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Nota/Note

Copper in *Citrus* production: required but avoided

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SUMMARY

Copper (Cu) deficiency affects young citrus trees mostly when fertilized with high nitrogen (N) rates and under reduced foliar applications of Cu-based fungicides. On the other hand, trees are prone to Cu toxicity resulting from excessive applications of fungicides to control foliar and fruit diseases in the orchards. Despite Cu present in pesticides is generally found in a water-insoluble form, which facilitates the formation of a pathogen protective layer on plant canopy surfaces, frequent use of these products causes an increases in Cu concentrations in soils and consequently root absorption of the metal. Copper deficiency reduces tree yield capacity by damaging many enzyme systems in plants, as well electron transport in the photosynthesis, whereas toxicity causes metabolic dysfunctions, mainly by increasing oxidative stress levels. This article summarizes the role of Cu nutritional disorders on nutrient status and production of citrus trees, as a basis for fine tuning health management of orchards required for superior plant productivity.

Index terms: *Citrus*, copper-based pesticides, plant nutrition, micronutrient.

O cobre na produção dos *Citrus*: necessário, porém prescindido

RESUMO

A deficiência de cobre (Cu) afeta plantas jovens de citros, principalmente quando adubadas com altas doses de nitrogênio (N) e sob reduzidas aplicações foliares de fungicidas cúpricos. Por outro lado, os citros estão sujeitos à toxicidade por Cu devido ao uso excessivo desses fungicidas para o controle de doenças foliares e de frutos nos pomares. Apesar do Cu nos fungicidas estar geralmente presente na forma insolúvel em água, o que facilita a formação de um filme de proteção contra o patógeno na superfície das folhas, o uso frequente desses produtos causa aumento na concentração de Cu no solo e consequentemente as raízes passam a absorver o metal. A deficiência de Cu reduz a produtividade das plantas por afetar a atividade de diversas enzimas, bem como o transporte de elétrons na fotossíntese, enquanto que a toxicidade causa disfunções metabólicas, principalmente pelo aumento dos níveis de estresse oxidativo. Este artigo apresenta os efeitos dos distúrbios nutricionais causados por Cu no estado nutricional e na produção de plantas cítricas, como base para o ajuste do manejo dos pomares de alta produtividade.

Termos de indexação: *Citrus*, fungicidas cúpricos, nutrição de plantas, micronutriente.

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INTRODUCTION

Nutritional disorders caused by copper (Cu) frequently affects citrus orchards, which can be characterized by two scenarios during citrus production: (i) deficiency in non-bearing trees grown in low fertility soils that receive high doses of nitrogen (N) intended to improve plant growth and early fruit yield (Mattos Junior et al., 2010); and (ii) toxicity in non-bearing as well bearing trees resulting from excessive applications of cupric-based pesticides used as preventive control of citrus diseases (Behlau et al., 2016; Silva Junior et al., 2016). The accumulation of Cu in soils resulted from such frequent use of these Cu-based pesticides has become a concern not only in citrus orchards (Discoll, 2004; Fan et al., 2011), but also in others such as grapevines (Martins et al., 2014) and tomatoes grown under protected cultivation (Sonmez et al., 2006).

To extend the understanding about the interaction of Cu on plant nutritional status and phytosanitary control programs, this review describes aspects about either Cu deficiency or toxicity affecting citrus production.

Phytosanitary management with copper-based pesticides

The copper-based pesticides are among the most commonly products used as a preventive control of diseases in citrus and many other crops, along with other horticultural practices (Adrees et al., 2015; Husak, 2015). In citrus, these pesticides are essentials to manage a range of diseases, such as citrus canker (*Xanthomonas axonopodi spv.* citri), citrus black spot (*Guignardia citricarpa* Kiely), scab disease (*Elsinoe fawcettii*), alternaria brown spot [*Alternaria alternate* (Fr.) Kiesler] and post bloom fruit drop (*Colletotrichum acutatum* Simmonds) (Bhatia et al., 2003; Laranjeira et al., 2005; Stein et al., 2007; Hendrix et al., 2013).

The Cu-containing pesticides are sprayed to build up a thin layer on tree canopy surfaces (leaves, woody parts, flowers and/or fruits) that can prevent pathogens infection. Despite Cu is essential to living organisms, when in high concentrations it triggers a large number of metabolic processes in microorganisms that inhibit infection and growth of the pathogen into the plant, resulted from its capacity to bind to sulfhydryl groups of protein compounds (active site), what then causes inactivation of proteins and enzymes and provides degradation of membranes and DNA of the pathogen (Noyce et al., 2006).

There is a wide range of copper-based pesticides available in agriculture systems, in which the most common forms are based on copper hydroxide [$Cu(OH)_2$], copper oxychloride ($ClCu_2H_3O_3$), cupric oxide (CuO), copper sulphate ($CuSO_4$) and copper carbonate [$Cu_2(OH)_2CO_3$] (Leite Junior, 1990; Gottwald & Timmer, 1995; Orbinovic et al., 2007 Husak, 2015). These are usually mixed into water to form suspensions, in which the insoluble Cu associated with adjuvants allows the metal to protect the surface of plant parts that can be infected by pathogens.

Although cupric-based pesticides do not present a curative or a systemic effect in plants, but a very efficient preventive one (Behlau et al., 2008), use of these products has increased based on the need to reduce the incidence of diseases in citrus orchards. In São Paulo (Brazil) and Florida (USA), eradication programs of canker symptomatic trees and those around the affected ones were revoked (Dewdney & Graham, 2014; Behlau et al., 2016). The eradication was considered an important strategy to prevent and control citrus canker. Currently, the removal of only the symptomatic tree is mandatory, but in São Paulo an area with a radius of 30 m has to be sprayed with copper-based products that must be repeated for every new vegetative flush growth of trees (Behlau et al., 2016). However, with the current modification of the referred legislation, the incidence of the disease in the São Paulo state's orchards is raising, varying from 0.14% of plots contaminated in 2009 to 0.44% in 2010, 0.99% in 2011 and 1.39% in 2012 (Behlau et al., 2016). Recently, the incidence of the disease was estimated in 15% of the plots located in São Paulo and south of Minas Gerais states (FUNDECITRUS, 2016).

The increment in disease incidence during the last years led the requirement to reformulate criteria and procedures for establishing and maintaining phytosanitary status for the citrus canker in Brazil. The Normative Instruction nº 37 was created in September 2016, which regulates procedures to reduce potential of inoculum, fruit transit permissions in Brazil and exportation abroad (MAPA, 2016). However, some characteristic of the Brazilian citriculture, such as large contiguous citrus grown areas, susceptible varieties, favorable climate for disease dispersion, as well as occurrence of other diseases, e.g. citrus black spot, has likely contributed to the continued increase in the use of Cu-based pesticides. Studies have shown the importance in reducing the quantity of Cu-based products used (Behlau et al., 2010; Scapin et al., 2015; Silva Junior et al., 2016), not only because of the high cost of treatment applications, but also about avoiding the induction of pathogen resistance (Behlau et al., 2013). Furthermore, by reducing Cu applications in the groves, we may expect to reduce adverse plant damages (Hippler et al., 2016) and environmental impacts caused by excess Cu in the environment (Fan et al., 2011, 2014).

Copper levels in soil and leaves of citrus orchards

An analysis of Cu concentrations in soil (0-20 cm soil depth; total of 4,867) and leaves samples (total of 5,604) collected from bearing citrus orchards in the Brazilian citrus belt (São Paulo State and Triângulo Mineiro) in 2015 revealed high levels of this micronutrient compared to the current interpretation of the results for soil (DTPA extraction, Abreu et al., 1997) and leaf (acid digestion, Bataglia et al., 1983) analyses (Figure 1).

Adequate levels of Cu in leaves occur between 10 to 20 mg kg^{-1}, expressed on the basis of leaf dry matter (Mattos Junior et al., 2012). With exception for the North region, the Brazilian citrus belt regions exhibit an expressive amount of leaf samples with leaf Cu >20 mg kg^{-1} (Figure 1). Furthermore, the Center and the South regions present the greatest frequency of leaf samples with >40 mg kg^{-1} of Cu, with levels reaching up to 600 mg kg^{-1} of this micronutrient. In fact, in bearing trees, the phytosanitary management of the orchards is carried out based on the disease occurrence, where the amount of metal foliar sprayed can reach 30 kg ha^{-1} yr^{-1} of Cu, that later reaches the soil by leaf run off (Fan et al., 2011).

The increment of Cu levels in soils has been to by the greatest levels of the element in leaves (Figure 1). Concentrations of Cu in soils >5.0 mg dm^{-3} of Cu are considered high for citrus production (Mattos Junior et al., 2012). The Center and South regions presented the highest

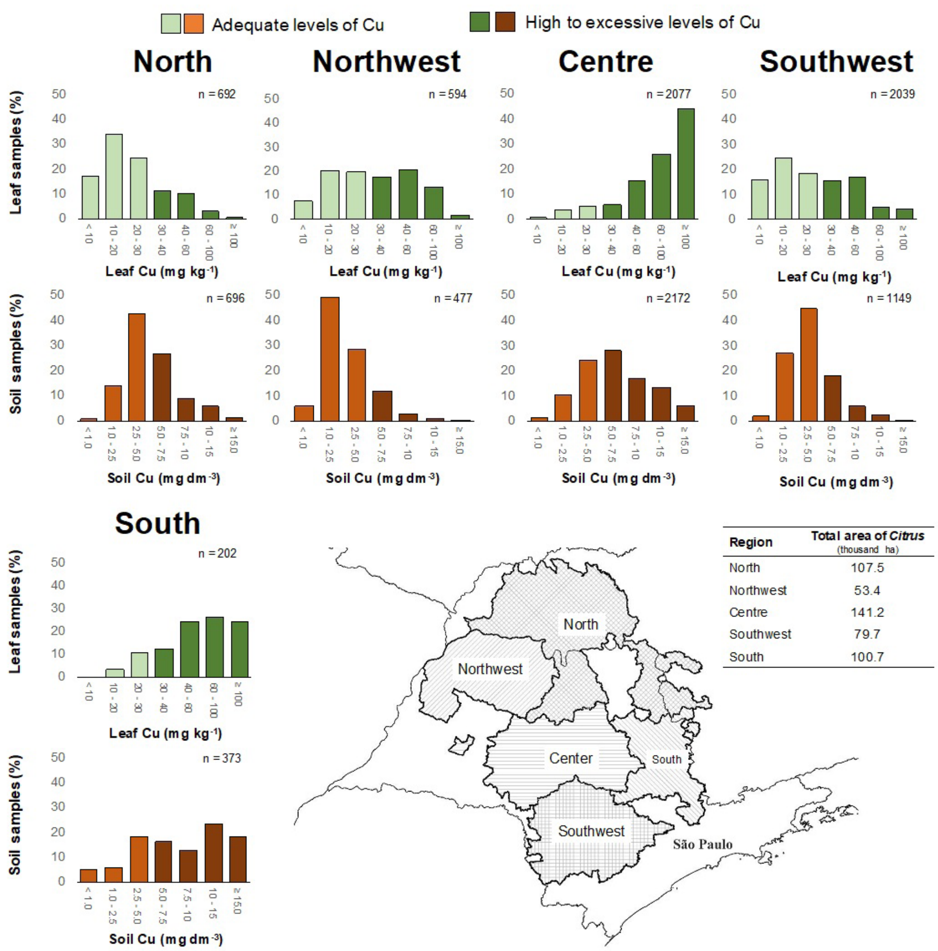

Figure 1. Soil copper (Cu) (0-20 cm depth layer) and leaf concentration surveys (n > 4000 samples) in citrus orchards of the "Brazilian citrus belt" (São Paulo State and Triângulo mineiro). (Map-figure adapted from Fundecitrus, 2016).

frequency of soil samples >10 mg dm^{-3}, with values up to 23 and 34 mg dm^{-3}, respectively for each region.

In Florida's orchards (USA), Cu accumulation in soils was linked to the age of the citrus groves as well to the increase in availability and distribution of the element with depth, which were dependent of pH and soil clay contents (Fan et al., 2011).

Copper deficiency in the plant nutritional status

Copper deficiency has been observed in vigorous plants, mainly in non-bearing trees, such as nursery and recently planted ones that present high demands for the nutrient associated to the supply of high doses of N (Mattos Junior et al., 2005, 2010). Copper availability for plants depends on pH, mineralogy, redox potential and organic matter (OM) of soils (Alva et al., 2000; Mouta et al., 2008). For example, the absorption of Cu by roots is limited in soils with high levels of OM due to the formation of highly stable complexes between this metal and humic acid (Toselli et al., 2009; Kalina et al., 2013). On the other hand, in replanted orchard areas, OM decomposition occurs quickly, due to soil ploughing and tillage what increases the availability of Cu to the new-planted trees (Toselli et al., 2009).

Copper deficiency has been verified by the appearance of visual symptoms (Mattos Junior et al., 2005), which in nursery trees are characterized by the formation of gum pockets in new branches resulted from tissue breaking down and consequent sap leakage from the xylem and phloem vessels (Figure 2A and 2B). Under severe Cu deficiency, die-back of stems is observed in trees, of which the Westin, a vigorous sweet orange variety, exhibits such problem more frequently in the field (Quaggio & Piza Junior, 2001). In non-bearing trees, these symptoms are characterized by the growth of branches that curve in "S" shape, leaf blades excessively developed and protruding veins on the underside (Figures 2C and 2D). These observations suggest that growth limitations occur and consequently potential maximum production of trees is affected. Therefore, information that allows the adequate diagnosis of the nutritional status and management recommendations for this nutrient in the orchards is still lacking.

Such deficiency symptoms are related to the role of Cu as a cofactor of polyphenol oxidase enzyme that is responsible for the lignification of plant tissues (Yruela, 2009). Copper is a nutrient that acts as a cofactor for many other enzymes, such as superoxide dismutase

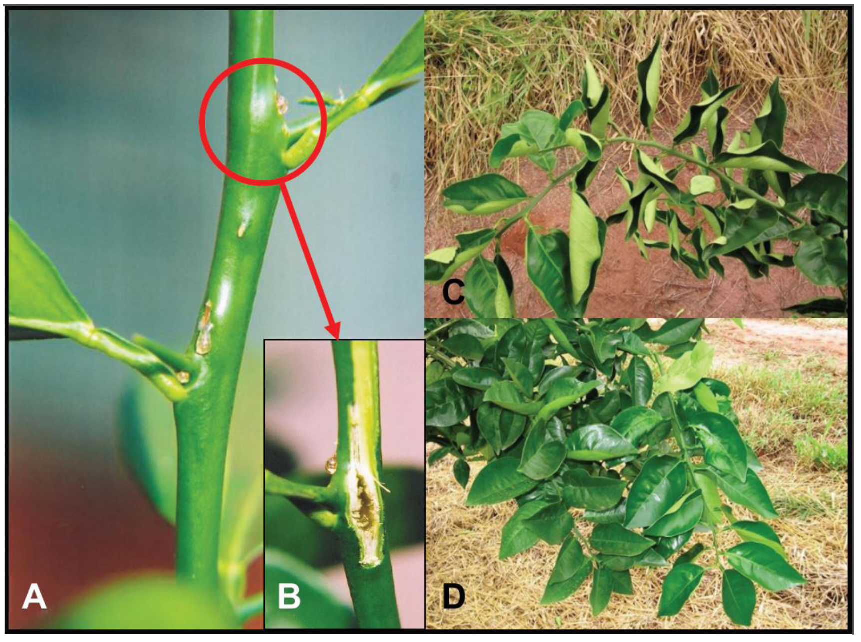

Figure 2. Symptoms of copper deficiency in nurseries trees (A) with formation of gum pockets in new branches (details in B) resulting from tissue breakdown and sap leakage, and in young trees in the field (C and D) showing long and vigorous twigs that grow tortuous. (Photos: adapted from Mattos Jr. et al., 2005).

(Cu/Zn-SOD), cytochrome oxidase, amino oxidase, laccase and plastocyanin, besides having an essential role in transcription and oxidative phosphorylation (Yruela, 2009). Copper deficiency in plants also results in reduced electron transport between the photosystems because of reduced synthesis of plastocyanin, proteins and chlorophylls (Ravet & Pilon, 2013).

The low Cu mobility in the plant phloem (Marschner, 2012) makes the supply of this nutrient necessary each flush of vegetative growth, similarly as observed for manganese (Mn) and zinc (Zn) (Boaretto et al., 2003).

Copper toxicity in the plant nutritional status

Excess Cu disturbs biochemical processes and causes inhibition of growth or abnormal development of plants (Zambrosi et al., 2013; Adrees et al., 2015; Hippler et al., 2016). It also interferes in the biosynthesis of the photosynthetic apparatus modifying pigmentation and membrane composition, causing oxidative stress due to the increased production of toxic oxygen free radicals (Yruela, 2009) and hydroxyl radicals (OH^-), from the Haber-Weiss reaction (Apel & Hirt, 2004). The majority of the deleterious effects of Cu on cellular metabolism are probably due to the inhibitory effects on several enzymes as a result of an irreversible association of Cu^{2+} at their active site (Adrees et al., 2015).

Symptoms of Cu toxicity in *Citrus* and other woody plants are still not clear, although in field conditions symptoms of iron (Fe) deficiency as interveinal chlorosis has been associated to the metal toxicity (Alva et al., 1993; Alva & Chen, 1995). Despite the toxicity of Cu reduces the uptake of Mn and Zn by the roots, it does not limit the absorption of Fe but reduces the transport of this metal from roots to leaves (Hippler et al., 2016). It suggests a specific interaction among these micronutrients, in which the same mechanism that limits Cu transport to the leaves does not discriminate Fe. Copper toxicity in the root medium disturbs also the uptake and accumulation of other nutrients (Mattos Junior et al., 2010; Hippler et al., 2016).

Nutritional and horticultural management to alleviate Cu-stress

In order to minimize the occurrence of Cu deficiency in non-bearing citrus trees, foliar sprays with soluble sources are likely more efficient to supply the micronutrient to plant growth compared to the soil supply. Copper is highly adsorbed to soil colloids, then its availability to plants will depend on solubility of fertilizer sources, clay and OM content of soils (Mouta et al., 2008; Komárek et al., 2009; Hippler et al., 2014, 2015). However, there is still a lack of information about micronutrient application for woody crops, comparing foliar and soil application, as well as, fertilizer sources. Utilization of rootstocks less responsive to Cu deficiency is also recommended in groves where Cu deficiency is more likely to occur (Alva et al., 1993; Hippler et al., 2016), e.g. in soils with low fertility or with reduced applications of cupric-based pesticides.

On the other hand, under Cu toxicity cultural practices are recommended to reduce the metal deleterious effects in orchards, such as: *i*) soil liming increases soil pH and consequently reduces the metal availability in the plant (Alva et al., 2000; Ambrosini et al., 2015); *ii*) increase OM content in the soil, once the metal has a strong interaction with organic compounds, also contributes with the reduction of metal availability to plants (Toselli et al., 2009); *iii*) use of tolerant rootstocks (Alva et al., 1993; Alva & Chen, 1995; Mattos Junior et al., 2010; Zambrosi et al., 2013; Hippler et al., 2016), mainly in groves to be planted in regions with historic of high incidence of citrus diseases, such as citrus canker or citrus black spot.

In fact, for both conditions of Cu-stress (deficiency or toxicity), a more balanced nutritional status is essential to reduce the described metal deleterious effects in plants. Despite symptoms of Cu deficiency are induced more severely with high levels of N fertilization, when plants are prone to toxic levels of this metal, high supply of N can improve levels of organic compounds into the plant, such as phytochelatins, chaperones and other organic compounds that reduce the metal availability and mobility inside the plant (Xiong et al., 2006). Furthermore, the adequate supply of calcium and/or phosphorous improve plant cell integrity reducing either the Cu absorption by roots or the damages caused by oxidative stress effects (Maksymiec & Baszynski, 1999; Zambrosi et al., 2013; Fan et al., 2014).

DIRECTIONS AND PERSPECTIVES

Balanced plant nutritional management along with sound phytosanitary managements strategies based in cupric fungicides use are essentials to achieve highest yields of citrus fruits. However, a better understanding about nutrient statuses of plants, horticultural performance of rootstock varieties and other cultural practices that contribute to alleviate damages caused by Cu nutritional disorders are still required in order to subside maximum yield where this nutrient limits plant growth.

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