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Phosphorus availability and rootstock affect copper-induced damage to the root ultra-structure of *Citrus*

Fernando César Bachiega Zambrosi[a,*], Geisa Lima Mesquita[b], Francisco André Ossamu Tanaka[c], José Antônio Quaggio[a], Dirceu Mattos Jr.[b]

[a] Centro de Solos e Recursos Ambientais, Instituto Agronômico, C.P. 28, 13012-970 Campinas, SP, Brazil
[b] Centro de Citricultura Sylvio Moreira, Instituto Agronômico, C.P. 04, 13490-970 Cordeirópolis, SP, Brazil
[c] Universidade de São Paulo, C.P. 09, 13418-900 Piracicaba, SP, Brazil

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ABSTRACT

The control of several citrus diseases requires continuous applications of fungicides containing copper (Cu) which favor to the accumulation of this metal in the soil. Therefore, the evaluation of how nutrient availability and rootstock interact with Cu toxicity in the citrus trees is required to maintain sustainability of fruit production in Cu-contaminated soils. Valencia orange trees on Sunki mandarin (SM) or Swingle citrumelo (SC) rootstock were grown in nutrient solutions combining adequate Cu (1.0 μmol L^{-1}), excess Cu (50.0 μmol L^{-1}), deficient phosphorus (P) (0.01 mmol L^{-1}) and sufficient P (0.5 mmol L^{-1}). The excess Cu reduced root and shoot growth, chlorophyll and relative water content in the leaves of the trees compared to those under adequate Cu supply. Furthermore, excess Cu caused severe damage to the root ultra-structure, characterized by the degeneration of the middle lamella and the presence of a thin and sinuous cell wall, as well as, starch accumulation in the plastids, disruption of the mitochondrial membranes and cellular plasmolysis. The damage caused by excess Cu in the cell wall and middle lamella on the root cells of SC was less severe than SM. Sufficient P supply improved the structure of the cell wall and middle lamella of trees subjected to excess Cu in comparison to P-deficient ones. Thus, the occurrence of more preserved cell wall and middle lamella supports the idea that sufficient P availability in the rooting medium and the use of SC rootstock might contribute to increase the ability of young citrus trees to cope with Cu toxicity.

1. Introduction

The excessive increase in the amount of copper (Cu) in agricultural soils has been associated with the continuous use of fungicides containing this metal (Mirlean et al., 2007; Nóvoa-Munñoz et al., 2007). In the case of citrus groves, successive applications of Cu fungicides occur for the control of foliar and fruit diseases, such as citrus scab (*Elsinoë fawcettii* and *Elsinoë australis*) and citrus canker (*Xanthomonas citri subsp. citri*) (Bettiol et al., 1994; Meneguim et al., 2007). Consequently, the accumulation, mobility, and availability of Cu in the soil have remained in proportion to the age of the citrus groves because annual doses of up to 30 kg ha^{-1} of Cu can be used (Fan et al., 2011). In this scenario, the accumulation of Cu in the soil as a result of the continuous use of fungicides becomes a constraint for citrus production due to the elevated phytotoxic potential of this element (Pätsikkä et al., 1998; Kukkola et al., 2000) and to the slow conversion of active forms of Cu into inactive forms in the soil (Pietrzak and Mcphail, 2004).

Copper toxicity in plants causes oxidative stress and compromises physiological and biochemical processes (Yruela, 2005), resulting in the inhibition of shoot and root growth (Kopittke and Menzies, 2006). An excess of Cu also leads to serious damage to the ultra-structure of the roots (Liu and Kottke, 2004; Panou-Filotheou and Bosabalidis, 2004). Since in Cu-contaminated soils the Cu toxicity primarily occurs in the roots because of the accumulation of the metal in the soil and the direct contact between Cu ions and the root surface, the sustainability of citrus production in these locals relies on strategies that can minimize Cu damage to the ultra-structure and growth of the roots. For instance, considering that P deficiency has a demonstrated influence on the intensity of abiotic stress in plants, including salinity (Qadar, 1998) and drought stress (Garg et al., 2004), appropriate management of P nutrition might be also important to minimize Cu toxicity in citrus. This notion is supported by the fact that reduced leaf area, photosynthesis, and root growth (Zambrosi et al., 2011, 2012a,b) and enhanced oxidative stress (Tewari et al., 2007) have been associated with P deficiency, which, in turn, might contribute to making plants more susceptible

* Corresponding author. Tel.: +55 19 21370752; fax: +55 19 32369119.
E-mail address: zambrosi@iac.sp.gov.br (F.C.B. Zambrosi).

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Table 1
Plant growth, leaf and root tissue concentrations of copper (Cu) and phosphorus (P), leaf relative water content (RWC) and SPAD readings of young citrus trees according to the supply of P and Cu in the nutrient solution.

P supply	Cu supply			*F* test[a]		
	Adequate Cu	Excess Cu	Average	P	Cu	P*Cu
RDW (g tree^{-1})						
Deficient P	17.4 bA	14.1 aA	15.8			
Sufficient P	22.5 aA	13.4 aB	18.0	*	*	*
Average	20.0	13.8				
NSDW (g tree^{-1})						
Deficient P	24.0 bA	4.6 aB	14.3			
Sufficient P	28.0 aA	3.9 aB	16.0	*	*	*
Average	26.0	4.3				
TSDW (g tree^{-1})						
Deficient P	42.6 bA	20.6 aB	31.6			
Sufficient P	50.8 aA	19.2 aB	35.0	*	*	*
Average	46.7	19.9				
Leaf Cu (mg kg^{-1})						
Deficient P	4.8	8.5	6.7			
Sufficient P	4.1	8.5	6.3	NS	*	NS
Average	4.5 B	8.5 A				
Root Cu (mg kg^{-1})						
Deficient P	29.1 aB	557.9 aA	293.5			
Sufficient P	18.1 aB	415.9 bA	217.0	*	*	*
Average	23.6	486.9				
Leaf P (g kg^{-1})						
Deficient P	1.4 bB	1.9 aA	1.7			
Sufficient P	1.9 aA	1.7 aA	1.8	*	*	*
Average	1.7	1.8				
Root P (g kg^{-1})						
Deficient P	1.3 bA	1.3 aA	1.3			
Sufficient P	3.2 aA	1.5 aB	2.4	*	*	*
Average	2.3	1.4				
RWC (%)						
Deficient P	88.6	82.5	85.6			
Sufficient P	91.4	81.9	86.7	NS	*	NS
Average	90.0 A	82.2 B				
SPAD readings						
Deficient P	80.1	52.7	66.4			
Sufficient P	78.9	53.8	66.4	NS	*	NS
Average	79.5 A	53.3 B				

P supply comparison: means (n = 3 or 6) followed by different lowercase letters within columns are significantly different by the *F* test ($p < 0.05$). Cu supply comparison: means (n = 3 or 6) followed by different uppercase letters across paired columns are significantly different by the *F* test ($p < 0.05$). Deficient P: 0.01 mmol P L^{-1}; sufficient P: 0.5 mmol P L^{-1}; adequate Cu: 1.0 μmol Cu L^{-1}; excess Cu: 50.0 μmol Cu L^{-1}. RDW: root dry weight; NSDW: new shoots DW; TSDW: total shoots DW.

[a] *F* test in the ANOVA of P supply (P) *vs* Cu supply (Cu) for each parameter evaluated. *$p < 0.05$ and NS = not significant ($p > 0.05$).

to abiotic stresses. As a result, there is a practical need to evaluate how the variation of P availability in the rooting medium affects the pattern of Cu toxicity in citrus trees since P deficiency is a common constraint to citrus production (Quaggio et al., 1998).

The selection of rootstock is also an important approach for the management of citrus groves based on the fact that rootstocks differ in regard to the adaptation to nutritional stresses (Garcia-Sancez et al., 2002; Pestana et al., 2005; Zambrosi et al., 2012b). Although, the deleterious effects of excessive Cu on growth and on nutrient uptake by citrus were previously reported (Alva and Chen, 1995; Alva et al., 1999; Mattos Jr. et al., 2010), we still find a lack of detailed information about the mechanism by which the rootstock influences Cu toxicity in the scion. Indeed, the characterization of such responses could help to better define the recommended rootstocks for cultivation in Cu-contaminated soils and more adequate management practices for citrus trees requiring the continuous application of Cu fungicides (*i.e.* groves under a high disease pressure).

Taking into account that the maintenance of improved cell ultra-structure is important for the tolerance of the plants to metals toxicity (Doncheva et al., 2009; Gzyl et al., 2009; Ali et al., 2013), our research was based on the hypothesis that more conserved root cell ultra-structure of citrus trees could be obtained with sufficient P supply in the rooting medium and with the use of Cu-tolerant rootstocks. Thus, the examination of the ultra-structure would contribute to improve the understanding about the effects of the excess Cu supply on the roots and also to define some practical strategies (*i.e.* nutrients management and rootstock selection) to increase the adaptation of citrus on Cu-contaminated sites. Accordingly, we aimed to investigate the interactions of P supply *vs* rootstock *vs* Cu supply in the rooting medium on the growth, water and nutritional status, and root ultra-structure of young citrus trees.

2. Materials and methods

The citrus trees were grown for 140 days, from August 2011 to December 2011, in an unshaded greenhouse with average day/night temperatures of 35 °C/23 °C. Uniform, 1 year-old nursery trees of Valencia sweet orange [*Citrus sinensis* (L.) Osbeck] on either Sunki mandarin (*Citrus reshni* hort. ex Tanaka, SM) or Swingle citrumelo [*C. paradisi* Macf. × *Poncirus trifoliata* (L.) Raf., SC] rootstock were obtained from a commercial nursery, bare-rooted and supported in non-draining pots containing 8 L of ¼ strength nutrient solution (NS) (Zambrosi et al., 2011) without P and Cu for plant establishment. Five days after transplanting, this ¼ strength NS was replaced with full-strength NS [in mmol L^{-1}, 9.6 N (11% as NH_4^+), 3.0 K, 4.5 Ca, 1.2 Mg, 1.2 S and, in μmol L^{-1}, 41.6 B, 54.0 Fe, 8.2 Mn, 2.5 Zn and 1.0 Mo] with variable Cu and P concentrations (see below). Each pot with NS contained one citrus tree and was equipped with a tube extending to the bottom through which air was continuously bubbled for the NS aeration. The solution pH during the experimental period was monitored and maintained close