372
Residue	20	1.02	1.819	13.150	66.177
CV (%)		28.56	27.03	4.34	10.172

ns - F not significant.
** - F significant at 1% of probability.
* - F significant at 5% of probability.

Table 4A – Table of variance analysis for the coloration index (CI), total titratable acidity (TTA), pH, electrical conductivity (EC), potassium leached (KL), total sugars (TS) and reducing sugars (RS) of beans harvested in the crop season 2010/2011

Means square								
Boron								
		CI	TTA	pH	EC	KL	TS	RS
	DF							
Treatment	5	0.013 ns	1.173 ns	0.008 ns	8.206 ns	0.019 ns	3.501 ns	0.0062 ns
Blocks	4	0.011	8.333	0.028	220.01	0.105	4.926	0.0116
Residue	20	0.011	4.573	0.005	34.573	0.268	2.476	0.0061
CV (%)		12.03	20.05	1.35	14.26	12.67	13.91	34.39
Copper								
		CI	TTA	pH	EC	KL	TS	RS
Treatment	4	0.011 ns	1.413 ns	0.008 ns	17.269 ns	0.032 ns	0.718 ns	0.0029 ns

Blocks	5	0.006	14.20	0.017	115.25	0.090	1.121	0.0019
Residue	20	0.009	7.08	0.001	31.745	0.031	2.745	0.0019
CV (%)		10.76	24.48	0.75	13.80	13.68	14.16	20.22
Zinc								
		CI	TTA	pH	EC	KL	TS	RS
Treatment	4	0.0096 ^{ns}	0.853 ^{ns}	0.0089 ^{ns}	14.586 ^{ns}	0.0306 ^{ns}	4.19 ^{ns}	0.005 ^{ns}
Blocks	5	0.0081	12.133	0.023	193.495	0.137	1.715	0.010
Residue	20	0.0096	3.653	0.006	45.891	0.032	3.871	0.006
CV (%)		11.06	17.27	1.45	16.70	14.13	17.24	35.65

^{ns} - F not significant.
** - F significant at 1% of probability.
* - F significant at 5% of probability.

Table 5A – Table of variance analysis for coloration index (CI), total titratable acidity (TTA), pH, electrical conductivity (EC) and potassium leached (KL) of beans harvested in the crop season 2011/2012

Means square						
Boron						
		CI	TTA	pH	EC	KL
	DF					
Treatment	4	0.143 **	87.400 ^{ns}	0.01 **	70.847 ^{ns}	0.098 ^{ns}
Blocks	5	0.054	187.983	0.0008	139.693	0.061
Residue	20	0.033	204.585	0.0023	59.532	0.086
CV (%)		31.89	31.12	0.86	13.47	14.28
Copper						
		CI	TTA	pH	EC	KL
Treatment	4	0.092 **	179.683 ^{ns}	0.0087 ^{ns}	95.462 ^{ns}	0.030 ^{ns}
Blocks	5	0.027	288.893	0.0022	56.194	0.213
Residue	20	0.022	243.972	0.0051	54.94	0.083
CV (%)		29.25	31.61	1.26	12.79	14.15
Zinc						
		CI	TTA	pH	EC	KL
Treatment	4	0.079 ^{ns}	42.967 ^{ns}	0.0083 ^{ns}	85.938 ^{ns}	0.142 ^{ns}
Blocks	5	0.051	249.424	0.0055	99.421	0.272
Residue	20	0.031	204.503	0.0044	84.046	0.110
CV (%)		31.29	30.15	1.17	15.86	15.91

^{ns} - F not significant.
** - F significant at 1% of probability.
* - F significant at 5% of probability.

Table 6A - Table of variance analysis for caffeine (Caf), trigonelline (Trig), sucrose (Suc), glucose (Glu), arabinose (Ara) and mannose (Man) of beans harvested in the crop season 2011/2012

Means square								
		Caf	Trig	Suc	Glu	Gal	Ara	Man
	DF	**Boron**						
Treatments	4	0.3703	0.02401**	2.922**	0.0146**	0.0028 ns	0.0003**	0.0003*
Blocks	5	0.0044	0.00061	0.128	0.0007	0.0025	0.00008	0.0001
Residue	20	0.0059	0.0026	0.135	0.0014	0.0027	0.00004	0.0001
CV (%)		5.85	5.54	6.04	14.32	42.61	19.23	6.59
Copper								
		Caf	Trig	Suc	Glu	Gal	Ara	Man
Treatments	4	0.3514**	0.0233**	2,218**	0,0089**	0,0012*	0,0004**	0,0005**
Blocks	5	0.0092	0.0016	0,224	0,0008	0,00006	0,00002	0,0001

Residue	20	0.0065	0.0018	0,133	0,0018	0,00038	0,00003	0,00004
CV (%)		6.16	4.63	6,14	16,78	17,06	19,39	4,06
				Zinc				
		Caf	Trig	Suc	Glu	Gal	Ara	Man
Treatments	4	0.3901**	0.0264**	2,9977**	0,01691**	0,0029^{ns}	0,00025**	0,0002**
Blocks	5	0.0039	0.0023	0,0620	0,00085	0,0031	0,00003	0,00007
Residue	20	0.0062	0.0022	0,1535	0,00134	0,0037	0,00001	0,00004
CV (%)		5.95	5.03	6,41	13,35	49,26	12,55	4,14

^{ns} - F not significant.
** - F significant at 1% of probability.
* - F significant at 5% of probability.

Table 7A – Table of variance analysis for 3-Caffeoylquinic acid (3-CQA), 4-caffeoylquinic acid (4-CQA), 5-caffeoylquinic acid (5-CQA), proanthocyanidin (Pro), polyphenol oxidase activity (PPO) and phenolic compounds (TP) of beans harvested in the crop season 2011/2012

				Means square			
				Boron			
		3-CQA	4-CQA	5-CQA	Pro	PPO	TP
	DF						
Treatment	4	0.0336*	0.0184^{ns}	0.0952**	1.891^{ns}	130.375$^{\lozenge}$	3.5832^{ns}
Blocks	5	0.0111	0.0142	0.0105	6.936	19.008	0.0879
Residue	20	0.0098	0.0213	0.0087	5.823	52.268	1.72
CV (%)		19.89	22.27	6.35	38.15	9.01	24.59
				Copper			
		3-CQA	4-CQA	5-CQA	Pro	PPO	TP
Treatment	4	0.0323**	0.0191^{ns}	0.10628**	1.995^{ns}	157.037**	4.024*
Blocks	5	0.0064	0.0061	0.00633	2.920	17.973	1.318
Residue	20	0.0065	0.0095	0.00635	5.940	26.157	1.387
CV (%)		16.10	14.37	5.45	37.40	6.25	22.67
				Zinc			
		3-CQA	4-CQA	5-CQA	Pro	PPO	TP
Treatment	4	0.0335**	0.0187^{ns}	0.1058**	2.427^{ns}	122.986*	1.695*
Blocks	5	0.0116	0.0138	0.0158	0.538	32.019	0.539
Residue	20	0.0065	0.0152	0.0079	4.631	32.112	0.627
CV (%)		16.25	18.28	6.08	31.92	7.11	14.10

^{ns} - F not significant.
** - F significant at 1% of probability.
* - F significant at 5% of probability.
$^{\lozenge}$ - F significant at 10% of probability.

Table 8A - Table of variance analysis for the contents of B, Cu and Zn in the index leaves, efficiency of the photosystem II (Fv/Fm), rate of electron transport (ETR), SPAD, polyphenol oxidase activity (PPO) and dismutase superoxide activity (SOD)

Mean square							
	DF	**Boron**					
		B content	Fv/Fm	ETR	SPAD	PPO	SOD
Regression	2	2461.59	0.568	320.289	2826.04	114.091	16590992.1
Residue	2	6.636	0.0007	0.156	14.620	0.086	93010.7
		Copper					
		Cu content	Fv/Fm	ETR	SPAD	PPO	SOD
Regression	2	695.769	0.883	215.909	2966.21	105.556	12072528.9
Residue	2	0.381	0.0004	0.629	1.863	0.051	178184.4

		Zinc					
		Zn content	Fv/Fm	ETR	SPAD	PPO	SOD
Regression	2	184.162	0.581	290.156	2816.16	161.864	9067174.3
Residue	2	0.782	0.0007	1.586	20.334	0.583	51472.8