Hippler, Franz Walter Rieger; Boaretto, Rodrigo Marcelli; Teixeira, Luiz Antonio Junqueira; Quaggio, José Antonio; Mattos-Jr, Dirceu de

Copper supply and fruit yield of young Citrus trees: fertiliser sources and application methods

Bragantia, vol. 77, no. 2, 2018, pp. 1-7
Instituto Agronômico de Campinas

DOI: 10.1590/1678-4499.2017125

DOI: http://dx.doi.org/10.1590/1678-4499.2017125

Copper supply and fruit yield of young *Citrus* trees: fertiliser sources and application methods

Franz Walter Rieger Hippler[1], Rodrigo Marcelli Boaretto[1]*, Luiz Antonio Junqueira Teixeira[2], José Antonio Quaggio[2], Dirceu de Mattos-Jr[1]

1.Instituto Agronômico - Centro de Citricultura Sylvio Moreira - Cordeirópolis (SP), Brazil.
2.Instituto Agronômico - Centro de Solos e Recursos Agroambientais - Campinas (SP), Brazil.

ABSTRACT: This study aimed to evaluate the fertiliser sources and application methods of copper (Cu) in citrus trees during the first years of fruit production. Two experiments were set up in an orchard with 3-year-old sweet orange trees, which were applied with three sources of Cu (nitrate, sulfate or EDTA) either via fertigation (Experiment 1) or via foliar sprays (Experiment 2). Regardless of the fertiliser source, Cu application via fertigation was not efficacious to increase the micronutrient concentration in leaves and, consequently, did not affect fruit yield. Conversely, foliar application of Cu, either as nitrate or sulfate, increased this nutrient level in leaves but when applied as copper nitrate, visual phytotoxicity symptoms were verified in leaves due to salt accumulation in the plant canopy, which reduced the fruit yield. Considering the plant growth and intensified phytosanitary management of the orchard with the application of copper-based products after the third year of the experiment, the effects of Cu treatments on fruit yield are expected to be negligible as the trees age.

Key words: micronutrient, nutritional status, fertigation, foliar application, citriculture.

*Corresponding author: boaretto@ccsm.br

Received: Apr. 11, 2017 – Accepted: Jul. 17, 2017

The demand for micronutrients by citrus trees increases in high yielding orchards. In this context, gains in fruit yields are possible in production systems that include (i) advanced soil management strategies for orchard establishment; (ii) improved tree planting densities; and adjusted (iii) phytosanitary management; (iv) fertilisation as a function of canopy and rootstock combination, and (v) nutrient doses applied in non-irrigated or irrigated areas (Mattos Jr. et al. 2010; (Mattos Jr. et al. 2017).

Copper (Cu) deficiency in citrus is commonly observed in non-bearing trees, during the first years after orchard establishment due to the increase in plant vigour by applications of high rates of nitrogen-containing fertilisers, as well as by the low application of copper-based pesticides (Mattos Jr. et al. 2010; Hippler et al. 2016; Hippler et al. 2017). Visual symptoms of Cu deficiency are characterised by plant growth with less lignified tissues of new plant parts, enlarged and S-shaped twigs and over-developed leaf blades with protruding veins on the underside (Camp 1938).

Foliar application is the main method used to supply metal micronutrients [Cu, manganese (Mn) and zinc (Zn)] in citrus. Such application is necessary, due to the low mobility of these nutrients in the phloem of every new vegetative flush and, consequently, their poor redistribution through the plant (Boaretto et al. 2003; Hippler et al. 2016; Hippler et al. 2017). Conversely, micronutrient application via fertigation can be split into several doses, which allows deficiencies or nutritional excesses in the orchards to be corrected during the plant growth (Zekri and Koo 1992). Additionally, under tropical soil conditions, the practice of fertigation enhances soil acidification in the wetting bulb, which likely increases the availability of Cu in the soil solution and favours the absorption of the metal by roots (Quaggio et al. 2010). Therefore, knowledge about the most appropriate fertiliser source and the application method are important to increase the efficiency of Cu utilisation and, hence, maximise the development and production of citrus.

This study aimed to evaluate the effects of application methods and Cu fertiliser sources on the nutritional status and fruit yield of young citrus trees. Therefore, two experiments were performed in a commercial orchard of sweet orange trees [*Citrus sinensis* (L.) Osb. 'Pera'] grafted onto Sunki mandarin (*C. reshni* hort. ex Tanaka) and fertigated by a single drip line with 0.6 m spaced drippers and a flow rate of 7.1 $L \cdot h^{-1}$. Irrigation was scheduled every 2 days and the amount of water applied was determined by measuring the evaporation using a Class A pan, the potential evapotranspiration and the crop evapotranspiration obtained, according to Allen et al. (1998). The orchard was planted at 7.0 × 2.9 m, in a total of 493 $trees \cdot ha^{-1}$, located in Colômbia-SP (lat 20°19'19.1"S, long 48°46'44.1"W; altitude of 560 m above sea level), in a sandy loamy soil (19.7% clay, 4.0% silt and 76.3% sand), pH ($CaCl_2$) 5.1, 68.5 $mmol_c \cdot dm^{-3}$ cation exchange capacity (CEC), and containing 23.5 $g \cdot dm^{-3}$ of organic matter and 4.4 $mg \cdot dm^{-3}$ Cu (DTPA-TEA pH 7.3) (Abreu et al. 1998). Adsorption curves for Cu in the soil were performed, according to Hippler et al. (2014), based on the Langmuir adsorption isotherm (Bradl 2004). The soil exhibited a maximum 703.1 $mg \cdot kg^{-1}$ adsorption capacity (b_L) and a binding energy (K_L) of 0.037 $L \cdot kg^{-1}$. The local climate is classified as Aw according to Köppen, with hot and humid summer and dry winter with average annual temperature of approximately 23 °C.

The experimental treatments consisted of three fertiliser sources containing Cu [$Cu(NO_3)_2$, $CuSO_4 \cdot 5H_2O$ or Cu-EDTA ($C_{10}H_{14}CuN_2O_8$)], which were applied either via fertigation (Experiment 1) or foliar sprays (Experiment 2). A Control treatment, common to both experiments, consisted of zero Cu application. Two years after beginning the study, phytosanitary management of the citrus orchard with copper-based pesticide sprays (Behlau et al. 2017) was conducted in all the experimental area. The experiments were established in a randomised block design, with four treatments and five blocks, with one replicate per treatment in each block. Individual plots comprised a line of 16 trees, with the 10 central trees considered for evaluation of treatment effects. Both experiments were done in the same area and the Cu treatment applications started in the third year after the orchard establishment. The experimental evaluations were performed in the following three years.

Copper applications in all treatments (except the Control trees) totalled 5 $kg \cdot ha^{-1} \cdot yr^{-1}$ of Cu. In Experiment 1, the Cu sources were applied via fertigation in a total of 20 applications per year (0.4 $kg \cdot ha^{-1}$ of Cu per application), between August (late Fall) and April of the subsequent year (Summer). In Experiment 2, the applications were performed in the same period as the first experiment but with 4 – 5 foliar sprays per year (1 – 1.25 $kg \cdot ha^{-1}$ of Cu per application), according to Quaggio et al. (2010). Orchard fertilisation, with the exception of Cu, followed the recommendations of Quaggio et al. (2010).

For both experiments, soil and leaves were sampled every year by the end of the Summer (March–April). In the 3 years of experimental evaluations, soil samples were collected from the 0 – 20 cm soil depth layer. Additionally, in the first and third years, such samples were also collected from the 20 – 40 cm soil depth layer. We used DTPA-TEA (pH 7.3) as the nutrient extractor (Abreu et al. 1998) to determine the Cu availability in the soil. Leaf samples were collected, as described by Mattos Jr. et al. (2017) and Cu concentration in the dry mass was determined according to Bataglia et al. (1983) by plasma emission spectrometry (ICP-OES, Perkin-Elmer 5100 PC, Norwalk, CT, USA).

Fruit yield (kg/tree) was evaluated in all 3 years, by harvesting fruits of the 10 central plants of the experimental plots. In the first and third years, fruit quality was assessed by sampling five oranges per tree, in a total of 50 fruits·plot^{-1}, to determine fresh weight, height and width, juice acidity, soluble solids (SS) (°Brix), SS/acidity ratio and SS yield/40.8 kg box, according to Redd et al. (1992).

In Experiment 1, Cu concentration in the soil was higher when applied via fertigation as a nitrate source in the first and the third years at the 0 – 20 cm depth (Figure 1), as well as in the third year at 0 – 40 cm depth layer (5.7 mg·dm^{-3}) compared to the other treatments (4.1 mg·dm^{-3}; $p < 0.05$; data not shown). Regardless of the fertiliser source, the application of Cu via fertigation did not increase the micronutrient level in leaves (Figure 1). Similarly, application of Cu-EDTA via fertigation in a sandy soil (pH 6.0) in Florida was not effective to increase the micronutrient levels in citrus leaves but was efficient for iron, Mn and Zn (Zekri and Koo 1992).

In this study, the absorption of Cu by roots was not likely limited by interactions between the micronutrient with the soil colloids due to the low K_L value (0.037 L·kg^{-1}) estimated by the Langmuir isotherm (Mouta et al. 2008; Hippler et al. 2014), as well as the low content of organic matter in the soil (23 g·dm^{-3}). Furthermore, despite the high b_L value (703.2 mg·kg^{-1}) for Cu in the soil of the present study, the accumulation of this micronutrient due to frequent additions causes adsorption sites to become more saturated, which reduces the affinity of the soil for the metal, in turn, increasing its concentration in solution and, consequently, its availability to the plants (Mouta et al. 2008). However, when Cu is taken up by roots, nutrient partitioning occurs mainly into the root tissue, which accounts for 60 – 80% of the total micronutrient content in the plant (Hippler et al. 2016). A similar pattern is observed for other micronutrients, such as Zn (Hippler et al. 2015) but not boron (Boaretto et al. 2003).

In Experiment 2, the Cu concentration increased up to 7.0 mg·dm^{-3} in the soil (0 – 20 cm depth layer) when applied as sulfate, via foliar sprays, in the second and third years of evaluation (Figure 2). However, no differences were verified in the Cu concentrations in soil at the 20 – 40 cm depth layer (3.0 – 4.0 mg·dm^{-3} Cu) in the first and third years (data not shown).

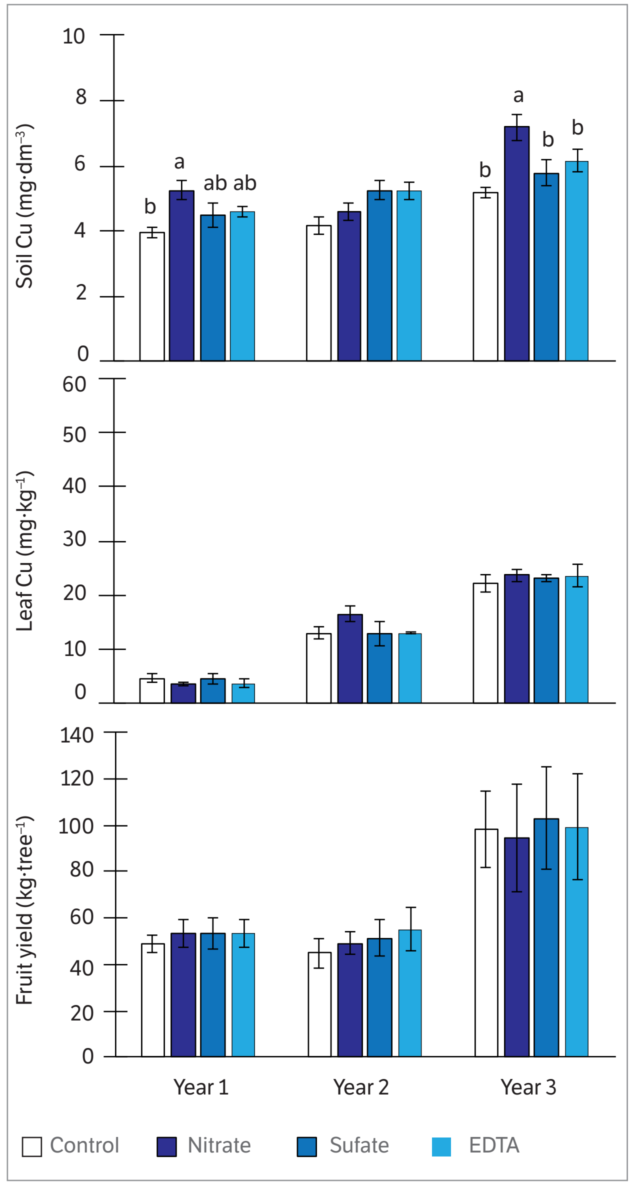

Figure 1. Concentration of copper (Cu) in the soil (0 – 20 cm layer) and in leaves, and fruit yield of sweet orange trees after application of different fertiliser sources of Cu via fertigation. Wiskers on the bars show the standard error of means. For the same year, different letters indicate significant differences between treatments according to Tukey's test ($\alpha = 0.05$).

Although Cu is not tightly bound to soil, this metal showed no significant concentration change in the 20 – 40 cm layer, as also observed for boron (Mattos Jr. et al. 2017). Furthermore, the Cu levels in leaves increased after application of Cu-nitrate in the first and second years (Figure 2). Although Cu-nitrate and -sulfate fertiliser sources are highly water-soluble, the accompanying ion influences the absorption of the metal by the leaf surface. For instance, when applied as the nitrate source, Cu absorption by leaves was higher compared to the sulfate source, as also verified for Zn and Mn (Boaretto et al. 2003). Moreover, when applied as the sulfate source, Cu might be easily washed out from the canopy by rainfall events and reach the soil surface (Fan et al. 2011), as evident from the increased Cu levels in the soil (0 – 20 cm depth layer; Figure 2). Application of Cu-EDTA increased the micronutrient concentration in the citrus leaves but did not affect soil concentration compared to the Control treatment (Figure 2).

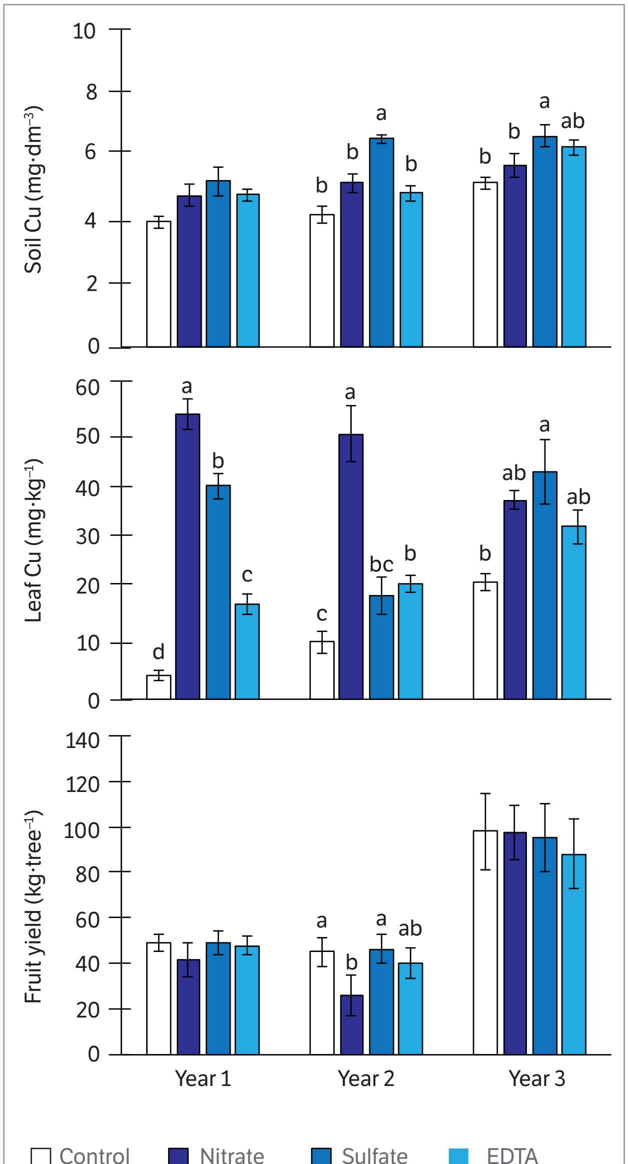

Figure 2. Concentration of copper (Cu) in the soil (0 – 20 cm layer) and in leaves, and fruit yield of sweet orange trees after application of different fertiliser sources of Cu via leaf sprays. Wiskers on the bars show the standard error of means. For the same year, different letters indicate significant differences between treatments according to Tukey's test ($\alpha = 0.05$).

The foliar application of Cu as nitrate source, provided the highest micronutrient content in the leaves in the first and the second year of evaluations (Figure 2), causing plant phytotoxicity that was verified by visual symptoms of leaf injury (Figure 3). These symptoms were characterised by the presence of chlorotic leaves that were necrotic at the border as a result of salt accumulation (Figures 3a,b), which caused severe leaf fall (Figures 3c,d). The deleterious effect of salt accumulation and consequent leaf drop-off from trees that received the Cu-nitrate as a foliar application, reduced fruit yield by approximately 40% compared to the other treatments in the second year of evaluation (Figure 2). However, the toxicity symptoms were not observed in the plant growth of the following year.

Copper levels increased in soil (0 – 20 cm) and leaves in both experiments in the third year, compared to the second year, when the phytosanitary management with copper-based pesticides application started ($p < 0.05$; Figures 1 and 2). Frequent spraying of copper-based pesticides in citrus orchards is required to control leaf and floral diseases, such as post-bloom fruit drop, *Alternaria* brown spot, black spot and citrus canker (Silva Jr. et al. 2016; Behlau et al. 2017). However, copper-based pesticides deliver Cu in an insoluble form, such as copper hydroxide or oxychloride (Behlau et al. 2017), which may hinder the metal uptake by plants (Boaretto et al. 2003; Favaro et al. 2017).

Copper concentration in fruits was quantified in the first year, but no differences were observed in Experiment 1, with Cu sources applied via fertigation (data not shown). In contrast, in Experiment 2, the Cu levels in fruits increased from 1.6 mg·kg^{-1} in Control plants to 3.2 and 3.0 mg·kg^{-1} when foliar was sprayed as sulfate and EDTA, respectively, and to 6.1 mg·kg^{-1} when applied as nitrate ($p < 0.05$; data not shown). Furthermore, the application of Cu reduced the SS/acidity ratio (Ratio in Table 1) of fruits in the first year (Table 1), regardless of the fertiliser source or the application method (fertigation or foliar), which is likely explained by the delay of fruit maturation compared to the Control trees (Quaggio et al. 2006). The foliar application of Cu, as nitrate and sulfate, in comparison to the Control plants, reduced the fruit weight in the third year but increased the

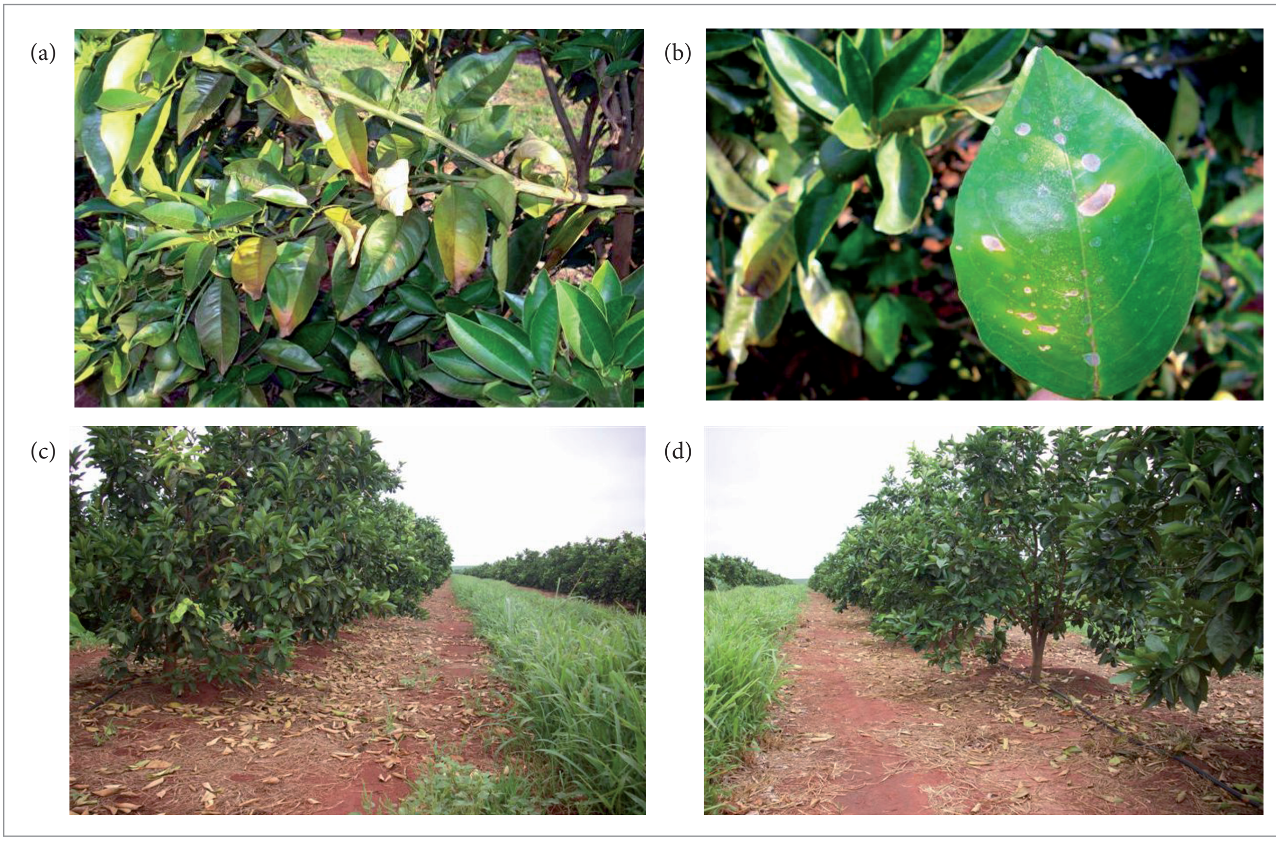

Figure 3. Visual symptoms of toxicity after application of copper nitrate in the second year with leaf burn (a) and necrotic symptoms (b), resulting in falling leaves (c) and (d).

Table 1. Fruit quality of sweet oranges at the first and third year after start the application of fertiliser sources of copper (Cu) via fertigation of foliar spray.

Application methods	Cu Fertilisers	Mass ($g \cdot fruit^{-1}$)	Height (cm)	Width (cm)	Juice (%)	Acidity ($g\ 100 \cdot mL^{-1}$)	SS[1] (°Brix)	Ratio	SS/box[2] (kg)
					Year 1				
Control	Nil Cu	233	8.5	7.5	50.5	0.58	9.0	15.6 aA[3]	1.8
Fertigation	Nitrate	230	8.6	7.4	52.6	0.63	8.6	14.1 b	1.9
	Sulfate	225	8.4	7.4	49.6	0.61	8.5	14.0 b	1.7
	EDTA	222	8.3	7.3	51.9	0.63	8.8	13.9 b	1.8
Foliar application	Nitrate	223	8.5	7.4	47.1	0.64	8.9	13.9 B	1.7
	Sulfate	221	8.4	7.4	53.1	0.63	9.0	14.3 B	1.9
	EDTA	230	8.4	7.5	51.9	0.63	8.6	13.6 B	1.8
					Year 3				
Control	Nil Cu	216 A	7.7	7.4	55.1	0.46	7.7	16.9	1.72 B
Fertigation	Nitrate	220.9	7.9	7.5	52.2	0.45	8.0	17.8	1.70
	Sulfate	213.1	7.8	7.4	56.6	0.48	7.5	15.7	1.73
	EDTA	214.9	7.7	7.4	56.1	0.48	8.0	16.7	1.84
Foliar application	Nitrate	208.9 B	7.7	7.4	55.3	0.47	8.2	17.4	1.90 A
	Sulfate	209.2 B	7.6	7.4	58.0	0.48	7.8	16.3	1.84 A
	EDTA	213.6 AB	7.7	7.3	56.6	0.46	7.8	17.0	1.81 AB

[1]SS = Soluble Solids; [2]SS/box – yield of soluble solids per box (40.8 kg); [3]Different lowercase letters in the same year are significantly different among the Cu sources applied via fertigation and different uppercase letter in the same year are significantly different among the Cu sources applied via foliar (Tukey's test, $p < 0.05$).

SS/box (Table 1), in agreement with the observations of Mattos Jr. et al. (2017), whereby, smaller fruits tended to exhibit a higher SS concentration than their larger counterparts.

Copper supply via fertigation is not effective to increase the levels of Cu in the leaves of young citrus trees. Foliar application of nitrate and sulfate sources of Cu are more efficacious to increase the foliar levels of the nutrient than Cu-EDTA. However, young trees with foliar application of soluble sources are prone to exhibit symptoms of salt toxicity, which results in fruit yield losses. After initiating the phytosanitary management with frequent application of copper-based pesticides, there is no need to apply fertiliser sources containing Cu.

ORCID IDS

F. W. R. Hippler
https://orcid.org/0000-0002-7545-001X
R. M. Boaretto
https://orcid.org/0000-0001-9959-7924
L. A. J. Teixeira
https://orcid.org/0000-0003-4890-7843
J. A. Quaggio
https://orcid.org/0000-0001-7432-9456
D. Mattos-Jr
http://orcid.org/0000-0002-6149-9189

REFERENCES

Abreu, C.A., Abreu, M.F., Andrade, J.C. and Raij, B. van. (1998). Restrictions in the use of correlation coefficients in comparing methods for the determination of the micronutrients in soils. Communications in Soil Science and Plant Analysis 29, 1961-1972. https://doi.org/10.1080/00103629809370085.

Allen, R. G., Pereira, L. S., Raes, D. and Smith, M. (1998). Crop evapotranspiration: guidelines for computing crop water requirements. Irrigation and Drainage Paper 56. Food and Agriculture Organization of the United Nations.

Bataglia, O.C., Furlani, A.M.C., Teixeira, J.P.F., Furlani, P.R. and Gallo, J.R. (1983). Métodos de análise química de plantas. Campinas: IAC. Boletim Técnico 78.

Behlau, F., Scandelai, L.H.M., Silva Jr., G.J. and Lanza, F.E. (2017). Soluble and insoluble copper formulations and metallic copper rate of control canker on sweet orange trees. Crop Protection, 94, 185-191. https://doi.org/10.1016/j.cropro.2017.01.003.

Boaretto, A.E., Muraoka, T. and Boaretto, R.M. (2003). Absorção e translocação de micronutrientes, aplicados via foliar, pelos citros. Laranja, 24, 177-197.

Boaretto, R.M., Quaggio, J.A., Mattos Jr., D., Muraoka, T. and Boaretto, A.E. (2011). Boron uptake and distribution in field grown citrus trees. Journal of Plant Nutrition, 34, 839-849. https://doi.org/10.1080/01904167.2011.544353.

Bradl, H. B. (2004). Adsorption of heavy metal ions on soils and soils constituents. Journal of Colloid and Interface Science, 277, 1-18. https://doi.org/10.1016/j.jcis.2004.04.005.

Camp, A.F. (1938). Symptomatology of deficiencies and toxicities of citrus. Proceedings of the Florida State of Horticultural Society, 51, 140-145.

Fan, J., He, Z., Ma, L.Q. and Stoffella, P.J. (2011). Accumulation and availability of copper in citrus grove soils as affected by fungicide application. Journal of Soils and Sediments, 11, 639-648. https://doi.org/10.1007/s11368-011-0349-0.

Favaro, M.A., Roeschlin, R.A., Ribeiro, G.G., Maumary, R.L., Fernandez, L.N, Lutz, A., Sillon, M., Rista, L.M., Marano, M.R. and Gariglio, N.F. (2017). Relationship between copper content in orange leaves, bacterial biofilm formation and citrus canker disease control after different copper treatments. Crop Protection, 92, 182-189. https://doi.org/10.1016/j.cropro.2016.11.011.

Hippler, F.W.R., Reis, I.M.S., Boaretto, R.M., Quaggio, J.A. and Mattos Jr., D. (2014). Características adsortivas de solos e o suprimento de zinco e manganês para os citros. Citrus Research & Technology, 35, 73-83.

Hippler, F.W.R., Boaretto, R.M., Quaggio, J.A., Boaretto, A.E., Abreu Jr., C.H. and Mattos Jr., D. (2015). Uptake and distribution of soil applied zinc by *Citrus* trees - addressing fertilisers use efficiency with ^{68}Zn labeling. Plos One. https://doi.org/10.1371/journal.pone.0116903.

Hippler, F.W.R., Cipriano, D.O., Boaretto, R.M., Quaggio, J.A., Gaziola, S.A., Azevedo, R.A. and Mattos Jr., D. (2016). *Citrus* rootstocks regulate the enzymatic and nutritional status and antioxidant system of trees under copper stress. Environmental and Experimental Botany, 130, 42-52. https://doi.org/10.1016/j.envexpbot.2016.05.007.

Hippler, F.W.R., Boaretto, R.M., Quaggio, J.A. and Mattos-Jr., D. (2017). Copper in *Citrus* production: required but avoided. Citrus Research & Technology, 38, 99-106. http://dx.doi.org/10.4322/crt.ICC067.

Mattos Jr., D., Ramos, U.M., Quaggio, J.A. and Furlani, P.R. (2010). Nitrogênio e cobre na produção de mudas de citros em diferentes porta-enxertos. Bragantia, 69, 135-147. http://dx.doi.org/10.1590/S0006-87052010000100018.

Mattos Jr., D., Hippler, F.W.R., Boaretto, R.M., Stuchi, E.S. and Quaggio, J.A. (2017). Soil boron fertilization: the role of nutrient sources and rootstocks in *Citrus* production. Journal of Integrative Agriculture, 16, 1609-1616. https://doi.org/10.1016/S2095-3119(16)61492-2.

Mouta, E.R., Soares, M.R. and Casagrande, J.C. (2008). Copper adsorption as a function of solution parameters of variable charge soils. Journal of the Brazilian Chemical Society, 19, 996-1009. http://dx.doi.org/10.1590/S0103-50532008000500027.

Quaggio, J.A., Mattos Jr., D. and Boaretto, R.M. (2010). Citros. In L. I. Prochnow, W. Casarin, S. R. Stipp (Eds.), Boas práticas para o uso eficiente de fertilizantes. (v.3, p.371-409). Piracicaba: International Plant Nutrition Institute.

Quaggio, J.A., Mattos Jr., D. and Cantarella, H. (2006). Fruit yield and quality of sweet oranges affected by nitrogen, phosphorus and potassium fertilization in tropical soils. Fruits, 61, 293-302. http://dx.doi.org/10.1590/S0103-90162011000300015.

Redd, J.B., Hendrix, D.L. and Hendrix Jr., C.M. (1992). Quality control manual for citrus processing plants. v.1. Safety Harbour: AGScience.

Silva Jr., G.J., Scapin, M.S., Silva, F.P., Silva, A.R.P., Behlau, F. and Ramos, H.H. (2016). Spray volume and fungicide rates for citrus black spot control based on tree canopy volume. Crop Protection, 85, 38-45. https://doi.org/10.1016/j.cropro.2016.03.014.

Zekri, M. and Koo, R.C.J (1992). Application of micronutrients to citrus trees through microirrigation systems. Journal of Plant Nutrition, 15, 2517-2529. https://doi.org/10.1080/01904169209364491.