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Differential response of citrus rootstocks to CuEDTA concentration in sand culture

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Abstract

Florida citrus groves that have been under continuous production for many years often have high levels of soil-fraction copper (Cu) from the use of Cu-containing fertilizers and pesticides. On such groves, citrus trees may develop Cu toxicity, a disorder that impacts both plant growth and nutrition. The objectives of this study were to investigate the growth and nutritional response of six citrus rootstock seedling varieties grown in sand in 3.8 L containers to increasing concentrations of Cu-ethylenediaminetetraacetic acid (CuEDTA). Citrus rootstocks included in the study were: 'Swingle' citrumelo [SC (*Citrus paradisi* × *Poncirus trifoliata*)], 'Volkamer' lemon [VL (*C. volkameriana*)], 'Cleopatra' mandarin [CM (*Citrus reticulata*)], 'Flying Dragon' trifoliate [FD (*P. trifoliata*)], 'US-812' [US812 ('Sunki' mandarin × 'Benecke' trifoliate)], and 'US-897' [US897 (CM × FD)]. Incorporated into a complete nutrient solution, Cu was supplied at 0.05, 0.25, 1.00 and 2.00 mg L^{-1}. Citrus rootstock but not Cu treatment was significant for root and leaf dry mass with FD and VL having the least and greatest total plant dry mass, respectively. Rootstock and Cu treatment was significant for root and leaf Cu. As a mean of Cu treatments, foliar Cu ranged from 4.05 µg g^{-1} (CM) to 7.74 µg g^{-1} (US812); and root Cu ranged from 30.18 µg g^{-1} (FD) to 61.08 µg g^{-1} (VL). Rootstock but not Cu treatment was significant for Ca, K, Mg, P, Fe, Mn and Zn. 'Volkamer' lemon had significantly higher levels of foliar Ca, K, and Mg than the other rootstocks; and along with US812, the highest level of foliar Fe. For all nutrients analyzed except for Mg, accumulation was greater in roots than in leaves. Magnesium, as a mean of rootstocks, accumulated equally in roots and leaves. Subjective visual observations of plants at harvest for nutrient disorder revealed that young terminal-growth leaves of VL and SC in the highest Cu treatment (2.00 mg L^{-1}) showed few to pronounced symptoms of a micronutrient-type disorder, respectively, that correlated with increasing Cu treatment. Based on visual symptoms in the highest Cu treatment (interveinal chlorosis and leaf/leaflet deformation/cupping), plants segregated as follows from greatest to least expression of the observed micronutrient-like disorder: SC > CM/FD > US812/US897 > VL.

Key words: Orange tree, grove, orchard, nutrient toxicity, nutrient deficiency, chelates

Introduction

Over many decades of citrus (*Citrus* sp.) production in Florida, USA, copper (Cu), an essential plant nutrient, has gone from a concern of deficiency to a concern of toxicity. Copper deficiency is characterized by large dark-green leaves, sinusoidal ('~' shaped) stem growth, progressing to die back of stems, development of necrotic areas on stems and fruit that exude clear amber-colored gum, and chlorotic new growth (Bradford *et al.*, 1964; Futch and Tucker, 2000; Zekri and Obreza, 2003). Reports of Cu deficiency in citrus date back to the mid eighteenth century (Camp and Fudge, 1939). This prompted growers to apply Cu-containing fertilizers even though Cu was also being introduced to groves (or orchards) as an agent to control plant pathogens. Even today Cu-based products are used as a bactericide to reduce the incidence of citrus canker (*Xanthomonas citri*) (Behlau *et al.*, 2010), and as a fungicide to control citrus scab (*Elsinoe fawcettii*), melanose (*Diaporthe citri*), and greasy spot (*Mycosphaerella citri*) (Stover *et al.*, 2004). Over many years of Cu-containing fertilizer and pesticide applications, Cu in soils of groves under continuous production in Florida rose to such a level that by the mid-1900's Cu deficiency was no longer a serious problem, but instead, Cu toxicity in citrus was being observed (Driscoll, 2004). Copper toxicity is characterized by death of fibrous roots, reduced plant growth (tree decline), and Fe chlorosis (Futch and Tucker, 2000; Reuther *et al.*, 1953). Copper-induced Fe chlorosis is an example of nutrient antagonism or interaction where one nutrient affects the uptake or translocation of another (Alva and Chen, 1995; Mann and Takkar, 1983).

The primary means by which citrus growers deal with excessive soil Cu in existing groves is by managing soil pH (Alva *et al.*, 2000; Fan *et al.*, 2011). Under reset situations in groves with excessive soil Cu, however, growers can also consider selecting rootstocks for Cu tolerance in the same way that rootstocks are selected for other abiotic and biotic factors related to soil physical and chemical properties, certain insect pests and plant pathogens, nutrition, environmental stress, plant size and vigor, fruit production and quality (Castle and Gmitter, 1999). There are numerous rootstocks used in citriculture, including rough lemon (*Citrus limon*), 'Volkamer' lemon (*C. volkameriana*), mandarin orange [*e.g.* 'Cleopatra' mandarin (*C. recticulata*)], sour orange (*C. aurantium*), trifoliate orange [*e.g.* 'Flying Dragon' (*Poncirus trifoliata*)]; 'Swingle' citrumelo [*C. paradisi* (grapefruit) x *P. trifoliata*] and 'Carrizo' citrange [*C. sinensis* (sweet orange) x *P. trifoliata*] (Stover and Castle, 2002; Wutscher, 1979). Several other new hybrid rootstocks released from the U.S. Department of Agriculture - Agriculture Research Service (USDA-ARS) are increasingly being used for commercial plantings in Florida, including 'US-812' [Bowman, 2001; Bowman and Rouse, 2006 ('Sunki' mandarin × 'Benecke' trifoliate)], and 'US-897' [Bowman, 2007 ('Cleopatra' mandarin × 'Flying Dragon' trifoliate)].

Citrus rootstocks have been reported to vary in tolerance to excessive soil Cu. For example, 'Swingle' citrumelo is said to be among the least tolerant citrus rootstocks to elevated levels of soil Cu (Castle *et al.*, 1988). Alva and Chen (1995) compared 'Swingle' citrumelo and 'Cleopatra' mandarin to increasing levels of soil Cu and found that these rootstocks differed in root and shoot Cu partitioning, with 'Swingle' citrumelo translocating more Cu to shoots. Therefore, the objectives of the study were to compare growth, nutrient uptake, and Cu-partitioning of six citrus rootstock varieties grown in sand-culture to increasing levels of CuEDTA fertilization.

Materials and methods

Growing conditions: Citrus rootstock seedlings 'US-812' (US812), 'US-897' (US897), 'Cleopatra' mandarin (CM), 'Flying Dragon' trifoliate (FD), 'Swingle' citrumelo (SC), and 'Volkamer' lemon (VL) were grown in cones (20 cm tall, tapered to base, 150 cm^3 volume) in a peat-based medium (Pro-Mix BX, Premier Tech Horticulture, Québec, Canada) for 6 months prior to transplant into 3.8 L containers in washed builders sand after first removing media from roots under a stream of tap water. A container-unit consisted of a pot-in-pot system with two layers of vinyl window screen sandwiched in between to prevent loss of sand through drainage holes. Sand in containers was washed prior to transplant as follows: leached with tap water followed by a 0.1 N HCl leach, and 6 h after, 3 leaches with deionized (DI) water. Plants were allowed to establish in sand for 28 days after transplant (DAT) before initiating Cu treatments. During this establishment period, half-strength base nutrient solution [BNS (described later) 600 mL per container] was applied on 5, 8, 11, 17, 20, and 23 DAT with DI water leach on 0, 14 and 26 DAT. Treatments were initiated 29 DAT and plants were harvested 137 DAT (108 days after initiating Cu treatments). Treatments (600 mL) were applied every 2 to 3 days on average with DI water leachings (600 mL) performed every 12-15 days. Deioninzed water leaches occurred just prior to a regular treatment application (*i.e.* on same day). The experiment was setup as a completely randomized design with six citrus rootstocks (CM, SC, VL, FD, US812, and US897), four Cu treatments (0.05, 0.25, 1.00, and 2.00 mg L^{-1} Cu) and six replications per rootstock-Cu treatment combination. The study was conducted in a double-walled polycarbonate-glazed greenhouse located in Fort Pierce, Florida, USA (27.41° North, 80.35° West) with growing temperatures over the course of the experiment averaging 16/27 °C night/day, respectively.

Nutrient solution and Cu treatments: A BNS was prepared in the lab and contained the following concentrations (mg L^{-1}) of essential plant nutrients: 200 nitrogen (N), 44 P, 166 K, 110 Ca, 49 Mg, 64 sulfur (S), 1 Fe, 0.5 boron (B), 0.5 Mn, 0.05 Zn, 0.02 Cu, and 0.05 molybdenum (Mo). Source reagents for preparing BNS were: KNO_3, $Ca(NO_3)_2$, NH_4NO_3, $(NH_4)_2H_2PO_4$, KH_2PO_4, $MgSO_4$, H_3BO_3, H_2MoO_4, FeEDTA, MnEDTA, ZnEDTA, and CuEDTA. Treatments consisted of CuEDTA disodium salt (Fluka Analytical, Steinheim, Germany) added to BNS to achieve a final Cu concentration of 0.05, 0.25, 1.00 and 2.00 mg L^{-1}. The BNS and CuEDTA stock solutions were prepared with DI water. The applied, unadjusted pH of treatment solutions was 5.86 ± 0.01.

Data collection, plant tissue harvest and analysis: At 29 (Cu treatments initiated) and 137 (harvest) DAT, trunk diameter was measured 3 cm above soil surface. At harvest, SPAD index readings (leaf greenness, an indirect measurement of leaf chlorophyll) were measured on young, recently-matured, and old-mature leaves. Leaves for nutrient analysis were collected and washed in sequence for approximately 10 to 15 sec in each of the following solutions: DI, 0.1 N HCl/0.01 % detergent (Tween-80, Fisher Scientific, Fair Lawn, NJ), and 3-DI water rinses. Roots, defined as where the first lateral root emerged from the trunk, were processed as described for leaves except that they were first washed clean of sand under a stream of tap water. Washed plant tissue was dried in a forced-air oven at 80 °C, weighed, and milled to pass a 20-mesh screen. Milled plant tissue was digested utilizing a closed-vessel microwave-assisted procedure according to USEPA method 3052 (1997). Briefly, 500 mg of leaf tissue was combined with 10 mL concentrated HNO_3 (trace metal grade) in a Teflon digestion vessel and processed in a microwave digestion oven (MARS 5, CEM Corp., Matthews, NC). Digestion conditions (internal) were 170 °C and 2068 kPa, held for 10 min following a 15 min ramp to these conditions. Digestates were transferred quantitatively to 100 mL volumetric flasks, brought to volume with DI water, filtered through Whatman 541 (Whatman Int., Kent, U.K.), and analyzed for Ca, K, Mg, P, Cu, Fe, Mn, and Zn by inductively coupled plasma-optical emission spectroscopy [ICP-OES (IRIS 1000 HR DUO IRIS, ThermoElemental, Franklin, MA)] according to USEPA method 6010B (1997).

Statistics: Data were analyzed by analysis of variance (ANOVA) to determine the main effects of rootstock and Cu treatment. Calculations were performed using the general linear model (GLM) procedure of SAS (ver. 9.2, SAS Institute, Cary, N.C.). Where appropriate, means were separated by LSD at P=0.05.

Results and discussion

Rootstock growth parameters: Copper treatment was not significant for leaf dry mass, trunk diameter, or SPAD; but rootstock was significant for these dependent variables. Young leaves, recently-matured leaves, and older-mature leaves, as a mean of rootstocks, had SPAD values of 41.30, 62.79, and 75.89, respectively. As a mean of leaf age, SPAD ranged from 56 (US812) to 63 (FD) (Table 1). This SPAD index range is normal for nutritionally-sufficient healthy leaves (Jifon *et al.*, 2005). Leaf and root dry mass was least and greatest for FD (15.02 g leaf, 4.51 g root) and VL (40.14 g leaf and 15.02 g root), respectively (Table 2). These data are consistent with the general growth characteristics for these rootstocks with FD being a dwarfing-type rootstock and VL, a fast-growing, vigorous-type rootstock. Root:leaf dry mass ratio varied from approximately 2 (CM) to 4 (US897) (Table 2). Syversten and Hanlon (2008) suggested that a root:shoot ratio between 2 to 4 is normal and desirable for citrus. Trunk diameter at the beginning of treatments (29 DAT) and at harvest (137 DAT) was least for CM and greatest for VL and SC which were not different (Fig. 1). As a percent increase in trunk diameter, FD increased the least (15 %) and VL the greatest (36 %). As mentioned previously, these data are consistent with the growth characteristics for these rootstocks (FD, dwarfing; VL,

Table 1. SPAD index readings for the mean of young, recently-matured and older-mature leaves for each Cu treatment for 'Cleopatra' mandarin (CA), 'Flying Dragon' (FD) trifoliate, 'Volkamer' lemon (VL), 'US-897' (US897), 'Swingle' citrumelo (SC), and 'US-812' (US812) citrus rootstocks. n = 24

Cultivar	Mean
CM	58.61bc[z]
FD	63.21a
VL	58.44bc
US897	61.90ab
SC	61.77ab
US812	56.02c

[z]Means followed by the same letter not significantly different $P \leq 0.05$).

Table 2. Dry mass (g) for root and leaf tissue, and root:leaf ratio based on dry mass as a mean of Cu treatment for 'Cleopatra' mandarin (CA), 'Flying Dragon' (FD) trifoliate, 'Volkamer' lemon (VL), 'US-897' (US897), 'Swingle' citrumelo (SC), and 'US-812' (US812) citrus rootstocks at harvest [137 days after transplant (108 days after initiating Cu treatments)]. n = 24

Cultivar	Dry mass (g)		Root: Leaf ratio[z]
	Root	Leaf	
CM	8.12 c[y]	15.68 c	1.9
FD	4.51 d	15.02 c	3.3
VL	15.02 a	40.14 a	2.7
US897	8.55 bc	31.82 b	3.7
SC	13.61 a	29.62 b	2.2
US812	10.55 b	30.35 b	2.9

[z]Statistics not performed on root:leaf ratio. [y]Means followed by the same letter not significantly different (LSD; $P \leq 0.05$)

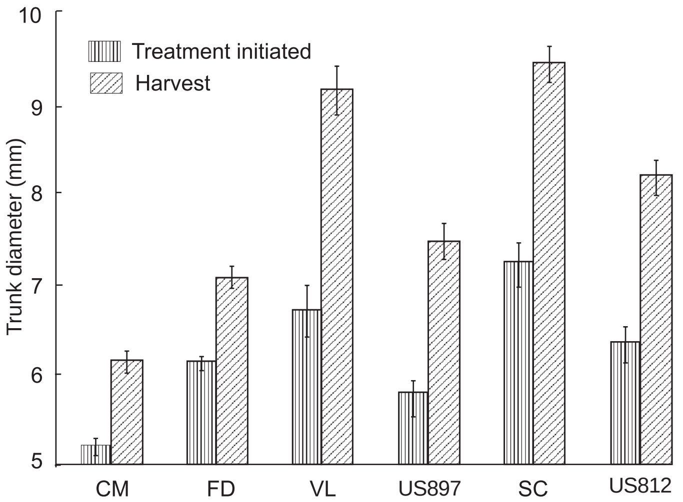

Fig. 1. Copper treatment was not significant for trunk diameter but rootstock was significant. Trunk diameter (mm) at 29 days after transplant [DAT (when Cu treatments were initiated)] and at harvest [137 DAT (108 days after initiating Cu treatments)]. Citrus rootstocks are: 'Cleopatra' mandarin (CA), 'Flying Dragon' (FD) trifoliate, 'Volkamer' lemon (VL), 'US-897', 'Swingle' citrumelo (SC), and 'US-812' citrus rootstocks. n = 24.

vigorous). Trunk diameter is important as it determines when a rootstock seedling can be budded with a scion; with FD requiring the longest period of time to reach this stage and SC and VL the least (Lynn Faulkner, personal communication, USDA-ARS, Fort Pierce, Florida, USA).

Leaf and root Cu: Rootstock and Cu treatment were significant for leaf and root Cu ($P < 0.001$). Data for these variables were statistically analyzed separately and presented in Tables 3 and 4. As a mean of rootstocks, leaf Cu ranged from 3.24 µg g^{-1} (0.05 mg L^{-1} Cu treatment) to 8.94 µg g^{-1} (2.00 mg L^{-1} Cu treatment), and root Cu ranged from 13.24 µg g^{-1} (0.05 mg L^{-1} Cu treatment) to 97.07 µg g^{-1} (2.00 mg L^{-1} Cu treatment). Leaf Cu levels were interpreted as sufficient, *i.e.* not deficient or excessive, for all rootstocks and Cu treatments except for the 0.05 mg L^{-1} treatment for CM and FD which were 2.91 µg g^{-1} and 2.87 µg g^{-1} Cu, levels interpreted as deficient (defined as < 3.0 mg L^{-1} Cu) based on Obreza *et al.* (2008). For all rootstocks, leaf and root Cu increased with Cu treatment level. The response was linear with r^2 values for leaves and roots of 0.9 or greater [values rounded up]. The slope of regression describes the response of rootstocks to increasing levels of Cu with the greater the slope, the greater the uptake of Cu into roots or leaves in response to increasing Cu treatment level. Slopes ranged from 1.06 (CM) to 4.19 (US812) for leaves and from 21.18 (FD) to 62.43 (VL) for roots. Differences in slopes between leaves and roots are due to greater Cu accumulation in root tissue. Due to differences in dry mass between rootstocks, Cu was also determined on a content basis. Based on content and as a mean of Cu treatments for a rootstock, for every 1 µg of leaf Cu there was 30 µg (lowest) and 61 µg (highest) root Cu for FD and VL, respectively (data not shown). These results are consistent with the work by Alva and Chen (1995) where it was found that Cu disproportionally accumulated more in roots than in leaves, and that citrus rootstocks differed in Cu translocation from roots to leaves.

Leaf and root Ca, K, Mg and P: Rootstock effect was significant for leaf and root Ca, K, Mg and P (P=0.05), but Cu treatment was not significant for these variables. Even though foliar levels of these macronutrients were statistically significant, their levels were interpreted as sufficient for normal citrus tree growth; averaging (as a mean of rootstocks) 1.69, 2.07, 0.26 and 0.19 % for Ca, K, Mg and P, respectively (Obreza *et al.*, 2008) (Table 5). Levels of Mg in roots (0.25 %) and leaves (0.26 %) (as a mean of rootstocks) were practically equal while levels of Ca, K and P accumulated more in roots than in leaves (Tables 5, 6). Volkamer lemon had statistically higher levels of Ca, K, and Mg in leaf tissue than for the other citrus rootstocks (Table 5).

Leaf and root Fe, Mn and Zn: Rootstock effect was significant for leaf and root Fe, Mn and Zn (P=0.05), but Cu treatment was not significant for these variables. For all rootstocks, the lowest levels of leaf Fe, Mn, and Zn was associated with CM (Table 5). Leaf Fe ranged from 40.94 µg g^{-1} (CM) to 87.20 µg g^{-1} (US897). Leaf Mn ranged from 22.77 µg g^{-1} (CM) to 48.18 µg g^{-1} (SC). Leaf Zn ranged from 10.51 µg g^{-1} (CM) to 16.40 µg g^{-1} (FD). Leaf Fe and Mn were interpreted as sufficient while Zn was considered low for normal citrus plant growth (Obreza *et al.*, 2008). Root Fe ranged from 260.29 µg g^{-1} (FD) to 435.37 µg g^{-1} (CM); root Mn ranged from 421.81 µg g^{-1} (SC) to 949.76 µg g^{-1} (FD); and root Zn ranged from 22.99 µg g^{-1} (VL) to 31.79 µg•g^{-1} (FD). Iron, Mn, and Zn accumulated at higher levels in roots than in leaves as was observed for Cu (Tables 3, 4, 5, 6). Root:leaf ratio based on concentration was approximately 2, 5, and 20 for Zn, Fe, and Mn, respectively.

Leaf symptomology: At harvest, plants were visually surveyed for nutrient disorder. In general, leaves of plants in the lowest Cu treatment appeared normal (Fig. 2). Young leaves in the highest Cu treatment (2.00 mg L^{-1} Cu), however, showed signs of differing degrees of interveinal chlorosis, leaf/leaflet cupping

Table 3. Leaf Cu for citrus rootstocks 'Cleopatra' mandarin (CA), 'Flying Dragon' (FD) trifoliate, 'Volkamer' lemon (VL), 'US-897' (US897), 'Swingle' citrumelo (SC), and 'US-812' (US812) at harvest, 108 days after initiating treatments. n = 6

Cu (mg L^{-1})	Leaf Cu (µg g^{-1})						Significance (P <)
	CM	FD	VL	US897	SC	US812	
0.05	2.91 c[z]	2.87 c	3.39 d	3.15 c	3.04 d	4.06 b	NS[y]
0.25	3.72 bc,C[x]	4.12 b,BC	5.71 c,A	5.02 bc,AB	5.73 c,A	5.59 b,A	0.001
1.00	4.35 ab,C	5.06 ab,C	7.76 b,B	7.45 ab,B	8.22 b,AB	9.73 a,A	0.001
2.00	5.21 a,B	5.83 a,B	11.17 a,A	9.76 a,A	10.07 a,A	11.59 a,A	0.001
r^2 of line	0.997	0.988	0.987	0.997	0.993	0.967	---
Slope of line	1.06	1.34	2.62	3.36	3.46	4.19	---
Significance (P <)	0.001	0.001	0.001	0.001	0.001	0.001	---

[z]Means followed by the same lowercase letter in the same column are not significantly different (LSD; $P \leq 0.05$). [y]NS, nonsignificant. [x]Means followed by the same uppercase letter in the same row are not significantly different (LSD; $P \leq 0.05$). Where no letters appear in a row, effect of Cu treatment was not significant.

Table 4. Root Cu for citrus rootstocks 'Cleopatra' mandarin (CA), 'Flying Dragon' (FD) trifoliate, 'Volkamer' lemon (VL), 'US-897' (US897), 'Swingle' citrumelo (SC), and 'US-812' (US812) at harvest, 108 days after initiating treatments. n = 6

Cu (mg L^{-1})	Root Cu (µg g^{-1})						Significance (P <)
	CM	FD	VL	US897	SC	US812	
0.05	15.30c[z]	9.38c	12.06c	13.10c	16.94c	12.56d	NS[y]
0.25	23.12c	21.87bc	33.33bc	29.50b	30.78c	29.23c	NS
1.00	63.57b,A[x]	35.65b,BC	59.00b,A	52.91b,C	63.43b,AB	49.38b,ABC	0.05
2.00	100.43a,B	53.81a,C	139.93a,A	76.32a,BC	104.23a,AB	108.26a,AB	0.01
r^2 of line	0.941	0.992	0.892	0.994	0.959	0.897	---
Slope of line	44.39	21.18	62.43	30.60	43.86	47.57	---
Significance (P <)	0.001	0.001	0.001	0.001	0.001	0.001	---

[z]Means followed by the same lowercase letter in the same column are not significantly different (LSD; $P \leq 0.05$). [y]NS, nonsignificant. [x]Means followed by the same uppercase letter in the same row are not significantly different (LSD; $P \leq 0.05$). Where no letters appear in a row, effect of Cu treatment was not significant.

Table 5. Leaf nutrients for citrus rootstocks 'Cleopatra' mandarin (CA), 'Flying Dragon' (FD) trifoliate, 'Volkamer' lemon (VL), 'US-897' (US897), 'Swingle' citrumelo (SC), and 'US-812' (US812) at harvest, 108 days after initiating treatments. n = 24

Cultivar	Macronutrients (%)				Micronutrients (µg g^{-1})		
	Ca	K	Mg	P	Fe	Mn	Zn
CM	1.48d[z]	1.75d	0.26c	0.18b	40.94d	22.77e	9.28e
FD	1.44d	2.13b	0.18d	0.20a	69.77c	27.27d	16.40a
VL	2.09a	2.38a	0.32a	0.19ab	81.23b	37.57b	10.51d
US897	1.48d	1.91c	0.29b	0.18b	87.20a	29.54d	11.57c
SC	1.89b	2.07b	0.25c	0.19ab	72.47c	43.18a	14.44b
US812	1.74c	2.19b	0.25c	0.20a	86.31ab	34.25c	14.10b

[z]Means followed by the same lowercase letter in the same column are not significantly different (LSD; $P \leq 0.05$).

Table 6. Root nutrients for citrus rootstocks 'Cleopatra' mandarin (CA), 'Flying Dragon' (FD) trifoliate, 'Volkamer' lemon (VL), 'US-897' (US897), 'Swingle' citrumelo (SC), and 'US-812' (US812) at harvest, 108 days after initiating treatments. n = 24

Cultivar	Macronutrients (%)				Micronutrients (µg g^{-1})		
	Ca	K	Mg	P	Fe	Mn	Zn
CM	2.45 a[z]	2.24 b	0.32 a	1.11 a	435.37 a	834.44 a	28.56 ab
FD	1.22 c	3.45 a	0.18 d	0.64 b	260.29 c	949.76 a	31.79 a
VL	1.92 b	2.63 b	0.25 b	0.72 b	337.14 b	455.63 c	22.99 c
US897	1.71 b	3.49 a	0.21 c	0.66 b	328.66 b	684.92 b	24.38 bc
SC	1.78 b	3.24 a	0.25 bc	0.64 b	313.98 b	421.81 c	24.37 bc
US812	1.68 b	3.24 a	0.26 b	0.76 b	337.15 b	649.48 b	26.63 bc

[z]Means followed by the same lowercase letter in the same column are not significantly different (LSD $P \leq 0.05$).

at the margins, or both (Fig. 2). 'Volkamer' lemon plants at the highest Cu treatment (2.00 mg L^{-1} Cu), in general, were normal with only very mild signs of these described symptoms (Fig. 2). The most pronounced symptoms were observed on SC. Young leaves of SC in the highest Cu treatment expressed interveinal chlorosis and upward leaflet cupping at the margins (Figs. 2, 3A). Leaflet cupping, some downwards, was also observed in CM at the highest Cu treatments (Figs. 2, 3B). These results support work by others that citrus rootstocks differ in response to applied/soil-available Cu and that SC is particularly susceptible to excess

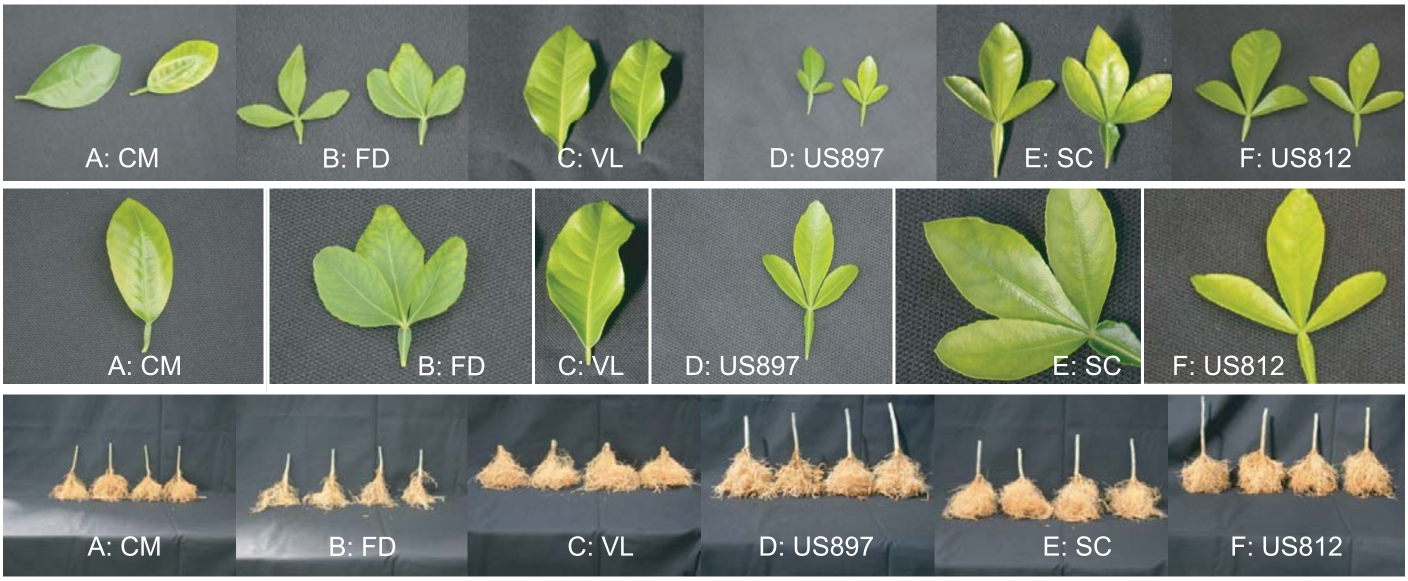

Fig. 2. Representative leaves and roots at harvest, 137 days after transplant (108 days after initiating Cu treatment) for citrus rootstocks (A) 'Cleopatra' mandarin (CM), (B) 'Flying Dragon' (FD) trifoliate, (C) 'Volkamer' lemon (VL), (D) 'US-897' (US897), (E) 'Swingle' citrumelo (SC), and (F) 'US-812' (US812) citrus rootstocks. Top row: leaves represent Cu treatments 0.05 mg L^{-1} Cu [left (lowest Cu treatment)] and 2.00 mg L^{-1} Cu [right (highest Cu treatment)]. Middle row: close up of 2.00 mg L^{-1} Cu treatment leaf shown in top row. Bottom row: roots represent, from left to right for each citrus rootstock, 0.05, 0.25, 1.00, and 2.00 mg L^{-1} Cu treatment.

Cu (Alva, 1993; Mozaffari *et al.*, 1996). The observed symptoms on terminal leaves are consistent with micronutrient (Cu, Fe, Mn, and/or Zn) deficiency. These symptoms disappeared on affected leaves as they matured [Fig. 3A, B (older leaves)] which have been noted for mild micronutrient disorders in citrus (Whiteside *et al.*, 1988). It is likely that Cu was the direct or indirect cause of these symptoms as they correlated with increasing levels of applied Cu. However, since symptomatic leaves were not specifically analyzed for micronutrient imbalance, this association is only be speculative and nonspecific to Cu, Fe, Mn, and/or Zn. Finally, there was no visual difference observed for roots between Cu treatments (Fig. 2).

A comparative study of six citrus rootstock seedlings to increasing levels of rhizosphere Cu [CuEDTA (0.05 to 2.00 mg L^{-1})] in sand culture at pH 5.86 was conducted for a period of 108 days. The study supports work by others that citrus rootstocks disproportionally accumulate more Cu in roots than in leaves, and that they differ in translocation of Cu from roots to leaves. Other nutrients analyzed (Ca, K, P, Fe, Mn, and Zn) also accumulated in roots more than in leaves. The exception was Mg which

Fig. 3. Young 'Swingle' citrumelo [A (SC)] and 'Cleopatra' mandarin [B (CM)] leaves showing interveinal chlorosis and leaflet (A) or leaf (B) cupping for the 2.00 mg·L^{-1} Cu treatment (the highest Cu treatment) at harvest 137 days after transplant (108 days after initiating Cu treatment).

accumulated nearly equally in roots and leaves (as a mean of rootstocks). In addition to plant nutrition, rootstocks were also subjectively assessed for nutrient disorder in relation to Cu treatment. Based on visual leaf symptoms on youngest leaves in the highest Cu treatment (2.00 mg L^{-1}), VL was the least, and SC the most susceptible to Cu with the other rootstocks falling in between these extremes as follows: (most visual symptoms) SC > CM/FD > US812/US897 > VL (least visual symptoms).

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