HortScience 59(8):1056–1064. 2024. https://doi.org/10.21273/HORTSCI17979-24

Evaluation of Different Container Types on Root Structure and Performance of Nursery-grown Citrus Plants

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Keywords. citrus greening, fibrous roots, propagation, root pruning, rootstock, root system

Abstract. **Health and quality of the root system are imperative to ensure the successful establishment of a citrus tree after transplant from the nursery into the field. Containerized citrus production in enclosed nurseries restricts root growth and can result in root circling and intertwining. This may hinder root expansion and result in root girdling after transplant, negatively affecting tree establishment and growth. The root structure of a transplanted citrus tree can also be affected by the container type used in the nursery. Containers with root-pruning properties like chemical pruning or air pruning reduce root circling and may produce superior root systems compared with regular, nonpruning containers. The aim of this study was to evaluate the effects of different nursery containers on root physiological and morphological traits and plant performance over 15 months of growth in the nursery. Three container types, chemical pruning containers, air-pruning containers, and standard nursery containers, were compared. The chemical pruning containers were standard citrus nursery containers with a mixture of copy hydroxide [$Cu(OH)_2$] and copper carbonate ($CuCO_3$) [10% copper (Cu)] applied to the inner wall. Pruning occurs upon contact of the roots with the Cu on the wall of the containers. The air-pruning containers were custom-sized Air-Pots in which pruning occurs on holes in the wall of the containers upon contact of the roots with the air. Two rootstocks, US-812 and US-942 (*Citrus reticulata* × *Poncirus trifoliata*), were included for comparison in the nongrafted stage and 12 months after grafting with 'Valencia' orange (*Citrus sinensis*). Chemical root pruning positively influenced tree height, shoot mass, leaf area, rootstock trunk diameter, and the nonfibrous root biomass. No differences among container types were observed for the fibrous root biomass, but chemical pruning produced more roots that were finer with a higher specific root length and a higher respiration rate. In contrast, air pruning produced more roots that were thicker compared with the other containers. Most of the leaf nutrients were lower in trees grown in the chemical pruning containers compared with the standard containers, except for Cu and zinc (Zn), which were highest in the former. Trees growing in air-pruning containers were not significantly different in growth from trees growing in standard containers.**

Commercial citrus trees are grown and grafted in nurseries before they are transplanted into the field. They are usually grown as grafted trees, combining the desired traits of the scion with the biotic and abiotic stress tolerance of the rootstock (Bowman et al. 2021; Goldschmidt 2014; Ollitrault and Navarro 2012). The scion/rootstock combination is selected based on their compatibility and other factors such as geographic and climatic suitability and market demand. There are 44 commercial citrus nurseries in Florida, propagating ∼3 million citrus trees annually (Rosson 2022). Until the 1980s, FL citrus nursery trees were grown mainly outdoors and transplanted bare-rooted (Castle 1987a). In 1997, to prevent the introduction of viruses and diseases to nursery stock, it became mandatory to grow citrus trees in enclosed structures (FDACS 2013). This, in combination with the advancement in greenhouse production systems, has led to the transition to containerized nursery production, which prevails to date (Marler and Davies 1987; Vashisth et al. 2020). The containerized production system increases the rate of survival, shortens the growing cycle, eases management, reduces transplantation shock, and reduces the risk of mechanical injury during transport (Allen et al. 2017; Grossnickle and El-Kassaby 2015; Marler and Davies 1987).

Up until 2022, Florida was the largest producer of citrus in the United States in terms of productivity and acreage (USDA 2022). However, Florida citrus production has been decimated by more than 80% since the discovery of the devastating disease Huanglongbing (HLB), also known as citrus greening (Bové 2006; Gottwald 2010). HLB is a bacterial disease for which there is no cure and, given that the disease is endemic to Florida (Graham et al. 2020), any field-grown citrus tree will eventually become infected. Fibrous root loss has been reported as one of the early consequences of HLB (Graham et al. 2013; Johnson et al. 2014; Kumar et al. 2018) accelerating tree decline. It is therefore imperative to direct citrus nursery practices toward the production of trees with high-quality root systems to prolong the health and productivity of a citrus grove.

Plant survival upon transplantation is contingent on its ability to adapt quickly to the new environment. This depends on multiple factors, especially the plant's root and shoot system morphology, nutrient and water uptake capacity, and available carbohydrate reserves (Grossnickle 2012; Marchioretto et al. 2020). Castle (1987b) and Marler and Davies (1987) emphasized that successful citrus grove establishment depends on the root development and expansion capacity of the nursery trees. Traditionally, citrus trees are grown in narrow, solid-walled containers of various shapes and sizes. These containers may promote root circling and the generation of pot-bound roots (i.e., matting or tangling of the roots), which can negatively impact tree establishment and growth after permanent transplantation in the field (Gilman 2009; Grossnickle and El-Kassaby 2015; South and Mitchell 2006). Since root deformations can persist after transplant, they may ultimately reduce the productivity and life expectancy of a tree, especially when subjected to adverse growing conditions such as HLB.

Root pruning can help generate a healthy root system by preventing root circling and girdling or "strangulation" and the formation of pot-bound roots that hinder tree establishment and growth after transplanting (Budiarto et al. 2019; Single and Single 2010; South and Mitchell 2006). Although the benefits of manual root pruning before transplanting on plant growth and performance have been reported, manual pruning causes injury and may result in considerable root loss (Geisler and Ferree 2011). It also requires additional labor and, therefore, increases the cost of production. Alternatives to mechanical pruning at transplant are chemical pruning and air pruning during the nursery stage. These alternative pruning methods were shown to improve root growth, reduce root deformations, and promote a robust root system in several tree species including apple (Elsysy and Einhorn 2022), red maple (Gilman et al. 2010), pine (Aldrete et al. 2002; Sword Sayer et al. 2009; Tsakaldimi and Ganatsas 2006), and oak (Mariotti et al. 2015).

Received for publication 21 May 2024. Accepted for publication 22 May 2024.

This research was supported by grants from the Citrus Research and Development Foundation (CRDF) and USDA NIFA Hatch project FLA-SWF-006160. We thank Lynn Stewart and Mark Crawford for providing nursery containers and inspiration for this project.

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Chemical root pruning involves coating the inside of the nursery container with root growth-inhibiting chemicals like $CuCO_3$ or $Cu(OH)_2$. This reduces lateral root growth and therefore circling of the roots along the container wall (Liu et al. 2016; Marchioretto et al. 2020; Montagnoli et al. 2022). These compounds can be mixed in a latex ink carrier, which controls the interaction between the Cu ions and the roots to prevent toxicity and ensure that root inhibition is localized to the root tip with little to no Cu translocating to other parts of the plant (Crawford 2003). Air pruning involves the use of specialized containers with walls designed to guide roots toward an air hole. On contact with the air, root tips will become dehydrated and consequently pruned. Like chemical pruning, the air pruning stimulates lateral root growth and root branching, promoting the formation of a dense fibrous root system within the substrate (Elsysy and Einhorn 2022; Feng et al. 2018).

When grown from seed, the citrus root system usually consists of a tap root with lateral roots originating from it. The root system is different when plants are generated from tissue culture or cuttings, in which case it contains numerous adventitious roots instead of a single tap root (Albrecht et al. 2017, 2020), which may be more prone to circling and intertwining. Vegetative propagation is important when seeds are not available or when rootstocks produce mostly zygotic embryos resulting in off-types (Albrecht et al. 2021; Bowman et al. 2021). Tissue culture propagation of rootstocks is increasingly being adopted in commercial citrus production and is common in other tree crops. This study investigates the effects of different nursery containers with different root-pruning properties (chemical pruning, air pruning, and no pruning) on the physiological performance, root structure, and biomass distribution of young citrus trees with two different, tissue culture–generated rootstocks. It is hypothesized that the containers designed to prune roots on contact with the container wall promote the formation of a healthier root system, and therefore plant growth, than the traditional containers used for nursery production of citrus in Florida.

Materials and Methods

Plant material

A greenhouse experiment was conducted at the Southwest Florida Research and Education Center, University of Florida, Immokalee, Collier County, FL, USA (26.462151, −81.435582), from Apr 2022 to Jul 2023. The maximum and minimum temperature of the greenhouse was 33.6 °C and 13.1 °C, respectively, during the time of experimentation, with a mean temperature of 23.9 °C. Plants received natural sunlight without any supplemental light. The rootstock cultivars used in this study were US-812 (*Citrus reticulata* 'Sunki' × *Poncirus trifoliata* 'Benecke') and US-942 (*C. reticulata* 'Sunki' × *P. trifoliata* 'Flying Dragon'). These rootstocks are among the top 10 propagated rootstocks in Florida, with US-942 ranking first and US-812 ranking sixth in 2022 (Rosson 2022). Three-month-old tissue culture–generated rootstock liners were provided by a commercial citrus nursery (Agromillora, Wildwood, FL, USA). After a 1-month acclimation period, the liners were transplanted into the experimental containers filled with Pro Mix BK25 potting medium (Pro Mix; Premier Tech Horticulture, Quakertown, PA, USA) in Apr 2022.

Nursery containers

Three types of nursery containers were evaluated: i) nonpruning, standard citrus nursery containers (Stuewe and Sons, Inc., Tangent, OR, USA), hereafter referred to as NPCs (nonpruning containers); ii) chemical pruning, Cu-coated containers, hereafter referred to as CPCs (chemical pruning containers), and iii) air-pruning containers (Air-Pot®, Caledonian Tree Co. Ltd, UK), hereafter referred to as APCs (Fig. 1). All containers were made up of black, high-density plastic. The NPCs were 13.5 inches (34.3 cm) long, with an upper square opening of 4 inches (10.2 cm), a bottom square of 2.8 inches (7.1 cm) containing five holes, and a volume of 2.65 L. For the CPCs, a latex-based mixture of $Cu(OH)_2$ and $CuCO_3$ (MicroKote, Ke-Coat LLC, Garrettsville, OH), was painted without dilution on the inner walls of the standard nursery containers according to the manufacturer's instructions. The total Cu in the mixture was 10% (w/v) with $Cu(OH)_2$ and $CuCO_3$ comprising 61% and 55.8% of the total Cu. The concentration was tested in a commercial citrus nursery setting before this study (Crawford M, personal communication). The APCs were custom-cut to be as close in size and volume of the NPCs and CPCs as possible and were 12 inches (30.5 cm) long with an upper and bottom diameter of 4.5 inches (11.4 cm), a circular, grid-like base elevated 1 inch (2.5 cm) above the greenhouse bench, and a volume of 2.66 L. Plastic net bottoms (Air-Pot Bros, Salem, OR, USA), 4.5 inches (11.4 cm) in diameter, were installed in the APCs to hold the potting medium. Figure 2 shows the fibrous root pruning at the interface of substrate and container wall when trees were grown in CPCs (Fig. 2A) and APCs (Fig. 2B), not evident in the NPCs (Fig. 2C).

Experimental design

The experimental design was a randomized split-plot block design with rootstock cultivar as the main plots and container type as subplots. Experimental units were arranged in six replications (blocks), with each replication containing five plants per rootstock/container type combination. There were 36 experimental units (6 replication × 2 rootstocks × 3 container types) and 180 plants in total.

In Jul 2022, 3 months after transplanting (MAT), one randomly selected tree from each experimental unit (replicate) was destructively sampled to assess root structures, plant biometrics, and fibrous root respiration. The remaining four trees in each replicate were budded with certified disease-free 'Valencia' (*C. sinensis*) budwood, clone 1–14–19, using the inverted T method (Albrecht et al. 2021). 'Valencia' clone 1–14–19 ranks first among the scions budded in Florida (Rosson 2022). Buds were unwrapped after 3 weeks, and 6-benzylaminopurine (5 mM) dissolved in Tween 20 (5%) and ethanol (100%) was applied to each bud to promote budbreak and shoot growth (Niedz and Bowman 2023).

Trees were irrigated three times a week by using an automated drip irrigation system (Irritol controller system, Riverside, CA, USA) until full saturation [0.5 gal/h (1.9 L/h), 20 min initially, and 25 min starting Jan 2023]. Trees were fertigated once a week using a water-soluble fertilizer mix (20% N, 8.7% P, 16.7% K, Peters Professional 20–20–20; The Scotts Company, Marysville, OH, USA). Fertigation was performed through the automated drip irrigation system by injecting the fertilizer to a final concentration of 400 ppm N. Fertigation was performed at the same flow rate and duration as the standard irrigation. Mineral oil (JMS Stylet oil, JMS Flower Farms, Inc., Vero Beach, FL, USA) was applied every 2 weeks to manage insects, fungal diseases, and mites. An additional insecticide/miticide (Avid; Syngenta, Greensboro, NC, USA) was applied every 2 months. Weeds were removed by hand periodically throughout the experiment.

Plant assessments

Tree size. Tree height and trunk diameter were assessed in nongrafted trees at budding (3 MAT) and in grafted trees at the end of the experiment (15 MAT), which was 12 months after budding. Tree height was measured from the surface of the potting medium to the top of the trees using a tape measure (Craftsman, Towson, MD, USA). Trunk diameters of nongrafted trees were measured at 5 cm above the surface of the potting mix at 3 MAT. Trunk diameters of grafted trees were measured at 2 cm above and below the graft union at the end of the experiment (15 MAT). Trunk diameters were measured with a digital caliper (Mitutoyo America, Aurora, IL, USA).

Tree biomass. The biomass of nongrafted trees was assessed at 3 MAT using one tree from each replicate. The biomass of grafted trees was assessed at 15 MAT using all four trees in each replicate. Trees were removed from the container and roots were washed with water to remove the potting medium. Trees were divided into shoot and root systems at the surface of the potting medium. The grafted trees were divided into scion and rootstock at the graft union and the scion was further divided into leaves and stems. The root system was further divided into fibrous roots ($<$1.5 mm diameter) and nonfibrous roots ($>$1.5 mm diameter). Shoot and root tissues were oven-dried at 65 °C until constant weight.

Stomatal conductance. The stomatal conductance was measured monthly on two grafted trees per replicate from Apr to Jun 2023

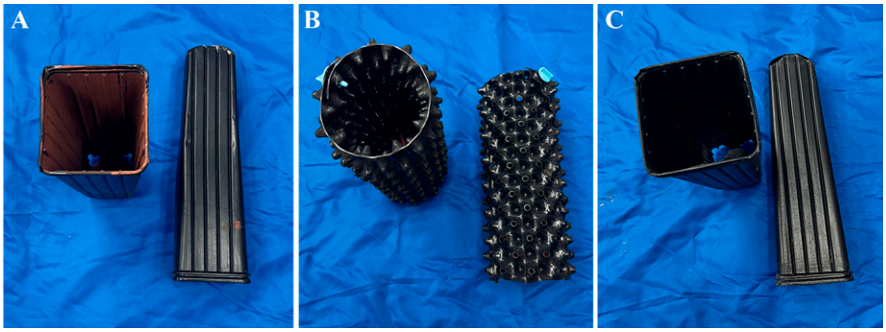

Fig. 1. Different container types used in the study. (**A**) Chemical (copper) pruning container, (**B**) air-pruning container, and (**C**) nonpruning (standard) nursery container.

(12–14 MAT), when the scion had produced at least eight mature leaves, by using a SC-1 leaf porometer (METER group, Pullman, WA, USA). The porometer was calibrated in the greenhouse and measurements were conducted between 9 AM and 12 PM using the "Auto" mode. A fully expanded leaf was chosen from a recently matured flush in the direction of sunlight. Midrib and large leaf veins were avoided during measurements.

Leaf area. At the end of the experiment (15 MAT), 10 mature leaves were randomly collected from each grafted tree and used for leaf area determination. Leaves were scanned at 300 dpi using a flatbed Epson V850 Pro scanner (Epson, Los Alamitos, CA, USA). The scanned images were processed using ImageJ 1.54d software (Rasband, National Institutes of Health, Bethesda, MD, USA) to obtain the leaf areas. Scanned leaves were placed in a paper bag and oven-dried at 65 °C until constant weight. The specific leaf area was determined as the ratio of the leaf area (cm^2) to the leaf dry weight (g).

Root hydraulic conductivity. Root hydraulic conductivity was measured on one grafted tree per replicate at the end of the study (15 MAT) using a high-pressure flow meter (HPFM) (Dynamax, Houston, TX, USA). De-ionized, degassed water was used for measurements. The shoot system was cut at 10 cm above the potting medium. The exposed stem was shaved with a razor blade and attached to the HPFM. Water flow pressure was adjusted to ensure proper flow of water into the root system and a quasi-steady state reading was taken for 1 min (Geng et al. 2022). The average hydraulic conductance (K) was calculated and expressed as $kg \cdot s^{-1} \cdot MPa^{-1}$. Root systems remained undisturbed during the measurements.

Root respiration. Fibrous roots were collected from each nongrafted tree at budding (3 MAT) and from grafted trees at the end of the experiment (15 MAT). Roots were pooled within each replicate, washed with water, and blotted dry. A subset of roots was immediately placed inside a 50-cm^3 respiration chamber (LI-COR Bioscience, Lincoln, NE, USA) and fibrous root respiration was measured using a LI-COR Li-850 CO_2/H_2O analyzer (LI-COR Bioscience). Gas exchange was recorded in mg CO_2/h for 3 min and the root respiration rate was expressed as mg CO_2/g/h.

Root length distribution and specific root length. A subset of fibrous roots collected from the grafted trees at 15 MAT as described previously was scanned at 300 dpi using the flatbed Epson V850 Pro scanner. The total root length and root length distribution across five different diameter ranges (0–0.3 mm, 0.3–0.6 mm, 0.6–0.9 mm, 0.9–1.2 mm, and 1.2–1.5 mm) were analyzed using RhizoVision Analyzer Interactive software (version 2.0.2) (Seethepalli and York 2020) following the algorithms in Seethepalli et al. (2021). The specific root length (SRL) was calculated by dividing the total root length by the root dry weight and expressed as $m \cdot g^{-1}$.

Leaf and root nutrients. Leaf and root macro- [nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S)] and micronutrients [boron (B), Zn, manganese (Mn), iron (Fe), Cu] of grafted trees were analyzed at the end of the study (15 MAT). Fully mature leaves were collected from all trees and pooled within each replicate. For root nutrient determination, a subset of fibrous roots collected at 15 MAT as described above was used. Samples were shipped to Waters Agricultural Laboratories,

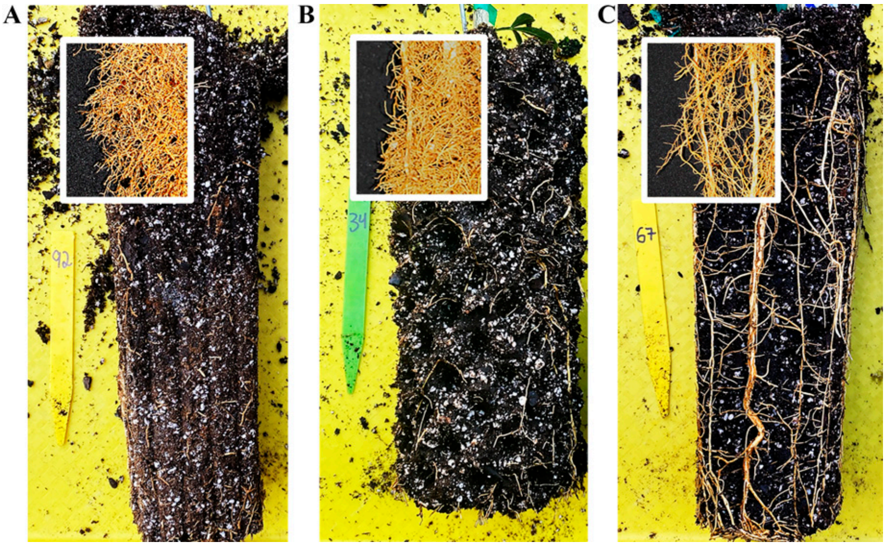

Fig. 2. Root systems of trees grown in chemical (copper) pruning (**A**), air-pruning (**B**), and nonpruning (standard) nursery containers (**C**). Note the fibrous root pruning at the interface of soil and container wall exhibited in chemical pruning and air-pruning containers, but not in nonpruning containers. Inserts show washed root systems.

Inc. (Camilla, GA, USA), where they were dried, ground to a fine powder, and analyzed using inductively coupled argon plasma emission spectroscopy after digestion in nitric acid and hydrogen peroxide (Isaac and Johnson 1985).

Statistical analysis

All statistical analyses were conducted in RStudio Version 4.3.1 (R Core Team 2023). A two-way analysis of variance (ANOVA) was performed for all variables. A linear mixed model was fitted for container type and rootstock as fixed factors and block and block × rootstock as random factors using the lmerTest (Kuznetsova et al. 2017). Before performing ANOVA, data were checked for normality and homogeneity of variance. If needed, data were log-transformed to meet the assumption of ANOVA. Post hoc comparison of significantly different means was calculated using Tukey's honestly significant difference test. All tests were performed with a probability level of $P < 0.05$.

Results

Nongrafted plants

There was no significant effect of the container type on the trunk diameter and plant height, which were 5.3–5.4 mm and 76–82 cm, respectively (Table 1), but there was a significant rootstock effect for both variables. US-812 had a significantly larger trunk diameter than US-942, whereas US-942 plants were significantly taller than US-812 plants. Plants grown in CPCs had the largest shoot biomass (27.3 g), whereas plants grown in APCs had the smallest (17.6 g). The fibrous root mass was 2.3–2.8 g and not significantly affected by the container type. However, plants grown in CPCs had a larger nonfibrous root biomass (4.37 g) than those grown in APCs (2.48 g). There was no significant effect of rootstock on the shoot, fibrous root, and nonfibrous root biomass. The container type did not influence the fibrous root respiration rate significantly, but US-812 had a significantly higher root respiration rate compared with US-942. There was no significant interaction between rootstock and container type for any of the variables measured.

Grafted trees

Tree size. The container type significantly affected tree height and rootstock trunk diameter, but not the scion trunk diameter (Table 2). Trees grown in CPCs were the tallest (114 cm), and trees grown in NPCs were the shortest (96 cm). Similarly, the rootstock trunk diameter was significantly larger in trees grown in CPCs (13.1 mm) than in NPCs (11.9 mm). Rootstock effects were also significant. US-942 produced taller trees with a larger scion and rootstock trunk diameter than US-812. There was no significant interaction between rootstock and container type for any of the variables measured.

Tree biomass. Leaf, scion, rootstock, and nonfibrous root biomasses were significantly influenced by container type and rootstock cultivar but not by their interaction (Table 3). Trees grown in CPCs produced the largest leaf biomass (17.5 g), scion biomass (16.1 g), rootstock biomass (13.9 g), and nonfibrous root biomass (18.0 g), whereas trees grown in NPCs produced the lowest (12.7 g, 11.2 g, 10.2 g, and 13.3 g, respectively). US-942 had a significantly larger leaf biomass (17.3 g), scion biomass (16.0 g), rootstock biomass (14.0 g), nonfibrous root biomass (19.9 g), and total root biomass (25.9 g) than US-812 (12.9 g, 11.0 g, 10.2 g, 10.4 g, and 14.9 g, respectively). There were no significant differences between container types for the fibrous root biomass.

The root-to-shoot system biomass ratio was not significantly affected by container type or the interaction with rootstock. However, US-942 had a significantly higher root-to-shoot system biomass ratio (0.52) compared with US-812 (0.45).

Stomatal conductance. There was no significant effect of container type, rootstock, and their interaction on the stomatal conductance measured in Apr, May, and Jun 2023 (Table 4). The highest stomatal conductance was measured in April and the lowest in June.

Leaf area. The container type and rootstock significantly affected the leaf area, but there was no significant interaction (Table 5). Trees grown in CPCs had the largest leaf area (50.6 cm^2/leaf), whereas those in NPCs had the lowest (44.2 cm^2/leaf). US-942 induced a larger leaf area (50.8 cm^2/leaf) than US-812 (44.4 cm^2/leaf). However, no differences in the specific leaf area were observed among container types, rootstocks, and their interactions.

Root hydraulic conductivity. The root hydraulic conductivity ranged from 2.3E-05 $kg \cdot s^{-1} \cdot MPa^{-1}$ to 7.7E-05 $kg \cdot s^{-1} \cdot MPa^{-1}$ and was not significantly affected by the container type, rootstock, or their interaction (Table 5).

Root respiration. The container type affected the root respiration rate significantly (Table 5). Fibrous roots of trees grown in CPCs had a higher respiration rate (1.06 mg CO_2/g/h) compared with trees grown in NPCs (0.74 mg CO_2/g/h). The rootstock and the interaction of container type and rootstock did not influence the root respiration rate significantly.

Specific root length. There was a significant difference in the SRL of trees grown in different container types (Table 5). Trees grown in APCs had a significantly lower SRL (21.6 m/g) compared with both NPCs (23.7 m/g) and CPCs (25.4 m/g). There was no significant rootstock effect nor interaction between container type and rootstock.

Root length distribution. The fibrous root length distribution across different diameter ranges was significantly affected by container type and rootstock except for roots with a diameter of 1.2 to 1.5 mm (Table 6). In general, roots in the 0.3- to 0.6-mm-diameter range accounted for the largest proportion of fibrous roots based on length (64% to 69%), and roots in the 1.2- to 1.5-mm-diameter range accounted for the smallest (1.6% to 2.2%). Trees grown in CPCs had a significantly higher proportion of 0- to 0.3-mm roots (8.6%) than trees grown in APCs (6.6%) and a significantly higher proportion of 0.3- to 0.6-mm roots (69%) than trees grown in APCs and NPCs (64%). APCs and NPCs produced significantly more roots in the 0.6- to 0.9-mm-diameter range (20%) compared with CPCs (15%). APCs produced a significantly higher proportion of roots in the 0.9- to 1.2-mm-diameter range (6.8%) compared with CPCs (5.1%). US-942 had a significantly higher proportion of thinner (0–0.6 mm) roots, and US-812 had a significantly higher proportion of thicker (0.6–1.2 mm) roots. There was no significant interaction

Table 1. Tree size, shoot and root biomass, and root respiration rate of nongrafted rootstock liners after 3 months of growth in chemical pruning, air-pruning, and nonpruning containers.

	Trunk diam (mm)	Plant ht (cm)	Shoot biomass (g)	Fibrous root biomass (g)	Nonfibrous root biomass (g)	Total root biomass (g)	Root respiration rate (mg CO_2/g/h)
Container type							
CPC	5.4	82	27.3 a	2.8	4.4 a	7.2	2.6
APC	5.4	81	17.6 b	2.3	2.5 b	4.7	3.7
NPC	5.3	76	19.8 ab	2.3	2.7 ab	5.0	3.1
P value	0.2592	0.2759	0.0393	0.3357	0.0202	0.0521	0.2499
Rootstock							
US-942	5.2 b	88 a	22.2	2.2	3.3	5.5	2.5 a
US-812	5.5 a	71 b	20.9	2.8	3.1	5.8	3.8 b
P value	0.0022	0.0081	0.6786	0.1051	0.6504	0.7151	0.0113
Container type × rootstock							
P value	0.3640	0.2791	0.3556	0.6391	0.8146	0.7865	0.6551

CPC = chemical (copper) pruning container; APC = air-pruning container; NPC = nonpruning (standard) container.

Different letters within columns indicate significant differences ($P < 0.05$) according to Tukey's honestly significant difference test.

Table 2. Tree height, scion trunk diameter, and rootstock trunk diameter of 'Valencia' trees grafted on different rootstocks after 15 months of growth in chemical pruning, air-pruning, and nonpruning containers.

	Tree ht (cm)	Scion trunk diam (mm)	Rootstock trunk diam (mm)
Container type			
CPC	114 a	9.4	13.1 a
APC	106 ab	9.3	12.8 ab
NPC	96 b	8.7	11.9 b
P value	0.0006	0.2654	0.0354
Rootstock			
US-942	111 a	9.9 a	13.5 a
US-812	99 b	8.4 b	11.8 b
P value	0.0230	0.0398	0.0172
Container type × rootstock			
P value	0.7271	0.7271	0.8965

CPC = chemical (copper) pruning container; APC = air-pruning container; NPC = nonpruning (standard) container.

Different letters within columns indicate significant differences ($P < 0.05$) according to Tukey's honestly significant difference test.

between the container type and rootstock for any diameter range.

Leaf and root nutrients. The container type significantly influenced the leaf nutrient content except for Ca, Mg, and B (Table 7). Trees grown in NPCs and APCs had more leaf N (3.4%), S (0.21% and 0.23%, respectively), Mn (41 ppm and 39 ppm, respectively), and Fe (99 ppm and 116 ppm, respectively) than trees grown in CPCs (3.2%, 0.18%, 31 ppm, and 82 ppm, respectively). The leaf K content was highest (3.0%) in trees grown in NPCs and lowest (2.8%) in trees grown in CPCs. Conversely, the leaf Zn content was higher in trees grown in CPCs (34 ppm) compared with trees grown in APCs (27 ppm). The leaf Cu content was higher in trees grown in CPCs (4.8 ppm) compared with APCs and NPCs (1.8 ppm and 2.3 ppm, respectively). There was a significant interaction between container type and rootstock for P. US-812 grown in NPCs induced the highest leaf P content (0.25%), whereas the lowest leaf P content was induced by US-942 (0.22%) and US-812 (0.22%) grown in CPCs (data not shown). The rootstock cultivar did not influence the leaf nutrient content except for Mg, S, and Mn, which were higher for US-812 than US-942.

Significant differences among container types and rootstocks were also found for the root nutrient content (Table 8). The root Mg, Zn, and Cu content was significantly higher in trees grown in CPCs (0.27%, 1418 ppm, 94 ppm, respectively) compared with APCs (0.23%, 732 ppm, 20 ppm, respectively) and NPCs (0.23%, 943 ppm, 38 ppm, respectively). US-812 roots had a higher K and Mg content than US-942 roots. There were no significant interactions between container type and rootstock.

Discussion

One year after grafting, trees grown in chemical pruning CPCs were taller and had thicker rootstock trunks than the trees grown in the other containers, following the same trend evident in the nongrafted plants. Trees growing in the CPCs also had the largest aboveground biomass, but the root-to-shoot mass ratio was unaffected by the container type. Root system differences were notable for the nonfibrous root mass, which was also largest when trees were grown in CPCs. In contrast, the fibrous root mass was unaffected by the container type. The effects of air pruning on biomass partitioning and other tree biometrics were similar to chemical pruning but less in magnitude and not statistically significant. Studies on other tree species reported inconsistent results for biomass partitioning and plant growth in nursery trees subjected to different root-pruning methods. For example, Dunn et al. (1997) reported no difference in tree height for three out of five Australian tree species after growing in nursery containers coated with a water-based solution of $CuCO_3$ (50 g/L), whereas the other two experienced growth reductions. Fernández et al. (2007) found no change in the root and shoot biomasses of *Eucalyptus globulus* Labill. after 7 months of growth in $CuCO_3 \times Cu(OH)_2$ (25 g/L) coated containers, whereas Aldrete et al. (2002) found an increased shoot and root dry weight and root collar diameter for *Pinus pseudostrobus* Lindl. and *Pinus montezumae* Lamb. trees when they were grown in polybags coated with a Cu-based formulation (Spin Out). Although Arnold and Struve (1993) found no difference in the total biomass and nursery seedling height for *Quercus* sp., the root biomass increased by the end of the study when trees were grown in $Cu(OH)_2$ (100 g/L) treated containers. Amoroso et al. (2010) found a smaller root biomass in little-leaf linden trees grown in air-pruning containers for one season compared with trees grown in conventional smooth-walled containers. Similarly, Elsysy and Einhorn (2022) reported a smaller root and larger shoot biomass in different apple cultivars grown in air-pruning containers compared with field-grown liners. Genetic differences, planting material, and growing conditions are likely responsible for the variable results encountered in different studies.

Table 3. Aboveground (shoot system) biomass and below-ground (root system) biomass of 'Valencia' trees grafted on different rootstocks after 15 months of growth in chemical pruning, air-pruning, and nonpruning containers.

	Shoot system			Root system			
	Leaf biomass (g)	Scion biomass (g)	Rootstock biomass (g)	Fibrous root biomass (g)	Nonfibrous root biomass (g)	Total root biomass (g)	Root-to-shoot system ratio
Container type							
CPC	17.5 a	16.1 a	13.9 a	5.8	18.0 a	23.7	0.49
APC	15.0 ab	13.3 ab	12.1 ab	5.7	14.2 ab	19.8	0.47
NPC	12.7 b	11.2 b	10.2 b	4.6	13.3 b	17.7	0.50
P value	0.0076	0.0129	0.0154	0.1561	0.0353	0.0547	0.5403
Rootstock							
US-942	17.3 a	16.0 a	14.0 a	6.0	19.9 a	25.9 a	0.52 a
US-812	12.9 b	11.0 b	10.2 b	4.7	10.4 b	14.9 b	0.45 b
P value	0.0003	0.0083	0.0360	0.0644	0.0022	0.0133	0.0391
Container type × rootstock							
P value	0.9721	0.8028	0.5032	0.8738	0.5230	0.8242	0.1204

CPC = chemical (copper) pruning container; APC = air-pruning container; NPC = nonpruning (standard) container.

Different letters within columns indicate significant differences ($P < 0.05$) according to Tukey's honestly significant difference test.

Table 4. Stomatal conductance of 'Valencia' trees grafted on different rootstocks after 12–14 months of growth in chemical pruning, air-pruning, and nonpruning containers.

	Stomatal conductance ($mmol·m^{-2}·s^{-1}$)		
	April (12 MAT)	May (13 MAT)	June (14 MAT)
Container type			
CPC	331	285	192
APC	340	266	192
NPC	280	255	200
P value	0.0719	0.6131	0.8889
Rootstock			
US-942	324	272	200
US-812	309	265	190
P value	0.616	0.7959	0.5889
Container type × rootstock			
P value	0.8562	0.5206	0.0515

MAT = months after transplant; CPC = chemical (copper) pruning container; APC = air-pruning container; NPC = nonpruning (standard) container.

Table 5. Leaf area, specific leaf area, root hydraulic conductivity, root respiration rate, and specific root length of 'Valencia' trees grafted on different rootstocks grown after 15 months of growth in chemical pruning, air-pruning and nonpruning containers.

	Leaf area (cm^2/leaf)	Specific leaf area ($cm^2·g^{-1}$)	Root hydraulic conductivity ($kg·s^{-1}·MPa^{-1}$)	Root respiration rate (mg CO_2/g/h)	Specific root length ($m·g^{-1}$)
Container type					
CPC	50.6 a	101	7.7E-05	1.06 a	25.4 a
APC	48.1 ab	101	2.3E-05	0.85 ab	21.6 b
NPC	44.2 b	102	5.6E-05	0.74 b	23.7 a
P value	0.0436	0.8965	0.5831	0.0408	<0.0000
Rootstock					
US-942	50.8 a	99	3.8E-05	0.91	24.7
US-812	44.4 b	104	6.6E-05	0.85	22.4
P value	0.0030	0.0695	0.7442	0.5721	0.0864
Container type × rootstock					
P value	0.0943	0.9857	0.3727	0.1528	0.3673

CPC = chemical (copper) pruning container; APC = air-pruning container; NPC = nonpruning (standard) container.
Different letters within columns indicate significant differences ($P < 0.05$) according to Tukey's honestly significant difference test.

Table 6. Fibrous root length distribution by diameter range of 'Valencia' trees grafted on different rootstocks after 15 months of growth in chemical pruning, air-pruning, and nonpruning containers.

	Proportion of roots per diam range (%)				
	0–0.3 mm	0.3–0.6 mm	0.6–0.9 mm	0.9–1.2 mm	1.2–1.5 mm
Container type					
CPC	8.6 a	69 a	15 b	5.1 b	1.8
APC	6.6 b	64 b	20 a	6.8 a	2.2
NPC	7.6 ab	64 b	20 a	6.1 ab	1.7
P value	0.0025	<0.0001	<0.0001	0.0104	0.1428
Rootstock					
US-942	9.2 a	69 a	16 b	5.1 b	1.6
US-812	5.6 b	64 b	22 a	6.9 a	2.2
P value	<0.0001	0.0075	<0.0001	0.0102	0.1293
Container type × rootstock					
P value	0.3964	0.1042	0.0601	0.7248	0.5402

CPC = chemical (copper) pruning container; APC = air-pruning container; NPC = nonpruning (standard) container.
Different letters within columns indicate significant differences ($P < 0.05$) according to Tukey's honestly significant difference test.

Based on the coordination model (Chen and Reynolds 1997), trees can balance the shoot-to-root biomass ratio to maximize the growth rate. It suggests that plant assimilates are allocated to either roots or shoots depending on source-sink relationships and specific growth-limiting factors. During the early developmental stages, the primary sinks are usually the roots and young leaves (Wardlaw 1990). In addition, resources can be reallocated as an adaptative response to different external stresses (Lynch et al. 2021). In our study, chemical root pruning seemed to have reallocated carbon to produce more nonfibrous, higher-order roots. Montagnoli et al. (2022) noted that the reallocation of fibrous root production to production of thicker, higher-order roots is a common response to Cu pruning. However, Montagnoli et al. (2022) also noted that root responses are influenced by the depth within the container. In contrast to our study, that study also measured a significant increase in the root-to-shoot ratio in response to Cu root pruning.

In addition to an increase in the nonfibrous root and shoot biomass, the leaf area per leaf was larger in trees grown in CPCs. A larger leaf area increases the tree's photosynthetic capacity and carbon assimilation (Milla and Reich 2007; Tholen et al. 2012). Thus, Cu pruning of citrus plants during the nursery stage may produce more vigorous trees, promoting growth and physiological performance after transplant into the field. For example, Arnold and Struve (1989) observed enhanced shoot growth in green ash (*Fraxinus pennsylvanica* Marsh.) and red oak (*Quercus rubra* L.) trees after two seasons of field growth when trees had been grown in containers treated with $CuCO_3$ (100 g/L); however, tree responses may be species-specific. A study by Struve (1993) found that red oak and scarlet oak (*Quercus coccinea* Muenchh.) trees produced in $Cu(OH)_2$ (100 g/L)-treated containers grew more during 3 years of field growth than untreated trees, whereas no effects were observed in sweetgum trees (*Liquidambar styraciflua* L.). In our study, in

Table 7. Leaf macro- and micronutrient content of 'Valencia' trees grafted on different rootstocks after 15 months of growth in chemical pruning, air-pruning, and nonpruning containers.

	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)	B (ppm)	Zn (ppm)	Mn (ppm)	Fe (ppm)	Cu (ppm)
Container type											
CPC	3.2 b	0.22 b	2.8 b	2.4	0.35	0.18 b	67	34 a	31 b	82 b	4.8 a
APC	3.4 a	0.23 ab	2.9 ab	2.6	0.36	0.21 a	66	27 b	39 a	99 a	2.3 b
NPC	3.4 a	0.24 a	3.0 a	2.6	0.37	0.23 a	68	30 ab	41 a	116 a	1.8 b
P value	0.0001	0.0118	0.0098	0.2285	0.1549	<0.0000	0.9756	0.0022	0.0001	0.0008	<0.0000
Rootstock											
US-942	3.4	0.23	2.9	2.5	0.34 b	0.19 b	66	30	34 b	102	2.3
US-812	3.3	0.23	3.0	2.5	0.39 a	0.22 a	67	31	40 a	96	3.5
P value	0.2850	0.2995	0.2852	0.9793	0.0193	0.0070	0.5463	0.4983	0.0057	0.5614	0.5880
Container type × rootstock											
P value	0.1564	0.0135	0.0655	0.2403	0.7998	0.3187	0.4565	0.4290	0.4798	0.1606	0.1581

CPC = chemical (copper) pruning container; APC = air-pruning container; NPC = nonpruning container.

Different letters within columns indicate significant differences ($P < 0.05$) according to Tukey's honestly significant difference test.

contrast to the leaf area, the specific leaf area was unaffected by the container type, suggesting no additional costs were required for trees to increase their leaf area while maintaining the photosynthetic capacity (Dwyer et al. 2014).

Although there was no difference in the fibrous root biomass among treatments, trees grown in CPCs had a higher proportion of finer, smaller-diameter roots and a higher SRL, while the other trees had a higher proportion of thicker, larger-diameter roots and a lower SRL. The SRL characterizes the root benefits (resource acquisition) to root cost (maintenance and construction) (Ostonen et al. 2007). The higher SRL found for trees grown in CPCs suggests that Cu pruning reduces the carbon expense while producing more finer roots that are more efficient in resource exploration and have a higher degree of plasticity (Eissenstat 1992; Lynch et al. 2021). Arnold and Struve (1989) observed that green ash and red oak root systems were more fibrous and evenly distributed throughout the growing medium in $CuCO_3$ (100 g/L)-treated containers than in nontreated containers. Similarly, Tsakaldimi and Ganatsas (2006) reported a more fibrous root system with significantly more laterals in *Pinus halepensis* Mill. seedlings grown in $CuCO_3 \cdot Cu(OH)_2$ (8.3 g/L and 33 g/L)-coated containers than in noncoated containers, and differences were more pronounced at when the Cu concentration was higher.

Trees with more fine roots can likely better adapt to changing soil environments, increasing the likelihood of successful establishment after transplant. Finer roots generally also have a higher respiration rate than coarser roots (Desrochers et al. 2002; Makita et al. 2009; Pregitzer et al. 2002). Accordingly, we found a significantly higher respiration rate for roots growing in the CPCs than in the other containers. The respiration rate represents the metabolic and physiological activity of the plant. A higher rate corresponds to more energy production to support growth and maintenance functions (Bahn et al. 2006; Han and Zhu 2021). Metabolically active roots are more efficient in nutrient and water uptake and, subsequently, plant growth (Bais et al. 2001). The positive effect of chemical pruning on the fibrous root physiology further underscores the potential benefits of this production system. Cu is an essential micronutrient and a cofactor for several proteins and enzymes involved in plant photosynthetic and respiratory processes (Burkhead et al. 2009), which may have contributed to the physiological effects measured in this study.

Under the growing conditions of this study all trees had optimum leaf macro- and micronutrient levels per the recommendations for citrus (Obreza et al. 2020). Nevertheless, trees grown in CPCs had less leaf N, K, S, Mn, and Fe than the other trees despite having a larger proportion of finer roots. Nutrient remobilization, especially of mobile nutrients, from leaves to roots as a response to the root pruning and the resulting regeneration of roots in combination with nutrient dilution due to the larger biomass may be responsible for this finding. Nutrient dilution in response to Cu treatments was also observed in *P. halepensis* seedlings, but only for Mg and Ca (Tsakaldimi and Ganatsas 2006). In contrast, trees grown in CPCs in our study had a higher leaf Cu and Zn content, and both metals were also increased in the roots. Cu and Zn are taken up and transported inside the plant by the same ZIP family transporters (Ajeesh Krishna et al. 2020), and Zn ions may have been absorbed by the roots along with the Cu released from the container walls. It should be noted that none of the trees showed Cu toxicity throughout the study. In contrast to the effects of container type on the nutrient balance, we did not measure any effects on the tree stomatal conductance and hydraulic conductivity, suggesting that differences in nutrients were not caused by any effects on the water balance and resource uptake capacity.

Both rootstocks used in this study are considered HLB-tolerant and are more productive with 'Valencia' scion than some other rootstocks in Florida (Bowman and Albrecht 2020; Bowman et al. 2016; Singerman et al. 2021). In the nongrafted stage, there were significant differences in tree height, trunk diameter, and the root respiration rate between the two rootstocks. US-942 was more vigorous but had a lower root respiration rate than US-812. Grafted trees on US-942 followed a similar pattern and were taller, with thicker trunks, more biomass, and more leaf area. The stomatal conductance, root hydraulic conductivity, and fibrous root biomass were

Table 8. Root macro- and micronutrient content of 'Valencia' trees grafted on different rootstocks after 15 months of growth in chemical pruning, air-pruning, and nonpruning containers.

	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)	B (ppm)	Zn (ppm)	Mn (ppm)	Fe (ppm)	Cu (ppm)
Container type											
CPC	2.8	0.41	2.2	1.1	0.27 a	0.16	18	1418 a	930	241	94 a
APC	3.0	0.38	2.5	1.1	0.23 b	0.18	19	732 b	1115	244	20 b
NPC	2.9	0.41	2.3	1.1	0.23 b	0.16	18	943 b	1074	255	38 b
P value	0.3274	0.4762	0.0977	0.9519	0.0062	0.2938	0.1881	0.0037	0.1439	0.7390	0.0005
Rootstock											
US-942	3.0 a	0.40	2.1 b	1.1	0.22 b	0.15 a	18	946	1025	257	38
US-812	2.8 b	0.40	2.5 a	1.1	0.27 a	0.18 a	19	1116	1055	236	62
P value	0.0463	0.7108	0.0009	0.2890	0.0002	0.0277	0.4530	0.3645	0.6797	0.1487	0.1180
Container type × rootstock											
P value	0.0948	0.7402	0.8644	0.9998	0.4699	0.077	0.5669	0.7242	0.4739	0.3583	0.0624

CPC = chemical (copper) pruning container; APC = air-pruning container; NPC = nonpruning (standard) container.

Different letters within columns indicate significant differences ($P < 0.05$) according to Tukey's honestly significant difference test.

similar for both rootstocks. Although rootstock liners differed in their root respiration rate, no difference was observed in grafted trees at the end of the study. The difference between rootstocks before grafting can be attributed to their inherent genetic variation in the absence of any influence from the scion. Grafting can influence plant physiological traits (Mauro et al. 2022; Rasool et al. 2020) and the graft union can act as a barrier for xylem and phloem translocation pathways (Martínez-Ballesta et al. 2010), which may have affected root respiration. The rootstock also influenced some of the leaf and root nutrients, which were generally lower in content in trees with US-942 despite it having a higher proportion of roots in the lower diameter range compared with US-812. The lower leaf nutrient concentration in US-942 may be the result of nutrient remobilization to support the larger growth.

Whether the positive attributes of root pruning will translate into any lasting differences in citrus tree health and growth under the HLB-endemic conditions in Florida remains to be determined. Field studies in other tree species and different environmental conditions have revealed mixed results. For example, the beneficial effects of Cu root pruning did not translate into a better field performance of *Eucalyptus globulus* trees (Fernández et al. 2007), while *Pinus halepensis* trees exhibited a larger stem volume 2 years after transplant (Tsakaldimi and Ganatsas 2006). Only minor differences in tree size, root length, and root volume were measured for Cu root-pruned *Pinus ponderosa* trees after 3 decades of field growth (Dumroese et al. 2022), whereas air root-pruned *Quercus bicolor* Willd. trees were larger and heavier than bare-rooted trees after 10 years of field growth (Sambeek et al. 2016).

Conclusion

Chemical root pruning of citrus trees in the nursery by using Cu-coated containers resulted in growth improvements compared with air pruning and no pruning, regardless of the rootstock cultivar. The larger biomass produced by the chemical root pruning suggests these trees have more available carbohydrate reserves for remobilization and immediate use upon transplant to the field. The improved fibrous root physiological traits (finer, and metabolically more active roots) may promote tree establishment by enhancing the nutrient uptake capacity after field transplant, as was demonstrated in other tree studies. Aside from the potential benefits for field establishment, the larger biomass induced by the chemical root pruning may enable nurseries to reduce the time needed to produce field-ready trees. Although air pruning induced some similar growth trends as Cu pruning, effects were not statistically different from no pruning. Regardless of the expected effects on tree establishment and field performance, self-pruning containers are likely advantageous over regular, nonpruning containers for use in long-term citrus nursery production systems, such as for maintenance of bud source trees and variety collections.

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